## CASE REPORT

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# Paternity-Testing on Paraffin-Embedded Abortion Tissue: Preparation of Fetal Cells May Be Indispensable

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**ABSTRACT:** In a case of sexual abuse, a paternity test was performed on paraffin embedded abortion material. STR typing was successful only after isolating fetal tissue from the abortion-material and separately extracting DNA from the excised fetal cells. Examination with five STRs led to a paternity index of 332, confirming the abuse that had resulted in pregnancy.

**KEYWORDS:** forensic science, parentage testing, DNA typing, short tandem repeat, abortion material, formalin-fixation, paraffin-embedded fetal tissue, TH01, vWA, FXIII, FES/FPS, ACTBP2

A young woman accused her stepfather of sexual abuse over a period of several years. Her pregnancy, which had been aborted in the 9th week three years ago could have been a result of this abuse. The defendant denied any sexual relationship with his stepdaughter, and the court ordered paternity testing on the basis of abortion tissue material that was still available embedded in paraffin for histological examination.

### Material and Methods

Histological sections of the paraffin embedded abrasio tissue were performed (10 μm, Azan-staining, not covered with glass), for demonstrating the presence of chorion cotyledons and decidua tissue (Fig. 1). Parts of the chorion cotyledons were scraped off and recovered from the slides using glass capillaries. This material, representing fetal tissue, was subjected to standard DNA-extraction procedures (1). In addition, DNA from blood samples of the accused and the young woman involved were extracted using Chelex 100 (2). The STRs TH01 (3), vWA (4), FXIII B (5), FES/FPS (5), and ACTBP2 (SE33) (3) were amplified by the polymerase chain reaction (PCR) applying fluorescent labeled primers, and typed using the capillary electrophoresis system ABI 310 Genetic Analyzer.

### Results

In the abrasio-tissue material chorion cotyledones were detected beside decidua tissue, indicating pregnancy (Fig. 1). Typing of the DNA extracted from the abrasio-tissue in toto revealed only maternal alleles (data not shown).

From the separated and recovered chorion cells, DNA was extracted, which enabled typing of this tissue in all STR-systems applied. These systems were also typed in the DNA from the woman and from her stepfather, the defendant. The STR profiles of the fetal material, the woman, and the defendant are shown in Fig. 2 and Table 1. The fetal tissue exhibited alleles that were consistent with genetic offspring of the woman and the defendant. By biostatistical calculation, based on frequencies of the alleles shared by the fetus and defendant, a paternity index of 332 was obtained. In the STR systems, TH01, vWA, and SE33, additional alleles of low signal-intensity were detected in the fetus DNA-profile, which could be attributed to the woman (Fig. 2).

### Discussion

It is well known that paraffin-embedded tissue can be typed using the polymerase chain reaction (6–8). However, problems may occur in cases where the tissue had been kept for fixation in formalin over a longer period. Treatment of tissue by formalin causes crosslinking of the DNA molecules preventing PCR analysis, whereas DNA degradation may play a minor role (9). However, it has been demonstrated that STR-typing is possible in different tissues that were treated with formalin for up to 70 days (10). In this case, obviously, no problems related to formalin-fixation occurred, probably because formalin fixation for pathological examination usually takes place for only 1–2 days.

In this case, PCR typing of the fetal cells from the abrasio material in toto was not possible because the ratio of fetal to maternal

TABLE 1—*STR-profiles of the examined samples. Alleles with lower signal intensity are shown in brackets.*

| | TH01 | vWA | FES | FXIII B | SE 33 |
|---|---|---|---|---|---|
| Woman | 7/9 | 17/18 | 8/11 | 10/10 | 23/24.2 |
| Fetal tissue | 7/8/(9) | 15/18/(17) | 8/11 | 9/10 | 23/28.2/(24.2) |
| Defendant | 8/9.3 | 15/16 | 10/11 | 9/10 | 19/28.2 |

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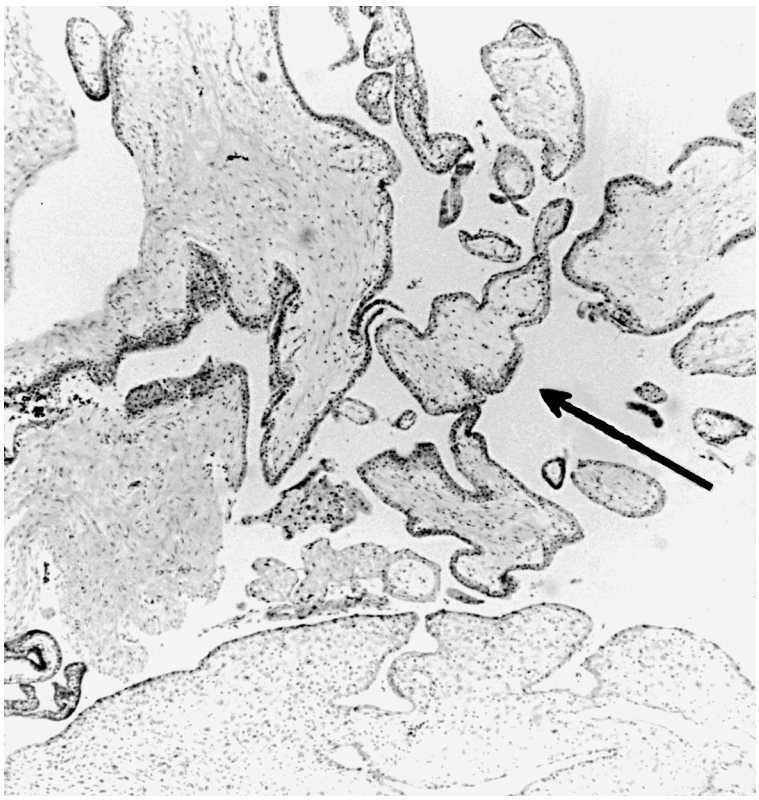

FIG. 1—*Histological section of the aborted material. One chorion cotyledon is marked with an arrow. Original magnification: 150 ×, stain: Trichrome-Azan.*

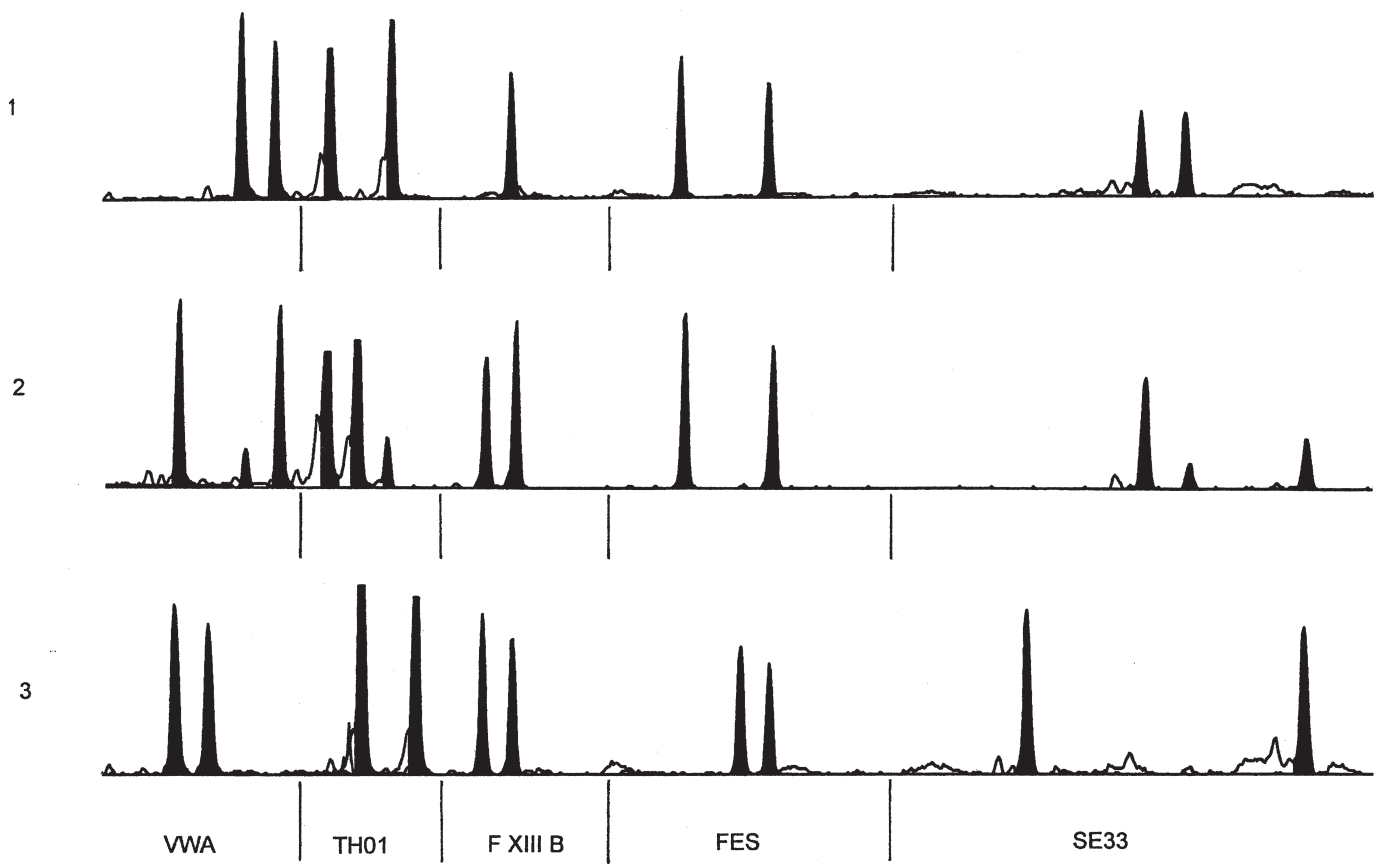


FIG. 2—*Combined electropherograms. STR systems are indicated. 1. woman, 2. fetal tissues, and 3. defendant.*

cells was too low (data not shown). Typing of DNA from fetal cells extracted together with maternal DNA using the abrasio material in toto may be successful, as shown by RFLP analysis (11), but is dependent on a relatively higher ratio of fetal to maternal cells.

In the present case, the separation of fetal cells was absolutely necessary to enable successful typing of the fetal DNA. The authors recommend a strategy for examination that includes visualization and preparation of fetal tissue when typing of the fetal DNA extracted out of total abrasio tissue fails.

Typing of the scraped off fetal tissue material showed additional alleles with a lower signal intensity in three STR-systems (TH01, vWA, and SE33) along with the major alleles. These alleles, representing the maternal alleles, are deriverd from coextracted maternal tissue due to incomplete separation of the fetal cells. However, these findings did not prevent interpretation of the results.

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