J Forensic Sci, Jan. 2003, Vol. 48, No. 1
Paper ID JFS2002021_481
Available online at: www.astm.org

CASE REPORT

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Detection of Fetal DNA in a Cell Pellet after Centrifugation of Mountant

ABSTRACT: In order to obtain fetal cells (e.g., for paternity cases) after abortion, the centrifugation of mountant (in our case formalin) may be tried when the DNA examination of the fixed tissue itself gives limited or no (or not enough) information. The fixed tissue was microscopically negative for fetal cells and gave no satisfactory results when examined for DNA. Centrifugation of approximately 50 mL of reddish colored formalin resulted in a cell pellet that was examined for DNA, which gave enough information to confirm a case of sexual abuse.

KEYWORDS: forensic sciences, paternity, fetal DNA, centrifugation, mountant

Paternity testing is regularly done with blood samples or other biological material obtained from the involved persons. If a pregnancy is the result of a sexual assault, an abortion will possibly be induced. Then, the DNA profile of the child must be clarified by the examination of fetal tissue from the abortion material (1–3). The differentiation of maternal and fetal tissue may be easy if macroscopically possible (2). Another possibility is a microscopically assisted isolation of fetal tissue prior to the examination (3).

Case Report

A 14-year-old girl was brought to the gynaecological clinic by an emergency transport because of massive vaginal bleeding (spontaneous abortion). Therapeutically, a curettage was done. This abortion material was sent to the Institute of Pathology, routinely fixed in formalin and microscopically examined. Fetal material was not identified, so it must be concluded that the fetus was lost during the spontaneous abortion. Initially, the girl alleged a colleague was the father, but later implicated her father. Because of this allegation, the tissue material was submitted to the laboratory. Comparative blood samples from the alleged father of the tissue material (who is additionally the father of the girl) and the girl herself were available.

Material and Methods

The tissue fixed in formalin for approximately three months was sent to our laboratory and classified as spongious and connective tissue (Table 1) (at total with a diameter of 8.5 cm, macroscopically identifiable as placental tissue), which resulted from the curettage and was fixed in approximately 50 mL now reddish formalin. Tissue samples of approximately 5 mm in diameter were used for DNA examinations. Since there was no macro- and microscopically identified fetal material in the red colored formalin solution, an additional examination was conducted. Therefore, the formalin was centrifuged (4000 rpm, 15 min) and the cell pellet, obtained after centrifugation, examined as well (Table 1). The DNA extraction was performed using a phenol/chloroform method (4). PCR was performed with the AmpFℓSTR®SGM Plus® Kit (Applied Biosystems, Weiterstadt, Germany), *gen*RES® MPX-2 Kit (Serac, Bad Homburg, Germany), and the PowerPlex® 16 Kit (Promega, Mannheim, Germany) according to the manufacturers' protocols on a 310 Genetic Analyser (Applied Biosystems, Weiterstadt, Germany). Quantification of DNA was not performed.

Results

The results of the DNA examinations are summarized in Table 1. The examination of the spongious and connective tissue did not provide enough data to give an expert's opinion on paternity.

The mixtures observed in the cell pellet confirm the presence of maternal and fetal DNA. The alleles that do not belong to the mother could be found in the DNA profile of the alleged offender. The homozygote and heterozygote states may not be the result of an allelic drop out, but could be a mixture, which is not detectable because of the similarities in the parents' DNA profile. The average peak height for the alleles was greater than 100 RFU, as a result, it appears that allelic drop out did not occur. A paternity index was not calculated, because it was not possible to determine definitely the fetal genotype.

Discussion

In cases of questioned paternity, the differentiation of maternal and fetal tissue to prevent a mixture or an overwhelming maternal state (3) could be a problem, which arises if abortion tissue is examined. In our case, the microscopical examination prior to the

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Received 25 Jan. 2002; and in revised form 10 April and 8 July 2002; accepted 8 July 2002; published 13 Nov. 2002.

TABLE 1—*Results of the DNA examinations; - = no amplification result, () = weak amplification result D2S1338, ACTBP2, Penta E, CSF1PO, Penta D, D16S539 and TPOX gave no result in all traces.*

	Amelogenin	D13S317	D3S1358	vWA	D7S820	D8S1179	D21S11	D18S51	D19S433	TH01	FGA	D5S818
Father	X, Y	8, 11	14, 18	16, 17	8, 10	12, 14	30	16, 19	15, 18.2	9.3	19, 20	13, 14
Daughter/mother	X	8, 12	14, 17	15, 16	9, 10	14	30	15, 16	14, 18.2	6, 9.3	19, 24	13, 14
spongious tissue	X	-	-	-	-	-	-		-	-	-	-
connective tissue	X	-	-	-	-	-	-		14, 15, 18.2	-	-	13, 14
Cell pellet	X	8, (11), 12	14, 17, 18	15, 16, 17	(9), (10)	14	30	(15), (16)	14, 15, 18.2	6, 9.3	19, 24	13, 14

DNA examinations showed no fetal tissue. The fetal tissue was most likely lost in a prior state of the spontaneous abortion, so the curettage only revealed residues of the early placenta. These tissue components did not contain microscopically identifiable fetal cells. The centrifugation of the formalin in which the tissue was stored for about three months prior to DNA examinations led to a clearly visible cell pellet, which was used for DNA examinations. These examinations showed an indisputable DNA mixture, which is only explainable by a mixture of maternal and fetal cells. The formalin must thus have contained fetal cells. The presence of maternal DNA in the cell pellet is explainable by detachment of cells from the compact tissue or by blood contamination when the fresh curettage material was given into the formalin. This may additionally be a source for fetal cells, even if the single cells or little cell groups could not be seen by microscopical examination.

The problems of DNA examinations of formalin fixed tissue, paraffin blocks, and stained tissue are generally known (5–7). Formalin leads to a DNA degradation (7). Fixation times of about three months may result in a complete loss of detectable nuclear DNA. Why the cell pellet gave a comparably good DNA examination result while the compact tissue was mostly negative for DNA must remain open for discussion. Possibly this is due to a higher absolute number of cells in the formalin compared to the used tissue samples.

As could be demonstrated, it may be helpful just to try even unusual methods. Without this additional information the expert opinion that the father of the girl could not be ruled out as the father of the unborn child would otherwise not have been possible.

References

1. Ludes BP, Mangin PD, Malicier DJ, Chalumeau AN, Chaumont AJ. Parentage determination on aborted fetal material through deoxyribonucleic acid (DNA) profiling. J Forensic Sci 1991;36:1219–23.
2. Wiegand P, Lorente J, Brinkmann B. DNA investigations on fetal material from paternity cases. Int J Legal Med 1991;104:277–80.
3. Zehner R, Lasczkowski G. Paternity-testing on paraffin-embedded abortion tissue: preparation of fetal cells may be indispensable. J Forensic Sci 2000;45:1332–4.
4. Old JM. Fetal DNA analysis. In: Davies KE, ed. Human genetic disease analysis. A practical approach. 1993; 2nd edition, IRL Press, Oxford New York Tokyo. 1–19
5. Banaschak S, Sibbing U, Brinkmann B. Identifizierung von Gewebsproben bei Verdacht auf Materialvertauschung. Pathologe 1997;18: 385–9.
6. Banaschak S, Rolf B, Brinkmann B. Influence of different staining techniques on the DNA analysis of histological sections. Int J Legal Med 2000;113:114–6.
7. Wiegand P, Domhöver J, Brinkmann B. DNA-degradation in formalinfixiertem Gewebe. Pathologe 1996;17:451–4.

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