## CASE REPORT

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# Superfecundation and Dual Paternity in a Twin Pregnancy Ending with Placental Abruption

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**ABSTRACT:** A case of superfecundation and dual paternity in a twin pregnancy is presented. Placental abruption developed at week 33 of gestation and the two boys had to be saved by emergency cesarean section. As they shared one placenta, had almost identical weight and had the same sex, they were assumed to be monozygotic. However, a subsequent paternity suit led to the conclusion, based on DNA-analysis, that the twin brothers had been fathered by two different men. Obstetrical implications are discussed.

**KEYWORDS:** forensic science, superfecundation, paternity testing, DNA-analysis, twin pregnancy, dual paternity, twinning, placenta

Traditionally, in some cultures the birth of twins was seen as a sign of particularly good fertility and thus good fortune; in other cultures it was seen as ominous proof that the mother of the twins had had sex with different men. Dizygotic twins may arise from a single copulatory event in which two ova are fertilized. When two ova from the same menstrual cycle are fertilized in two separate copulatory events with the same partner, leading to dizygotic twins, this is termed monopaternal superfecundation. Two fertilized ova from the same menstrual cycle from two copulatory events with different partners are termed hetero- or dipaternal superfecundation. Fertilization of an ovum in the presence of an existing pregnancy is termed superfetation.

The suspicion of dual paternity in a twin pregnancy is part of folklore and myth in many cultures (1). Until the advent of red-cell blood typing (2) and later HLA-typing (3,4), only rare anecdotal cases were reported in which anthropometric and racial features of a set of twins were so strikingly different that dual paternity could be assumed (5). Recently, several cases of dual paternity have been confirmed by DNA analysis (6,7).

### Case History

A 26-year-old healthy Caucasian, gravida II para II, last menstrual period 21 June 1995, expected date of confinement 28 May 1996, had regular checkups with her gynecologist, who documented concordant growth of both infants as measured with repeat ultrasound examinations. At 31 wk, 2 d of gestation she developed premature contractions and was referred to the Innsbruck University Ob/Gyn Department. Ultrasound showed a vital twin gestation with two males with a diamniotic monochorial placenta. Discrepancy in estimated fetal size was less than 10%. Doppler flow measurements of fetal vessels were normal. The patient received intravenous tocolysis with magnesium sulfate as well as betamethasone to induce lung maturation. Contractions soon ceased, the patient remained hospitalized. On the last day of week 33 of gestation, the patient reported a sudden onset of pain in the abdomen and a "strange" feeling with cold sweat accompanied by sudden vaginal bleeding. Ultrasound and CTG showed bradycardia in both twins. Emergency cesarian section was performed because of suspected placental abruption; a diagnosis that was confirmed during the operation by the presence of large retroplacental blood clots.

Two live infants were delivered: (1) male, 1715 g, 46.5 cm, 32 cm head circumference, arterial pH 7.17, APGAR 4/8/8; (2) male, 1630 g, 43 cm, 34.5 cm head circumference, arterial pH 7.20, APGAR 4/7/8. Both infants were transferred to NICU and dismissed from hospital after three weeks, both having gained weight above 2000 g. Examination of the placenta showed a monochorial, diamniotic placenta with velamentous insertion of the umbilical cord of twin 1 and no vascular anastomoses between the two halves. Histology showed normal placental development for the time of gestation. Both infants are developing normally and are aged 3 years at the time of this writing.

#### *Paternity Proceedings*

In the course of divorce proceedings between the infants' mother and her husband, the latter claimed that he had reason to doubt his paternity of the twins. At the time of assumed conception the marriage had been legally intact. Since the infants' birth, the couple had separated and the woman was living with her lover. However, the woman claimed she had not had sex with her lover during the period in late spring 1995 in which the twins were probably conceived. Nine months after the infants' birth, the court ordered a combined serological and molecular-biological paternity investigation.

#### *Blood Group, Plasma Factor and Enzyme Factor Analysis*

Blood samples were available from mother, husband, lover, boy 1 and boy 2. Serological examination consisted of analysis of ery-

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throcyte membrane blood group systems (ABO, MN, Rhesus, Duffy), a plasma protein system (Gc-Iso group specific component) and erythrocyte enzyme (Iso phosphoglucomutase 1 - System - $PGM_1$) using serological and chemical procedures.

*DNA-RFLP Analysis*

DNA examination of the samples was performed using single locus probes for DNA-RFLP examination. DNA was isolated from blood samples by digestion with Proteinase K (Boehringer Mannheim) and subsequent extraction with phenol/chloroform. Restriction was performed with Hinf I. DNA fragments were separated by electrophoresis in 0.8% agarose-gel. Fragments were subsequently transferred to a fixed layer (blot on nylon membrane). DNA typing was performed with four single-locus probes marked with chemoluminescent dye (Table 1).

*DNA-PCR Analysis*

Because of the equivocal nature of the results of the serologic and RFLP tests (see below), DNA-PCR examination of the STR systems was performed using 5 ng of DNA that were amplified by multiplex PCR (Hum THO1, HumFES/FPS, HUMF13A1, and HUMvWFA31/A) and electrophoresed as reported previously. To further ascertain the dual paternity of the two infants, human Y-linked chromosomal STR markers were investigated from the four male blood samples (husband, lover and both boys). We investigated DYS19 and the tetrameric microsatellite DYS389 (I,II).

## Results

Serological examination of blood group systems showed that the husband was probably the father of boy 2 but his paternity of boy 1 could be excluded by differences in the Rh and MN systems, three of four DNA-RFLP analyses (Table 1) as well as 2 of 4 STR examinations (Table 2).

TABLE 1—*RFLP examinations: the husband's paternity of boy 1 could be excluded in three out of four loci; similiarly the lover's paternity of boy 2 could be excluded.*

| Sample Source | YNH24 | MS43A | MS31 | g3 |
|---|---|---|---|---|
| Locus name | (D2S44) | (D12S11) | (D7S21) | (D7S22) |
| Mother | 2.95/3.72 | 9.04/9.04 | 6.45/8.07 | 2.90/3.27 |
| Boy 1 | 3.73/4.19 | 9.05/9.79 | 7.05/8.08 | 3.27/10.41 |
| Lover | 2.62/4.17 | 8.28/9.96 | 5.66/7.19 | 9.80/10.78 |
| Boy 2 | 3.73/4.38 | 8.10/9.05 | 7.61/8.10 | 3.27/8.24 |
| Husband | 4.174.38 | 4.56/8.12 | 5.67/7.61 | 6.62/8.26 |
| Exclusion of husband's paternity of boy 1 | no | yes | yes | yes |
| Exclusion of lover's paternity of boy 2 | yes | no | no | no |

TABLE 3—*As Y-chromosomes are transmitted from father to son, examination of Y-chromosomal loci showed a clear kinship between the two boys and their respective fathers.*

| | DYS19 | DYS389 I* | DYS389 II* |
|---|---|---|---|
| Boy 1 | 15 | 12 | 29 |
| Lover | 15 | 12 | 29 |
| Boy 2 | 15 | 14 | 30 |
| Husband | 15 | 14 | 30 |

* Nomenclature according to ISFH recommendations.

Examination of bi-allelic Y-chromosomal STR DYS389 supports this result as the husband shares haplotype with boy 2 (i.e., 12/29, Table 3) whereas the lover shows the same haplotype as boy 1 (14/30). It was not possible to detect any differences using DYS 19 as both men and both boys share the same genotype.

The probability of the husband being the father of boy 2 was established as 99.999957 (Pat Index 2,373,369). The probability of the lover being the father of boy 1 was established as 99,999962 (Pat Index 2,629,057).

## Discussion

Heteropaternal superfecundation can occur when two or more ova are released in the same menstrual cycle, fertilized in separate copulatory events with two men, and subsequently are successfully implanted in the uterine cavity. Dizygotic twins by the same father may be a consequence of monopaternal superfecundation. There are numerous factors favoring superfecundation: more dizygotic twins are conceived during the summer (8). Genetic modeling of dizygotic twins has also demonstrated the probable existence of a gene for double ovulation that has been calculated to have a gene frequency of 0.3 and a penetrance of 0.03 per female carrier (9). In another line of reasoning, it has been argued that both high coital rates and favorable erotic circumstances—both of which would apply in the early stages of marriage (or in that precarious stage of a marriage when a lover is yet undiscovered)—will lead to an increase of dizygotic twinning rates by provoking and accelerating ovulations (10,11). Similar conditions apparently prevailed in the case described.

Our case presents some unique features thanks to exact ultrasound documentation of the pregnancy, the histological workup of the placenta and the availability of samples from all five subjects concerned. The course of pregnancy was by no means exceptional, with almost concordant growth curves of the two male twins. The circumstances surrounding birth were, however, dramatic and the infants could only be saved because placental abruption had taken place in hospital. Retrospectively, the morphologically single pla-

TABLE 2—*STR examinations: the husband's paternity of boy 1 could be excluded in two out of four loci.*

| Sample Source | HUMFES/FPS | HUMTHO1 | HUMF13A1 | HUMvWA31/A |
|---|---|---|---|---|
| Locus name | 15q25-qter | 11p15-15.5 | 6p24-p25 | 12p12-pter |
| Mother | 10/12 | 8/9 | 4/7 | 14/17 |
| Boy 1 | 11/12 | 9/9.3 | 6/7 | 14/16 |
| Lover | 10/11 | 9.3/9.3 | 6/6 | 16/18 |
| Boy 2 | 10/10 | 8/9.3 | 4/6 | 14/15 |
| Husband | 10/12 | 9.3/9.3 | 6/7 | 14/15 |
| Exclusion of husband's paternity of boy 1 | yes | no | no | yes |
| Confirmation of lover's paternity of boy 2 | no | yes | yes | no |

centa must have resulted from the early fusion of the two separate placentae. Had there not been a paternity suit there would have been no reason to order state-of-the-art DNA analysis of the infants and their parents and the two boys would have been assumed to be monozygotic.

Velamentous insertion of the umbilical cord is deleterious to fetal well-being, resulting in higher rates of stillbirth and early neonatal death. Placental abruption is a dreaded emergency in any pregnancy. Whether the fact that the twins had two different fathers had any causal relationship with placental pathology is open to speculation.

The birth of twins or triplets sired by different fathers is rare in humans with less than 30 cases reported worldwide in the past 200 years. However, a future increase of detected cases has been predicted (12): frequency of concurrent sexual partners has increased since the 1960s and generous prescription of ovulation-inducing drugs (known to cause superovulation) for fertility treatment is widespread. Accidental superfecundation during in-vitro fertilization procedures, when sperm from different donors are mixed, has received widespread attention in the popular press both in the U.S. and in Europe. Based on laboratory data, Wenk et al. have suggested a rate of heteropaternity of 2.36% in all dizygotic twins (12). With access to DNA analysis becoming increasingly widespread, in a few years this issue, which has fascinated humanity since biblical times, will be clarified (13). Until now, heteropaternal twinning was considered an almost impossible rarity. Establishing that 1 out 40 pairs of dizygotic twins were sired by different fathers may put coniderable strain on all twins and their parents.

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