

## AN UNUSUAL CASE OF FAILED VASECTOMY CONFIRMED BY DNA PROFILING

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### Introduction

The increasing use of DNA polymorphisms in recent years has greatly improved the ability of parentage testing to confirm paternity in those cases where the named man is not excluded from fathering the child. The high discriminating power of such systems is of great benefit in cases where other evidence suggests that the man is not the father. Such a case is presented here where the putative father had undergone an apparently successful vasectomy.

Vasectomy is a safe and effective means of sterilisation. The success of the operation is monitored by the examination of semen samples at suitable intervals post-operation. Failed vasectomies are identified by the presence of spermatozoa in the semen and they can be classified according to the time post-operation that sperm are identified and the condition of those sperm.

The majority of failures are identified in the first sperm counts carried out post-operation. Late failures can be defined as those in which spermatozoa, in any condition, are identified after the operation has been declared a success. This normally means after two consecutive semen samples have been completely free of spermatozoa.(1,2)

These late failures can be further divided into those in which high levels of active spermatozoa are observed and those in which non-significant numbers of non-motile spermatozoa are present. The mechanism by which these latter "technical" failures occurs is unclear but they have previously been reported as being of no clinical significance (3) and no confirmed associated pregnancies have been identified in the studies cited.

### Case History

A 30 year old man (DP) and his 25 year old wife (JP) had decided that their three child family was complete and in December 1987 DP underwent a vasectomy under local anaesthetic. Semen samples at 12 and 18 weeks post-operation were clear of spermatozoa and he was told that the vasectomy had been successful.

Two and a half years later, in 1990, JP became pregnant. Repeated examinations of DP's semen showed the presence of small numbers of non-motile spermatozoa; a pattern normally thought to be indicative of infertility. No motile spermatozoa were observed in five specimens. The assumption of many of their associates was that JP had been unfaithful, which she always denied. DP was convinced of his wife's fidelity. A summary of these events is shown in table 1.

After an uneventful pregnancy, a healthy male child was born on April 10 1991. It was decided to carry out tests including DNA profiling to confirm the paternity of the child.

These tests provided no evidence that DP was not the father of the child. A relative chance of paternity of over 99.99% was calculated. Such a figure is normally considered to offer virtual proof of paternity, provided that a close relative of the man is not a possible father. In order to exclude this possibility it was decided to test DP's brother, to which DP, JP and the brother BP all willingly consented. The brother was duly excluded from paternity on two of the five DNA tests used.

## Methods

Sperm counts were carried out by Dr T. Kelly of the Dept of Clinical Microbiology, Mayday Hospital, Surrey. Semen samples were stored for a maximum of two hours at room temperature. The whole film was scanned at 400x magnification by two members of staff.

Paternity testing was performed at LHMC. Conventional grouping was carried out using the following systems: ABO, Rh, MNSs, Fy, K, Lu AK, ADA, EAP, Glo, ESD, PGM, Hp, Gc, Pi, Tf, PLG and  $\alpha$ 2HSG. DNA profiling tests were performed using the methods described by Syndercombe Court *et al* (6). Six single locus probes (Mucin, MR24/1 (Amersham), YNH24, TBQ7, 3' $\alpha$  HVR (Promega) and MS43a) were utilised.

## Results

### Sperm counts

The results of the semen examinations are summarised in table 1. They show that no motile spermatozoa were observed in seven separate examinations over a period of three years, both before and after JP's pregnancy.

**Table 1. Summary of semen examinations**

Time post-op	Observations
12 weeks/ 18 weeks	Initial semen examinations. Spermatozoa not seen
28 months	JP confirmed pregnant
2½ years	5 further semen examinations. No spermatozoa seen in 2 samples. Few non-motile spermatozoa seen in 3 samples
3 years	Child Born

### Paternity testing results

#### Conventional tests:

The results of the conventional red cell antigen, serum protein and red cell enzyme tests do not exclude DP from paternity of the child (results not shown). They indicate a relative chance of paternity of about 94%.

The conventional tests also fail to exclude the man's brother, BP, from possible paternity of the child.

#### DNA Profiling tests:

The results of the DNA profiling tests are shown in table 3.

These results do not exclude DP from paternity of the child using fragment size matching criteria established in this laboratory (10). They indicate a relative chance of paternity of about 99.99%. When combined with the figure from the conventional typing this figure is increased to over 99.999%. Such figures are usually considered to offer virtual proof of paternity.

The results for the man's brother, BP exclude him from paternity on the MR24/1 and TBQ7 systems. In both these tests the child has a fragment which must have been inherited from its true father which BP does not possess.

**Table 3. DNA profiling test results (observed fragment sizes, kb)**

	Probe					
	Mucin	YNH24	MR24/1	TBQ7	MS43a	3'α HVR
DP (Husband)	5.01 <u>4.62</u>	<u>3.66</u>	4.36 <u>3.39</u>	4.18 <u>1.41</u>	<u>9.23</u> 4.31	<u>2.93</u> 2.24
JP (Mother)	4.47 1.92	4.25 2.83	4.83 4.41	4.49	7.52 4.66	4.02 1.37
C (Child)	<u>4.65</u> 1.94	<u>3.72</u> 2.84	4.83 <u>3.40</u>	4.51 <u>1.42</u>	<u>9.24</u> 4.67	4.03 <u>2.96</u>
BP(Brother)	4.98 <u>4.61</u>	<u>3.70</u> 3.60	4.31 4.22	5.57 3.56	<u>9.29</u> 7.70	ND

## Discussion

This case demonstrates the value of DNA profiling in establishing beyond doubt the paternity of a child in a situation where other factors strongly suggest a different outcome. It was important that a high relative chance of paternity was obtained to counter this *à priori* evidence. For the same reasons it was also important to exclude the man's brother from possible paternity, and again the high discriminating power of the DNA systems employed enabled this to be the case.

This is an important demonstration of fertilisation by a man deemed infertile by the criteria usually accepted for defining a successful vasectomy.

Failed vasectomies are normally characterised by the time post-operation at which the failure is observed and by the nature and numbers of any spermatozoa seen. This case belongs to a very small subset of failures where initial sperm counts were zero and only later (in this case following the pregnancy of the man's wife) were any spermatozoa identified. Even at this later stage the spermatozoa seen were very few and were all non-motile and would normally satisfy criteria for infertility. Alderman (2) states that when such sperm characteristics were observed he would "cautiously advise" that the operation had been a success. This is supported by Edwards and Farlowe (3) who showed no associated pregnancies in their study of 200 such technical failures. Schmidt (4), however, disagrees and would "find it difficult to believe that these patients [showing the presence of any spermatozoa of any kind] are totally safe".

This single case does nothing to detract from the view that men with very few non-motile spermatozoa following vasectomy are likely to be infertile but clearly demonstrates that this conclusion is not immutable.

## References

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