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CASE REPORT

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Paternity testing after pregnancy termination using laser microdissection of chorionic villi

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Abstract We report an unusual case of paternity testing from residues of chorionic villi 5 weeks after pregnancy termination. The autopsy of a 32-year-old female homicide victim revealed the presence of intact chorionic villi at the former placenta implantation site. Fetal cells were selectively isolated by laser-induced microdissection of the remaining villi to avoid contamination with maternal DNA. Simultaneous amplification of 12 STR loci in 2 PCR reactions resulted in a combined probability of paternity of 99.94%. This case demonstrates that laser-assisted microdissection and multiplex STR typing provide tools for paternity testing performed on endometrial mucosa long after the product of conception was removed by therapeutic abortion.

Keywords Paternity testing · Laser microdissection · STR typing · Abortion

Introduction

The use of PCR-based DNA analysis has enabled paternity testing to be performed on chorionic villi, amniocytes and products of conception. In the event of pregnancy after rape, paternity determination to identify the perpetrator can be performed prenatally by biopsy (Hammond et al. 1995; Reshef et al. 1999), by amniocentesis (Nata et al. 1993) or by using abortion material as the DNA source (Wiegand et al. 1991). Paternity testing on fetal tissue after homicide of a pregnant woman may provide a link to the identity and motivation of suspects (Ludes et al. 1991). In most cases DNA isolation and amplification from biopsy and abortion material or tissues taken during autopsy can be performed with standard protocols even if putrefaction is present (Mangin and Ludes 1991). When the aborted material is not available after pregnancy termination, chorionic villi still present at the former implantation site may provide fetal DNA for paternity testing until placental site involution is complete.

We investigated a case of homicide 5 weeks after induced abortion using laser microdissection (MOMeNT microbeam microdissection of membrane-mounted native tissue) to selectively obtain fetal DNA from chorionic villi at the former placental site without contamination by maternal cells. The results of paternity determination from tissue obtained by this technique were compared with those from conventional DNA isolation from paraffin-embedded sections.

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Case report

A 32-year-old female was found dead in her apartment some hours after leaving work. Post-mortem findings confirmed strangulation as the cause of death. In the uterine cavity a small (1 × 1 cm) dark coloured area slightly protruding into the lumen was detected in the mucosa. Macroscopically no fetal tissue was visible. The cut surface showed necrosis alternating with small hemorrhages and macroscopically vital tissue (Fig. 1).

Microscopical examination revealed a small number of intact chorionic villi surrounded by decidua, neutrophilic infiltrates, hemorrhage and necrosis (Figs. 2 and 3). These findings were consistent with the diagnosis of abortion in the first trimester of gestation. The gynecologist who had performed the legal abortion 5 weeks before death by vacuum aspiration without subsequent curettage, reported that according to his records the gestational age was 9 weeks. The victim had not named the father of the fetus.

Materials and methods

The formalin-fixed tissue was embedded in paraffin and 5-µm sections (n = 5) were placed onto a specifically designed 1- to 3-µm-thick stretched polyethylene membrane which was mounted onto a glass slide and fixed with nail polish (Fig. 4). After heating the slide for 1 h at 40 °C and deparaffinising in xylol for only 1 min in order to avoid detachment of the section, routine hematoxylin and eosin (HE) staining was performed. The membrane-tissue unit was

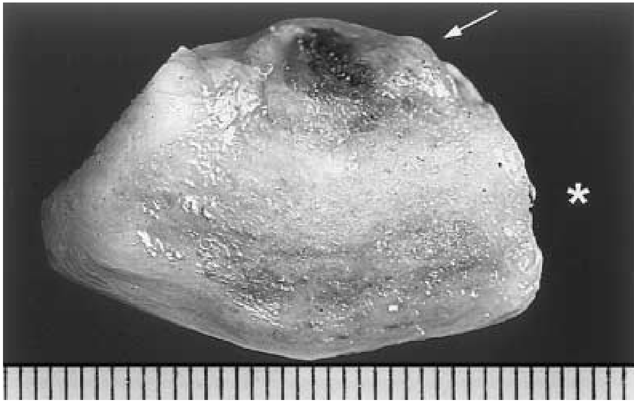

Fig. 1 Cut surface of the uterus wall with myometrium (*) and placenta implantation site *(arrow)*

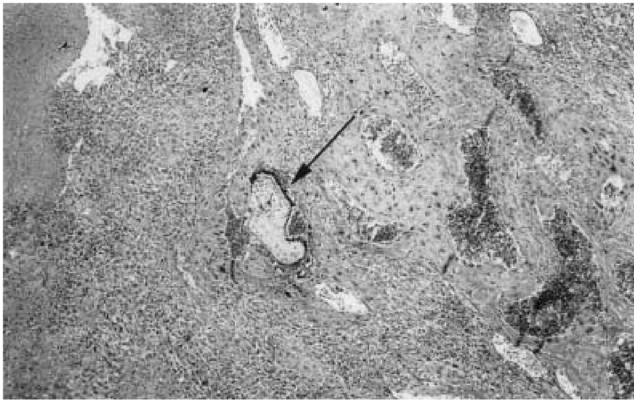

Fig. 2 Chorionic villus *(arrow)* surrounded by decidual cells and necrosis *(left side)*. Magnification × 40

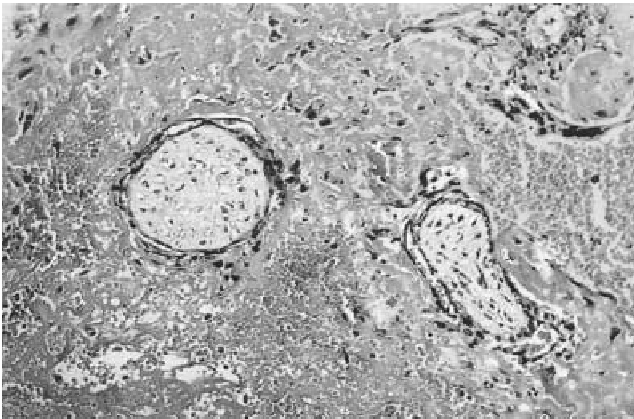

Fig. 3 Immature chorionic villi at higher magnification (× 100). The stroma shows only few vessels consistent with first-trimester stage

then placed upside-down on a 0.17-mm-thick glass slide. The chorionic villi and control areas from the decidua were dissected with a 337-nm-pulsed laser microbeam coupled with a microscope. The dissected pieces of the support membrane with the attached chorionic villi were pierced with a 30-gauge needle and transferred into a PCR tube using a computer-controlled micromanipulator (Fig. 5).

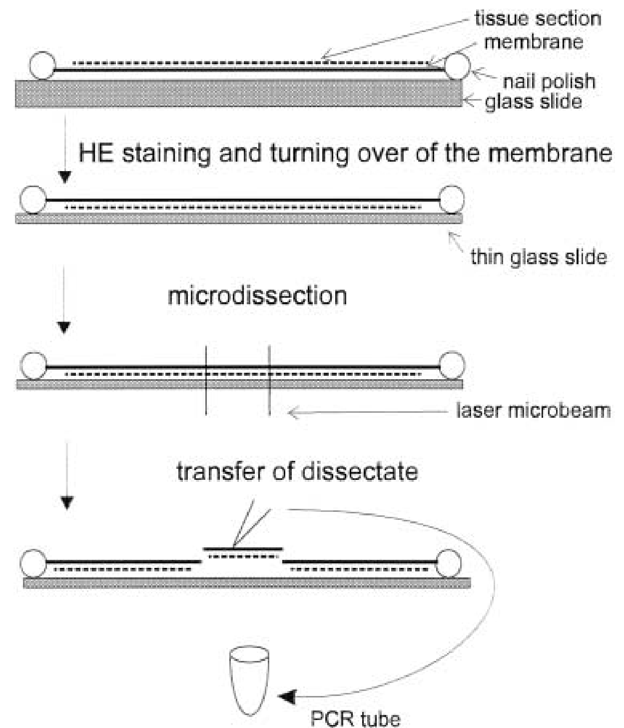

Fig. 4 Schematic representation of the method for laser microdissection (MOMeNT)

The tissue was digested for 3 h at 56 °C using a standard lysis buffer and proteinase K (2 mg/ml). After digestion the enzyme was heat-inactivated for 10 min at 95 °C and the extract was directly used for PCR.

In a second assay five sections were mounted on glass slides and stained with HE. The villi-containing region (2 × 2 mm) was manually cut out, placed in Tris buffer and boiled in the microwave for 1 min. After removing the paraffin and centrifugation, the supernatent was digested with proteinase K.

DNA from a blood sample obtained during autopsy of the victim and from a blood sample taken from a suspect was isolated using standardised chelex extraction.

PCR was performed using the PCR amplification kits AmpFlSTR Profiler and Profiler Plus (Perkin Elmer) on a GeneAmp PCR System 2400 (Perkin Elmer). PCR conditions were as recommended by the manufacturer except that the cycle number was increased to 35 instead of 28, to enhance sensitivity. Of the DNA extracted from the chorionic villi and the decidual tissue, 10 μl was used, corresponding to about 50–100 cells, in a reaction volume of 25 μl.

Electrophoresis and analysis of the amplification products were carried out on an ABI Prism 310 Genetic analyzer using GeneScan Analysis 2.1. software.

Results

The allele data are presented in Table 1. No amplification was seen with D7S820 and D18S51 (Profiler Plus kit). There was clear matching of the DNA pattern obtained from the chorionic villi with the pattern of the maternal DNA and the genotype of the suspect. The combined probability of paternity was calculated to be 99.94% for Caucasians. Amplification of DNA isolated from the mi-

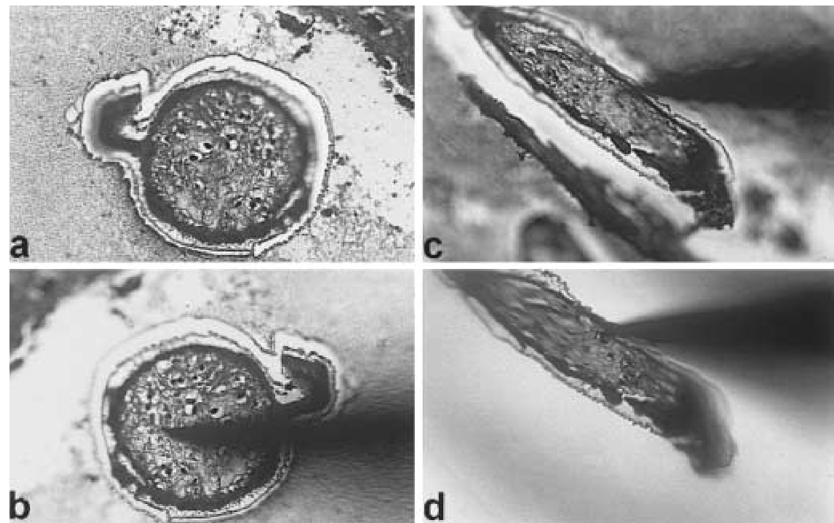

Fig. 5 Photographic documentation of villus microdissection, **a** dissection track around a chorionic villus, **b, c, d** piercing of the dissectate with a 30-gauge-needle

Table 1 STR typing results from micro-dissected chorionic villi show complete matching with maternal and paternal DNA patterns (*n.d.* not detected)

System	Sample			
	Chorionic villi		Mother	Alleged father (suspect)
	Profiler	Profiler plus		
FGA	21/23	21/23	21/24	22/23
vWA	16/17	16/17	16/18	17/17
D13S317	11/13	11/13	11/13	11/12
D5S818	10/12	10/12	10/11	12/12
D7S820	8/12	n.d.	8/10	10/12
D3S1358	17	17/17	17/18	15/17
CSF1PO	12/12	–	12/12	11/12
TPOX	8/11	–	8/8	11/11
TH01	9/9.3	–	6/9.3	6/9
D18S51	–	n.d.	16/19	12/16
D21S11	–	28/30	30/31.2	28/31.2
D8S1179	–	13/13	13/14	13/14
Amelogenin	X	X	X	X/Y

cro-dissected decidual cells revealed exclusively the maternal alleles.

Analysis of the DNA isolated by manual trimming of the sections resulted in amplification of the maternal alleles (as presented in Table 1) and fetal DNA could not be detected.

Discussion

Paternity determination from placental tissue (e.g. chorionic villi biopsy) has been performed in prenatal medicine since DNA typing was available (Strom et al. 1996). The chorionic villi represent the outermost fetal cells which are in direct contact with the maternal surface. Although the involution of the normal human placenta site after abortion has been studied by only a few investigators (Benirschke and Kaufmann 1995), chorionic villi were reported to persist for 4–5 weeks after abortion at the former placenta implantation site (Anderson and Davis 1968; Reyniak et al. 1975). This is consistent with our results confirming that intact fetal tissue can indeed be found in the uterus long after the product of conception was removed.

In previous case reports, post-abortion paternity testing was performed on aborted material by southern blotting (Dobosz et al. 1990; Ludes et al. 1991; Wiegand et al. 1991; Jiang and Wei 1993). With the introduction of single locus probes (Wiegand et al. 1991) and PCR-based STR-analysis (Noble et al. 1991) into prenatal paternity, testing, contamination with maternal cells was ruled out as a limiting factor. However, with conventional trimming of HE-stained sections in order to diminish the amount of surrounding maternal tissue we were not able to co-amplify fetal alleles. The preferential amplification of the abundant maternal DNA obviously inhibited the detection of fetal alleles. The use of laser-assisted microdissection (MOMeNT, Böhm et al. 1997; Schutze et al. 1997) originally developed for obtaining homogenous tumour cell populations in cancer research, allowed the selective isolation of chorionic villi from paraffin-embedded and HE-stained sections with a high precision due to the short wavelength of the laser (Fig. 5).

The yield of DNA isolated from an estimated total number of only 200–300 fetal cells present in the microdissected chorionic villi, limited the possible number of PCR reactions. Therefore we performed co-amplification of 12 sensitive and reliable STR loci with a combined probability of exclusion of > 0.99999999 (Hantschel et al. 1999; Kupferschmid et al. 1999; Neuhuber et al. 1999). Staining with HE prior to laser microdissection obviously did not affect PCR amplification (Banaschak et al. 2000). Each allele obtained from the chorionic villi DNA could be assigned either to the maternal or to the paternal DNA proving the fetal origin. The overlap of six STR loci between the multiplex reactions served as internal control.

Our results demonstrate that laser microdissection can be a useful high performance tool in paternity testing when the selective analysis of small cell populations from

histological sections is required and that combining this technique with highly sensitive multiplex PCR, paternity determination is possible up to 5 weeks after abortion from only microscopically visible fetal cells still present at the former placenta implantation site.

These unexpected findings were of great value in revealing the relationship between the victim and a man (the biological father) who was strongly suspected of having committed the crime.

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