PREGNANCY LOSS

Transcervical Embryoscopy in Missed Abortion

THOMAS PHILIPP^{1,3} and DAGMAR K. KALOUSEK²

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Purpose: The present study was conducted to determine the usefulness of transcervical embryoscopy in diagnosing localized and systemic defects in embryonic morphogenesis of missed abortions.

Methods: The study population consisted of 24 women with the final diagnosis of missed abortion. Prior to the instrumental evacuation of the uterus a rigid hysteroscope was passed transcervically into the amniotic cavity to obtain a detailed view of the embryo. Karyotyping was attempted in all cases included in this study.

Results: An embryo could be visualized in 19 cases. Ten embryos showed multiple developmental defects.

Conclusions: In cases of early failure of pregnancy, embryoscopy permits visualization of the embryo in utero, unaffected by the damage usually caused by its instrumental evacuation or spontaneous passage. This technique can be a helpful tool for pathologists and geneticists in enhancing their understanding of human embryonic malformations, but more importantly, it improves clinical care and follow-up, especially in cases of repeated abortions.

KEY WORDS: embryoscopy; karyotype; missed abortion; morphologic defects in embryos.

INTRODUCTION

About 20% of clinically recognized pregnancies are aborted, the majority of these being early spontaneous pregnancy losses before 12 gestational weeks (1). It is generally accepted that more than one-half of all embryonic losses are associated with an abnormal karyotype (2). Additionally, it is known that these abortuses show a high rate of phenotypic abnormalities (3). The increasing use of high-frequency endovaginal probes has enabled the physician to diagnose the failure of pregnancy prior to spontaneous passage of the products of conception. This allows for the possibility of elective dilation curettage (D&C), followed by submission of the products of conception for karyotyping and detailed morphological and genetic examination of early spontaneous abortion specimens by a pathologist.

Morphological examination of the conceptus consists of gross and microscopic examination of the gestational sac and the embryo and sampling for cytogenetic studies. It provides important information concerning the developmental age, morphology, and genetic makeup of the conceptus. This information serves as a basis for counseling parents, especially when early pregnancy loss is a repeated event. Unfortunately, more than one-half of early abortion specimens are difficult to evaluate morphologically because they are either incomplete or damaged, due mainly to the instrumental evacuation of the uterus (4,5).

Currently, the only method which allows direct visualization of the embryo in utero is embryoscopy. The procedure documents developmental events prior to instrumental evacuation, and the technology

¹ Ludwig Boltzman Institute of Gynecology and Obstetrics, Danube Hospital, Langobardenstrasse 122, 1220 Vienna, Austria. Fax: ++43 1 28802 3880. E-mail: thomas.philipp@smz.magwien.gv.at.

² Cytogenetic Laboratory, Department of Pathology, B.C. Children's Hospital, 4480 Oak Street, Vancouver, B.C. V6H 3V4, Canada. Fax: +604 875 3601. E-mail: d.kalousek@cw.bc.ca.

³ To whom correspondence should be addressed.

has also been used to confirm congenital anomalies detected by ultrasonography in the early second trimester (6).

The present study was conducted to determine the usefulness and limitations of transcervical embryoscopy in diagnosing localized and generalized defects in the embryonic morphogenesis of missed abortions. The embryoscopic findings are supplemented by the results of cytogenetic analysis in most cases.

MATERIALS AND METHODS

The study population consisted of 24 women who were admitted to the Danube Hospital (Vienna) between April 1999 and January 2000 with the final diagnosis of missed abortion and who were scheduled for elective D&C. The study was approved by the ethics committee of the hospital and informed consent for embryoscopy was obtained from the patients.

Transvaginal ultrasonograms were performed using Logiq @ 200 (GE Medical Systems) with a 7.5-MHz transvaginal probe. A missed abortion was diagnosed on ultrasound as an embryo without cardiac activity, based on Hansmann's definition (15). Only failed pregnancies with an embryo measuring 30 mm or less in crown-rump length (CRL) were included. Procedures performed on failed pregnancies showing an anembryonic sac (seven cases) or a dead fetus (one case) were excluded. The gestational age was determined from the last menstrual period (LMP) to the day of curettage. The actual developmental age (DA) of the embryos was derived from their CRL, measured ultrasonographically, and their developmental stage was evaluated embryoscopically (7). The difference between the actual developmental age and the gestational age usually reflects the duration of embryonic retention in utero after its death.

Embryoscopy as well as subsequent curettage was performed under intravenous general anesthesia. The examinations were carried out by two obstetricians with a particular interest in endoscopic techniques. The patient was placed in a dorsal lithotomy position. A speculum was inserted into the vagina, which had been cleansed with betadine solution. After careful dilatation of the cervix, the rigid hysteroscope (12° angle of view with both the biopsy and the irrigation working channel; Circon Ch 25–8 mm) was passed transcervically into the chorionic cavity. A continuous normal saline flow was used throughout the procedure (pressure, 40–120 mm Hg) to help distend

and clean and, thus, provide a clear view. The chorion was opened with a microscissor (Ch 7–2 mm) and the embryo was first viewed through the amnion. Then the amnion was carefully opened with the microscissor to get a detailed view of the embryo. All procedures were viewed on a TV monitor by connecting a videocamera (3-CCD Colour Camera-Circon Microdigital III) to the eyepiece of the endoscope and were recorded for later analysis.

The embryoscopic findings were classified into four categories:

- (a) normally developed embryos,
- (b) growth-disorganized embryos,
- (c) embryos with isolated or multiple external defects, and
- (d) macerated embryos.

Growth-disorganized embryos were further subdivided, based on their degree of disorganization (8). The diagnosis of isolated or combined localized external defects was made after evaluation of the embryonic developmental stage by an experienced embryopathologist.

Karyotyping was attempted in all cases included in this study. The chorionic villi were cleansed from decidual contamination and analyzed, using standard Q-banding cytogenetic techniques. Comparative genomic hybridization in combination with flow cytometry (CHG/FCM) was performed from the paraffin-embedded placental tissue in all cases in which the traditional cytogenetic analysis failed to provide the results (9).

RESULTS

The embryoscopy procedure required an average of 13 min (range, 5–25 min). No uterine perforation or maternal morbidity was observed. An embryo could be visualized in 19 cases. In three cases, the embryo was viewed only through the amnion (cases 2, 7, and 10 in Table I). Table I provides a summary of the embryonic morphology and cytogenetic findings in these cases. In four cases, the embryo could not be visualized due to the inability to detect the embryonal structure after opening the chorion. In one case the view was obscured by bleeding.

The average age of the women experiencing missed abortion was 30 years (range, 19–40 years) and the average in utero retention time was 2 weeks (range, 0–4 weeks). Four patients had a history of repeated early spontaneous abortions (cases 5, 7, 10, and 16 in Table I).

Table I. Summary of Embryoscopic and Cytogenetic Findings in 19 Missed Abortions

Case No.	GA ^a	DA ^b	CRL ^c	Morphology	Karyotype
1	10	6	16	Macerated intact normal embryo	69,XXY ^d
2	12	8	25	Normal embryo with nuchal edema and eye pigment diffusion indicating early degeneration	Not available
3	12	6	20	Maceration, microcephaly with facial dysplasia, severely retarded limb development	Not available
4	12		8	GD 3	47,XY,+16 ^d
5	10		9	GD 4	46,XY ^d
6	12	7	19	Maceration, microcephaly with facial dysplasia, retarded limb development, midline brownish subcutaneous pigmentation	46,XX ^d
7	8	6	10	Macerated, partially fragmented, otherwise normal embryo	46,XX ^d
8	10	4	5	Normal embryo with upper limb bud and prominent tail	46,XY ^d
9	9	4	3	Macerated, abnormal embryo with a kink or twist in the abdominal area	46,XX
10	11		11	Embryo with recognizable head, eyes, mouth, trunk, and rudimentary limbs	47,XX,+15 ^d
11	13	8	22	Microcephaly, fusion of the mouth to the chest, retarded limb development	45,X
12	12		20	Absence of the head, fusion of the discolored, pigmented embryonal neck area to the amnion, retarded limb development	Tetraploidy ^d
13	13	7	22	Maceration, microcephaly with facial dysplasia, severely retarded limb development	45,X
14	10	8	22	Abnormal embryo with delayed limb development	45,X
15	10	4	5	GD3	Not available
16	9	5	8	Normal embryo	Not available
17	7		5	GD2	46,XX
18	12	7	20	Macerated embryo, severe microcephaly, facial dysplasia, retarded limb development, intrauterine fragmentation	45,X
19	10	6	15	Severe microcephaly, median upper cleft lip, facial dysplasia, severely retarded upper limb	46,XX,-13,+ t (13q;14q)

^a Gestational age (weeks).^b Developmental age (weeks).^c Crown-rump length (mm).^d Examined by CGH/FCM.

Normal embryonic development was diagnosed in five cases (cases 1, 2, 7, 8, and 16 in Table I) (Fig. 1). Embryonic growth disorganization of various levels (GD2–4) was detected in four embryoscopies. For these embryos the developmental age could not be established since it is known that such embryos do not have a normal pattern of development.

In the case of the GD2 embryo, an excessively large amniotic sac adhering to the chorion was found. The embryonic tissue was attached to the internal aspect of the amnion, showing no recognizable external features and no retinal pigment.

The GD3 embryos showed an absence of limb buds, but retinal pigment and a filiform body stalk were present. Both the chorionic sac and the amniotic sac were disproportionately large for the size of the embryo.

The stunted GD4 embryo had a discernible head, trunk, and limb buds. The limb buds showed a marked retardation in development and the development of the facial structures was virtually absent.

Seven embryos showed signs of maceration which in some cases mimicked focal defects such as open

neural tube defects and umbilical cord stricture. One embryoscopy (case 18 in Table I) revealed a macerated and fragmented embryo consisting of the separated embryonic head and trunk. Fragmentation occurred prior to embryoscopy, because two embryonic parts could be seen on the ultrasound examination performed before the procedure.

Ten cases showed multiple localized developmental defects (cases 3, 6, 9–14, 18, and 19 in Table I); the defects included microcephaly (Fig. 2), facial dysplasia, facial clefts, fused mouth, and retarded limb development. One embryo (case 12 in Table I) had an unusual defect not previously described in the literature at this developmental stage; this embryo showed marked retarded upper and lower limb bud development (Fig. 3). The caudal pole could be easily distinguished. The cephalic pole, clearly identified on ultrasound, was completely adherent to the amnion. Morphologically, the pattern of malformation was highly suggestive of an amniotic adhesion. Cytogenetically, this embryo had a tetraploid complement.

Of the 19 cases studied embryoscopically, a successful cytogenetic evaluation was performed in 15.

Fig. 1. A normal embryo, stage 14, measuring 8 mm in crown-rump length. Note the prominence of the forebrain (O), branchial arches (1, 2, 3), and limb bud (X) and tail (T).

Chromosomal abnormalities were found in 9 of 15 cases and included mostly numerical aberrations such as monosomy X (4 cases), trisomy 16 and 15, triploidy, and tetraploidy. An inherited structural chromosome anomaly leading to chromosomal trisomy was observed once.

Chromosome abnormalities were found in the category of morphologically normal embryos in 1 case (triploidy) of 5 and in 7 cases of 10 in the category of embryos showing combined localized defects.

Fig. 2. A degenerated abnormal embryo with microcephaly and delayed development of both the upper (UL) and the lower (LL) limbs, for a crown-rump length of 19 mm. The umbilical cord shows a cyst (C). The karyotype is normal (46,XX).

Fig. 3. An abnormal embryo 20 mm in length with an amniotic adhesion (arrows) of the damaged cephalic pole and retarded upper (UL) and lower (LL) limb development. Umbilical cord (U). the karyotype shows tetraploidy.

DISCUSSION

The demised embryo often cannot be subjected to detailed pathological examination because of secondary damage occurring during instrumental evacuation of the uterus. Previous reports showed the diagnostic potential of transcervical embryoscopy in the first trimester.

Cullen *et al.* (10) performed embryoscopies in 100 patients (gestational ages ranging from 5 to 13 weeks) scheduled for elective termination of pregnancy. An embryo was visualized through the amniotic membrane, in all cases except four. In this study the equipment included a rigid fiberoptic endoscope (outer diameter, 2–3.5 mm) passed transcervically under ultrasound guidance. Failure to visualize the embryo was due to the inability to penetrate the chorion with the tip of the scope.

In our series of 24 missed abortions, all embryos except 5 were visualized. We obtained good results by adjusting the pressure of the distension medium on demand: after passing the gently dilated cervical canal, a high pressure of about 120 mm Hg was used to distend the uterine cavity and detect the implantation site of the pregnancy. As ultrasound guidance was not available in our series, embryonal structure could not be visualized in four cases. Care was taken to open the chorion with a microscissor to enter the chorionic cavity, whose fusion usually occurs at between 10 and 12 weeks of gestation. Using the microscissor

prevented the membranes from tenting, separating the chorion from the uterine wall, but bleeding that obscured the examiner's vision did occur once.

Inside the chorion cavity we usually reduced the pressure of the distension medium to 40–60 mm Hg to prevent the amnion from collapsing and the embryo from spinning. External morphological examination of the embryo was first done through the transparent amniotic membrane. Since amniotic rupture need not be avoided in cases of failed pregnancies, the hysteroscope was then passed into the amniotic cavity after opening this membrane with a microscissor in most cases. The documentation of further delicate embryonic details could be better achieved inside the amniotic cavity. In this context, special emphasis was placed on observing the limb development. Most likely due to these three steps in the examination, the mean time required to perform embryoscopy in the present study, namely, 13 min, was longer than that reported in a previous study (10).

As our rate of failure to visualize embryos measuring 6 mm or less was relatively high, further studies demonstrating the usefulness of applying hysteroscopes of a smaller diameter, using a wide-angle lens, are needed.

Pathologic investigation of early spontaneous abortion specimens can clarify the developmental age and morphology of the product of conception. Unfortunately, pathological examination is difficult to perform after instrumental evacuation, as these specimens are often incomplete. If an embryo is present, it is either severely damaged or fragmented.

In a pathological study of 1794 specimens from spontaneous first-trimester losses, a large number of specimens consisted of ruptured/fragmented sacs (690) or fragmented/degenerated embryos (134), which could not be evaluated meaningfully (4).

Although most embryos were severely macerated at the time of embryoscopy, all could be fully evaluated morphologically by embryoscopic examination, as damage to the especially fragile embryonic tissue occurring either during spontaneous passage of the conceptus or due to instrumental evacuation of the uterus is avoided. We were able to demonstrate that this obvious drawback of pathological examination of early spontaneous abortion material can be avoided by performing embryoscopy prior to D&C.

The tetraploid conceptus is usually associated with the most severe form of embryonic growth disorganization, namely, intact empty sacs (5,11). One tetraploid embryo showed, on embryoscopy, retarded

limb development, an umbilical cord, and a broad fusion of a damaged cephalic portion to the amnion (Fig. 3). The intact cephalic pole of the embryo could not be visualized on the embryoscopy but it was documented by vaginal ultrasonography. We investigated in our embryoscopies the chorion and the amniotic cavity stepwise as described above, whenever possible. Detection of abnormal adhesions of the amnion to the chorion or of the embryo to the amnion—as in this case—is a major advantage of stepwise embryoscopy.

In our study, a successful cytogenetic analysis could be performed in 15 of 19 cases. Comparative genomic hybridization in combination with flow cytometry (CHG/FCM) was performed from the paraffin-embedded placental tissue in eight cases where the traditional cytogenetic analysis failed to provide the results. Chromosomal abnormalities were monosomy X (four cases), trisomy (three cases), and polyploidy (two cases). As our study was limited to abortuses with ultrasonographic evidence of an embryo, blighted ova were excluded. It has been shown that karyotypes of monosomy X are much more frequently seen among abortuses with ultrasonographic evidence of embryos than among blighted ova (12,13). Thus, the exclusion of blighted ova in our study might have resulted in a relative predominance of monosomy X among abnormal karyotypes.

The methodology of cell culture and analysis of chromosomes is well established and has been accepted as a clinical diagnostic technique for the past four decades. It is well recognized that this technique has limitations—especially, the relatively high rates of tissue culture failure, maternal contamination, and culture artifacts (9).

As expected, we found a high proportion of chromosomal abnormalities among embryos with localized developmental defects. This finding is similar to that in other reports: Kalousek *et al.* (5) investigated 3912 specimens from spontaneous first-trimester losses. Among 279 embryos with localized defects, 88% were chromosomally abnormal.

Since there is a much higher proportion of chromosomal abnormalities among embryos with localized developmental defects than in morphologically normal embryos, the detection of multiple developmental defects highly suggests a chromosomal disorder. It has been shown that couples with a history of repeated spontaneous abortion and documented aneuploid losses have a better chance for a normal pregnancy than couples with losses showing a normal diploid karyotype (14).

Additional investigations will be needed to show whether embryoscopy of missed abortions will be of increasing importance in specific cases, such as repeated pregnancy loss, to provide morphological evaluation of embryonic development and to sample viable embryonic tissue, e.g., amnion, for chromosomal or molecular cytogenetic analysis.

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