Embryoscopic and cytogenetic analysis of 233 missed abortions: factors involved in the pathogenesis of developmental defects of early failed pregnancies

T.Philipp1,4, K.Philipp1, A.Reiner2, F.Beer2 and D.K.Kalousek3

1Ludwig Boltzmann Institute of Clinical Gynecology and Obstetrics and 2Cytogenetic Laboratory, Department of Pathology, Danube Hospital, Langobardenstrasse 122, 1220 Vienna, Austria and 3Cytogenetic Laboratory, Department of Pathology, B.C. Children’s Hospital, 4480 Oak Street, Vancouver BC, V6H 3V4, Canada

4To whom correspondence should be addressed. E-mail: thomas.philipp@wienkav.at

BACKGROUND: While chromosomal abnormalities are often the cause of missed abortions, other defects could be involved, which might be screened for by transcervical embryoscopy. METHODS: A total of 272 patients with missed abortion underwent transcervical embryoscopy prior to dilatation and curettage, together with cytogenetic analysis of chorionic villi, using either standard G-banding cytogenetic techniques or comparative genomic hybridization in combination with flow cytometry analysis. RESULTS: Visualization of the embryo or early fetus (12 cases) was successful in 233 patients, and karyotyping in 221. Among 233 examined cases, 33 had normal external features, 71 were classified as growth-disorganized and 129 had either isolated or multiple defects, including holoprosencephaly, anencephaly, encephalocele, spina bifida, microcephaly, facial dysplasia, limb reduction defect, cleft hand, syndactyly, pseudosyndactyly, polydactyly, various forms of cleft lip and an amniotic adhesion. Of the 165 cases with an abnormal karyotype, there were 46 grossly disorganized embryos, 98 multiple defects, six single defects and 15 morphologically normal cases. Of the 56 cases with a normal karyotype, there were 20 grossly disorganized embryos, 16 multiple defects, four single defects and 16 morphologically normal cases. CONCLUSIONS: A total of 75% of the cases with missed abortion had an abnormal karyotype, 18% had a morphological defect with a normal karyotype, while no embryonic or chromosomal abnormality could be diagnosed in 7% of the cases. Correlation of morphological and cytogenetic findings in spontaneous abortion specimens could provide valuable information for genetic counselling and prenatal care in future pregnancies in couples with a history of repeated pregnancy loss.

Key words: chromosome abnormalities/developmental defects/missed abortion

Introduction
Approximately 15% of all clinically recognized pregnancies are spontaneously aborted and ~60–70% of these are attributable to detectable chromosome abnormalities (Tariverdian and Paul, 1999).

Although the incidence of first trimester losses is high, spontaneous abortion material is often poorly described from a developmental perspective. More than one-half of early spontaneous abortion specimens contain no embryonic/fetal parts. If an embryo is present at all, it is often either severely damaged or fragmented (Kalousek, 1987). Transcervical embryoscopy in cases of missed abortion is a new technique that allows direct visualization of the dead embryo in utero, unaffected by the damage caused by either instrumental evacuation or spontaneous passage.

With respect to the various possible aetiologial factors of developmental defects in early abortion specimens, cytogenetic analysis is an important component in the assessment of human malformation in early failed pregnancies. The detection of aneuploidy/polyplody provides a causal explanation for the observed developmental defect and also indicates that the risk of recurrence of the observed developmental defect and chromosomal abnormality in these couples is not substantially increased (Warburton et al., 1987).

We have previously reported the detection of 48 growth-disorganized embryos in cases of embryoscopically examined missed abortion (Philipp and Kalousek, 2002). Ten selected cases of embryonic neural tube defect documented that the technique of embryoscopy offers the possibility of accurately diagnosing developmental defects in cases of early pregnancy loss (Philipp and Kalousek, 2001a,b,c).

The objective of this study was to estimate the frequency of a chromosomal abnormality or hitherto unexplained mechanism in the pathogenesis of external structural abnormalities of the
first trimester conceptus. Indications for more extensive morphological examination of first trimester abortion specimens are discussed.

Materials and methods
A missed abortion was diagnosed in a total of 272 patients. The condition was established by sonography and the women were scheduled for elective dilatation and curettage (D&C) at the Danube Hospital, Vienna between April 1999 and September 2002. All of these cases were included in the present study which was approved by the ethics committee of the hospital. Informed consent for embryoscopy was obtained from all patients. The diagnosis of missed abortion was based on sonographic demonstration of an embryo or early fetus without cardiac activity on transvaginal ultrasonography (7.5 MHz transvaginal probe). The threshold separating embryos from fetuses was set at 30 mm crown–rump (CRL), which corresponds to ~8 completed weeks of development.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Total specimens</th>
<th>Total specimens successfully karyotyped</th>
<th>Specimens with abnormal karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Normal</td>
<td>33</td>
<td>14.2</td>
<td>31</td>
</tr>
<tr>
<td>Growth disorganization</td>
<td>71</td>
<td>30.5</td>
<td>66</td>
</tr>
<tr>
<td>Combined defects</td>
<td>119</td>
<td>51.1</td>
<td>114</td>
</tr>
<tr>
<td>Isolated defects</td>
<td>10</td>
<td>4.3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>233</td>
<td>100</td>
<td>221</td>
</tr>
</tbody>
</table>

Table I. Summary of specimen morphology and karyotypic outcome in 233 missed abortions

*Percentage of total number of specimens with that morphology.
*Percentage of each morphological category successfully karyotyped.
*Percentage of each morphological category with an abnormal karyotype.

Figure 1. Close-up of a 4 mm crown–rump length growth-disorganized (GD) embryo. The GD 2 embryo showed no recognizable external structures after the amnion (A) was opened. Cytogenetically, trisomy 16 (47,XX,+16) was diagnosed.

Figure 2. A growth-disorganized (GD) 2 embryo, with a crown–rump length of 6 mm, in the intact amniotic sac (A). The yolk sac (Y) is clearly discernible. A normal karyotype was diagnosed cytogenetically (46,XY).

Accurate diagnosis of a specific defect present in an embryo or early fetus depends on correct evaluation of the developmental stage. The term gestational age, used in clinical terminology and ultrasound, was not used in this study of missed abortions, as most of these specimens were retained in utero. Instead, the term the developmental age (DA) was used. The actual DA was derived from the CRL, measured by ultrasonography, and from the developmental stage assessed by embryoscopy (Moore, 1993).

All patients were given general anaesthesia and placed in a dorsal lithotomy position. After careful dilatation of the cervix, the rigid hysteroscope (12° angle of view with both biopsy and irrigation working channel, Circon Ch 25–8 mm) was inserted transcervically into the uterine cavity and the implantation site of the pregnancy was visualized. Continuous normal saline flow was used throughout the procedure (pressure ranging from 40 to 120 mmHg) to clean the operative field. The chorion was opened with microscissors (CH 7–2 mm) and the embryo was initially viewed through the amnion. The amnion was then carefully opened using the microscissors to obtain a
detailed view of the embryo. A complete examination of the conceptus included visualization of the head, face, dorsal and ventral walls, limbs and umbilical cord. All procedures were viewed on a TV monitor by connecting a video camera (3-CCD Colour Camera, Circon Microdigital III) to the eyepiece of the endoscope, and were recorded for future analysis. Video-documentation of embryoscopically detected abnormalities helped investigators to cooperate with an experienced embryopathologist.

The embryoscopic findings were classified into four categories: (i) normal development; (ii) growth-disorganized embryos; (iii) specimens with multiple external defects; (iv) specimens with isolated external defects. Growth-disorganized embryos were further subdivided, based on their degree of disorganization (Poland et al., 1981).

After evacuation of the uterus, chorionic villi were separated from decidual contamination and blood clots, cultured and analysed cytogenetically using standard G-banding cytogenetic techniques. Comparative genomic hybridization in combination with flow cytometry analysis (CGH/FCM) of paraffin-embedded or frozen placental tissue was performed in 51 cases in which traditional cytogenetic analysis had failed to provide results (Lomax et al., 2000).

Results

The procedure of embryoscopy required an average of 10 min (range 3–25). A complete anatomical survey was possible in 233 cases.

In 15 cases the embryonic structure could not be identified after the chorion had been opened and in 24 cases a complete evaluation of the embryo was not possible because the investigator’s vision was obscured. The causes were bleeding, a tight amniotic sac, or a short umbilical cord closely attaching the embryo to the chorionic plate and therefore hindering the examination.

Table I provides a general description of 233 studied cases. Fifteen of these were early fetuses, with a CRL of >30 mm

Figure 3. Lateral (A) and close-up of the face (B) of a trisomy 4 (47,XX,+4) growth-disorganized (GD) embryo. The GD4 embryo 11 mm in length shows a small head and a dysplastic face. There is evidence of upper (UL) and lower (LL) limb growth retardation relative to the crown–rump length.

Figure 4. Lateral view of a growth-disorganized (GD) 4 embryo with a crown–rump length of 10 mm. Retarded upper (UL) and lower (LL) limb bud development is visible; no facial structures can be seen. U = umbilical cord. A normal karyotype was diagnosed cytogenetically (46,XY).

Figure 5. Lateral view of a microcephalic embryo 16 mm in length with fusion of the face to chest and retarded limb development. The karyotype showed tetraploidy (92,XXYY).
range 32–57). Table I shows that no external abnormalities were found in 33 cases (14%), whereas abnormal development was seen in 200 (86%) missed abortions. Among the abnormal cases, embryonic growth disorganization (GD2–4) was reported in 71 cases. GD2 embryos showed embryonic tissue 3–5 mm in length. These conceptuses had no recognizable external embryonic landmarks and no retinal pigment (Figure 1 and Figure 2). GD3 embryos were ≤10 mm long, lacked limb buds but retinal pigment was often present. A cephalic and caudal pole could be distinguished. GD4 embryos had a CRL of >10 mm with a discernible head, trunk and limb buds. The limb buds showed marked retardation in development and the development of the facial structures was highly abnormal (Figures 3 and 4).

A total of 119 cases showed no disorganization of development, but had severe combined developmental defects such as: (i) fusion of the face to the chest in combination with microcephaly and retarded limb development (13 cases) (Figure 5), (ii) severe microcephaly, facial dysgenesis, retarded limb development and often a short umbilical cord (41 cases) (Figures 6 and 7), (iii) microcephaly and retarded limb development (Table I).
development (32 cases) (Figure 8) and (iv) specific developmental defects similar to those seen in fetuses or newborns (30 cases) (Figures 9, Figure 10 and Figure 11). These specific defects were all associated with other developmental defects such as microcephaly, facial dysgenesis, delayed limb development and face-to-chest fusion, and included holoprosencephaly (one case), anencephaly (two cases), encephalocele (10 cases), spina bifida (10 cases), various forms of cleft lip (three cases), limb reduction defect (two cases), cleft hand (one case) and an amniotic adhesion (one case).

In three cases amniotic bands caused combined defects which were discernible on embryoscopy. The spectrum of defects seen in one embryo and two early fetuses with amniotic band syndrome included constrictions of the digits, pseudo-syndactyly due to wrapping of fingers and toes, umbilical cord stenosis, gastrochisis and omphalocele.

Ten specimens had isolated developmental defects (Figure 12) including anencephaly (one case), microcephaly (two cases), polydactyly (one case), limb reduction defect (one case) and retarded development of the limbs (five cases).

Of the 233 cases studied on embryoscopy, a successful cytogenetic evaluation was performed in 221 cases (95%; Table I). A total of 165 (75%) specimens were abnormal, of which 101 (61%) were trisomic, 37 (22%) monosomic X, 19 (12%) polyploid and eight (5%) were structural chromosome anomalies. Trisomies for all chromosomes with the exception

---

**Figure 8.** Anterolateral view of a microcephalic 45,X embryo with a crown–rump length of 25 mm. Distinct grooves are formed between the fingers, but the digits are not separated and the upper limbs are not bent at the elbows, indicating retarded development for an embryo of this size.

**Figure 9.** Lateral view of a triploid embryo (69,XXY) 15 mm in length. A large neural tube defect involving the lumbosacral area (arrow) is present. There is evidence of upper limb growth retardation relative to the crown–rump length. The face is fused to the abdominal wall. The dark brown area in the frontal region is due to necrosis. Herniation of the mid-gut into the umbilical cord is still physiological at this stage of development.

**Figure 10.** (Case 8, Table III.) Lateral (A) and posterior view (B) of an embryo with a crown–rump length of 28 mm. Note the absence of normally developed eyes of the microcephalic embryo (A). A spina bifida involving the lumbar area (arrow) is present (B). The karyotype was normal (46,XY).
of chromosomes 1, 5 and 19 were observed. The most common trisomy was 15 (17 cases), followed by trisomies 16 (16 cases), 21 (15 cases), 22 (14 cases), 14 (seven cases), 13 (five cases), 8 (five cases) and 9 (five cases). Correlations of morphology and specific cytogenetic findings are shown in Table II.

The highest rate of chromosome anomalies was found in the category of 119 conceptuses with combined developmental defects. A successful cytogenetic evaluation in this subgroup was performed in 114 cases. Chromosomal abnormalities were found in 98 cases (86%; Table I). Specific cytogenetic findings among abortuses with severe combined developmental defects are listed in Table II.

Among the 71 grossly disorganized embryos, 66 could be analysed cytogenetically. Of these, 46 (70%; Table I) were cytogenetically abnormal; the data are shown in Table II.

The lowest rate of chromosomal abnormality was found in phenotypically normal specimens and in specimens with isolated defects (see Tables I and II). Of 33 cases with normal external features, 31 could be analysed cytogenetically. Cytogenetic results showed abnormality in 15/31 (48%) cases of chromosomal abnormalities were found in 98 cases (86%; Table I). Specific cytogenetic findings among abortuses with severe combined developmental defects are listed in Table II.

The highest rate of chromosome anomalies was found in the category of 119 conceptuses with combined developmental defects. A successful cytogenetic evaluation in this subgroup was performed in 114 cases. Chromosomal abnormalities were found in 98 cases (86%; Table I). Specific cytogenetic findings among abortuses with severe combined developmental defects are listed in Table II.

Among the 71 grossly disorganized embryos, 66 could be analysed cytogenetically. Of these, 46 (70%; Table I) were cytogenetically abnormal; the data are shown in Table II.

The lowest rate of chromosomal abnormality was found in phenotypically normal specimens and in specimens with isolated defects (see Tables I and II). Of 33 cases with normal external features, 31 could be analysed cytogenetically. Cytogenetic results showed abnormality in 15/31 (48%) cases.
in this subgroup. Six of 10 specimens with isolated defects showed chromosomal abnormalities (60%).

**Discussion**

The morphological features of a consecutive series of 233 missed abortions are described in this report.

Of 165 cases with an abnormal karyotype, 150 (91%) showed abnormal development (46 GD embryos, 98 multiple defects, six single defects) and in 15 cases no external embryonic abnormalities could be detected on embryoscopy. The grossly abnormal development documented by embryoscopy in the majority of these aneuploid specimens suggests a severe disturbance in their early development and shows that early stages of human development are particularly vulnerable to genetic disorders.

Of the 56 cases with a normal karyotype, no external embryonic abnormalities could be detected in 16 cases, whereas amniotic bands (cases 2, 10, 13; Table III) interfered with normal embryonic development in three cases.

Thus, there were 37 cases (20 growth disorganized embryos, 13 specimens with multiple developmental defects and four cases with isolated defects) with an apparently normal karyotype and a maldevelopment similar to that resulting from aneuploid syndromes, without the diagnosis of a specific pathogenetic mechanism. Table III provides a detailed morphological description of 13 cases with combined defects (cases 1, 4, 6–9, 11, 12, 14, 15, 18–20) and four specimens with isolated defects (cases 3, 5, 16, 17) and an apparently normal karyotype.

Embryonic development is a precisely choreographed event of programmed developmental steps, requiring many genes to regulate growth and morphogenesis. The grossly abnormal development documented by embryoscopy in these cases with apparently normal chromosomes was as severe as that resulting from an aneuploidy. They might have been due to genetic lesions that prevent normal embryogenesis and are undetectable by the techniques used in the present study.

These factors are usually not considered to be aetiologically related to early pregnancy loss, as there has been a tendency in the past to assume that if no laboratory test confirms the presence of a genetic disorder, one should search for non-genetic causes.

Embryoscopy in cases of missed abortion might reveal subtle morphological abnormalities undetectable by ultrasound (Blaas, 1999) and expand the diagnostic spectrum used for the evaluation of reproductive loss. This technique could establish a highly characterized cohort of abortion specimens with apparently normal chromosomes as a starting point for further detailed genetic studies. Such studies are needed to reach a better understanding of embryopathogenesis and, consequently, of early pregnancy loss itself.

Whether embryoscopy and cytogenetic studies should be offered to all women with missed abortion is debatable. This policy has the advantage of providing comprehensive aetiological data, but has the disadvantage of requiring an invasive
procedure and of inducing extra costs for the management of a condition with a low risk of recurrence.

However, a detailed embryoscopic examination of the dead embryo is likely to be useful in couples who have experienced recurrent abortion. In such cases, chromosome analysis is generally recommended (Wolf and Horger, 1995), and an elevated risk of birth defects in subsequent pregnancies was recorded (Khoury and Erickson, 1993). Therefore, transcervical embryoscopy could be indicated prior to D&C or medically induced abortion (Blanch et al., 1998; Lelaider et al., 1993).

### Table III. Summary of embryoscopic and clinical data of 16 specimens with severe combined developmental defects, and four embryos with isolated developmental defects and an apparently normal karyotype

<table>
<thead>
<tr>
<th>Case no.</th>
<th>CRLa (mm)</th>
<th>Karyotype</th>
<th>Description</th>
<th>Maternal age (years)</th>
<th>Parity</th>
<th>Spontaneous abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>19</td>
<td>46,XX</td>
<td>Macerated microcephalic embryo with retarded limb development, mid-line brownish pigmentation in the frontal region, umbilical cord cyst</td>
<td>28</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>2c</td>
<td>35</td>
<td>46,XX</td>
<td>Early fetus with amnion adhesion at the tip of the nose, strands of amnion wrapped around the terminal phalanges of both feet</td>
<td>30</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>46,XY</td>
<td>Macerated microcephalic embryo with no other apparent abnormalities</td>
<td>40</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>46,XY</td>
<td>Macerated embryo, severe microcephaly, facial dysplasia, absence of cervical flexion, retarded limb development, bilateral cleft lip</td>
<td>37</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>46,XY</td>
<td>Macerated well-preserved embryo with generalized oedema, severe microcephaly and an unusually large physiological umbilical hernia</td>
<td>38</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>46,XY</td>
<td>Microcephalic embryo closely attached to the amnion, fusion face to the chest, retarded limb development</td>
<td>24</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>46,XX</td>
<td>Macerated embryo, severe microcephaly, facial dysplasia, retarded limb development</td>
<td>32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>46,XY</td>
<td>Microcephalic embryo with no eyes, large open neural tube defect of the lumbar spine</td>
<td>35</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>46,XX</td>
<td>Microcephalic embryo with a dysplastic face and retarded limb development</td>
<td>29</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>46,XX</td>
<td>Fine amniotic bands wrapping the digits of both hands, umbilical cord structure, gastrochisis</td>
<td>17</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>21</td>
<td>46,XY</td>
<td>Microcephaly, parietal encephalocele, limb reduction defect affecting all limbs</td>
<td>23</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>46,XX</td>
<td>Macerated microcephalic embryo with a dysplastic midface and a large frontal encephalocele</td>
<td>35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>39</td>
<td>46,XX</td>
<td>Early fetus with a large omphalocele, strands of amnion wrapped around the terminal phalanges of the right hand, constricting band around the umbilical cord</td>
<td>20</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>46,XY</td>
<td>Microcephaly, fusion of the face to the chest, retarded limb development</td>
<td>30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>46,XX</td>
<td>Microcephaly, fusion of the face to the chest, retarded limb development</td>
<td>29</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>24</td>
<td>46,XY</td>
<td>Transverse limb reduction defect affecting digit IV of both hands</td>
<td>28</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>22</td>
<td>46,XX</td>
<td>Anencephaly with spinal rachischisis</td>
<td>34</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>46,XY</td>
<td>Macerated embryo, severe microcephaly, facial dysplasia, retarded limb development</td>
<td>31</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>11</td>
<td>46,XX</td>
<td>Microcephalic embryo with retarded limb development and a large neural tube defect involving the lumbosacral area</td>
<td>37</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>46,XX</td>
<td>Microcephaly, fusion of the face to the chest, retarded limb development</td>
<td>24</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

aCrown–rump length.
bAlso reported in Philipp and Kalousek (2001c).
cAlso reported in Philipp and Kalousek (2001a).

### References


Blanch, G., Quenby, S., Ballantyne, E.S., Gosden, C.M., Neilson, J.P. and Holland, K. (1998) Embryonic abnormalities at medical termination of...


Submitted on May 24, 2002; resubmitted on September 23, 2002; accepted April 16, 2003