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Forensic Paternity Testing With Mixed Fetal DNA Samples

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Abstract

Single source STR DNA profiles are preferably used in paternity testing and forensic casework. In this paper we present paternity testing cases, where conceptus samples contain a mixture of both maternal and fetal cells/tissues. Using autosomal STR and Y-chromosomal STR profiling we were able to successfully use the mixed DNA profile to prove paternity. Such a course of action was only applied to cases where it was impossible to separate maternal and fetal tissues and resampling was not viable.

Keywords: Mixed DNA profile, Paternity Testing, Conceptus, Fetal Tissue, Maternal Tissue.

Paternity testing and forensic casework rely on STR DNA typing to supply strong evidence, so that the culprit is brought to justice[1]. The number of paternity cases arising due of sexual offences is on the rise. Often, unwanted pregnancies, caused as a result of sexual offence, are aborted for the benefit of the victim. In India, for special cases that involve survivors of sexual abuse, minors, victims of rape, and disabled women, the abortion/ termination of pregnancy is allowed up to 24 weeks, according to the MTP Amendment Act 2021. In certain cases, where abortion is done in the early stages of pregnancy -when the fetus has yet to take complete form - the product of conception collected by the medical professional contains tissue of the conceptus (fetus) and tissue from the uterus of the mother. For successful DNA paternity testing, where a single STR DNA profile is the norm, fetal samples should be devoid of any maternal tissues adhering to it; otherwise, mixed DNA profiles containing both the alleles of the fetus and the mother are obtained. However, it has been observed that, even after taking precautions, it is very difficult to differentiate between fetal and maternal tissues during sample collection in the early stages of pregnancy.

Single source STR DNA profiles are always preferred in analysis of paternity as well as forensic casework. A single source STR profile has one allele, called a homozygotic condition, or two alleles, called a heterozygotic condition, on a particular locus; whereas the presence of two or more alleles at a single locus is a characteristic of a mixed STR profile[2] (Table 1).

Mixed DNA STR profiles are generally encountered in forensic cases, with a history of murder or sexual assault, where contribution from multiple sources is possible. Mixed profiles are not encountered in paternity testing, as the samples are generally obtained directly from the source. This study spanning over the course of 14 years reports paternity cases - both paternity inclusion and paternity exclusion- where a mixed conceptus STR DNA profile was obtained. Since re-collection of the sample was not a possibility, analyzing the obtained mixed STR DNA profile was the only course of action. Y-chromosome STR profiling was used along with autosomal STR profiling to further prove paternity for male conceptus samples. The Y-chromosome is used in forensic DNA analysis to trace paternal lineages, using various markers available on the Y Chromosome [6,7]. The Y-chromosome is paternally inherited and haploid; hence, the chromosome does not recombine with any other chromosome during meiosis (except for the pseudoautosomal region) and is passed from father to son, genetically unchanged, except for mutations [8].

One such instance of analysis of mixed sample in paternity cases have been reported earlier. In a real paternity testing case, the alleged father deliberately tried to manipulate his DNA sample to avoid being identified as the biological father. During routine paternity testing, the alleged father's buccal (cheek swab) sample showed an unusual mixed DNA profile. STR analysis revealed: More than two alleles at several loci and an abnormally high X-chromosome peak as compared to the Y-chromosome peak at the Amelogenin marker, suggesting the presence of female DNA. Laboratory contamination was ruled out through repeated testing, review of controls, and reanalysis by a second technician. Researchers suspected that the alleged father had intentionally introduced another person's biological material into his mouth before sampling. When recalled for a second sample collection, he admitted that he had carried his wife's saliva in a plastic bag and placed it in his mouth immediately before the swab was taken. A new buccal swab produced a normal single-source DNA profile. The subsequent paternity analysis showed that the alleged father could not be excluded as the biological father [3].

In the above-mentioned case, re-collection of reference sample from the source was possible, whereas re-collection of samples in the cases presented in this paper was not a possibility, as it was an aborted sample of early-stage pregnancy.

Another case, that deals with proving paternity, with mixed maternal and fetal sample in which the researchers tested a new genetic marker system called DIP-STRs to detect cell-free fetal DNA (cffDNA) circulating in a pregnant woman's blood. The goal was to enable non-invasive prenatal testing without procedures like amniocentesis. Fetal DNA makes up only about 10% of the DNA in maternal plasma, making it difficult to distinguish from the mother's DNA. Existing methods often require expensive technologies such as next-generation sequencing. The researchers tested 28 DIP-STR markers in 48 pregnant women. Collected blood samples across the first, second, and third trimesters and used allele-specific PCR to selectively amplify paternally inherited fetal DNA that differs from maternal DNA [9].

DIP-STRs were originally developed for forensic analysis of mixed DNA samples (10). DIP-STR genetic markers were originally designed, to improve the analysis of highly unbalanced DNA mixtures, a common problem in forensic science where a tiny amount of a suspect's DNA is hidden within a much larger amount of another person's DNA (e.g., victim DNA or touch DNA). A DIP-STR marker combines: a Deletion/Insertion Polymorphism (DIP) and a Short Tandem Repeat (STR). Using allele-specific PCR primers, these markers selectively amplify DNA that contains a unique DIP allele, allowing the minor contributor's DNA to be detected even when present at a 1:1000 ratio relative to the major contributor.

Numerous samples are submitted to our laboratory for paternity analysis, with varied histories of crime against women. A number of victims choose to abort the pregnancy that has taken place as a result of crime against them. The victim lodges a complaint against the accused in the police station, and DNA analysis of the aborted product of conception or fetus emerges as strong evidence against the perpetrator for the investigating authorities.

Materials and Methods:

In all cases, referred in this paper, the abortus is collected by the medical officer, properly sealed, preserved on ice, and submitted to our laboratory for STR DNA Analysis and paternity testing. On receipt of the tissue samples, they are stored at -20°C, until they are processed for DNA extraction. The reference samples (mother and alleged father) are collected in 2.5 ml centrifuge tubes containing 0.5 M EDTA, provided by the laboratory. Reference blood and tissue samples are processed, and DNA is extracted using the robotic EZ1 Advanced (QIAGEN, Limberg, Netherlands) Extraction System, using the protocol as recommended by the manufacturers. [4,5]

PCR amplification was performed using various commercially available autosomal DNA analysis PCR kits -- AmpF*l*STR(R) Identifiler(R) Kit / Global Filer TM PCR Amplification Kit (Applied Biosystems, Foster City, CA), PowerPlex® Fusion 6C System (Promega, USA)

and Amp*l*STR(R)YfilerTM Kit (Applied Biosystems, Foster City, CA) and PowerPlex® Y23 System (Promega, USA).

Capillary electrophoresis was performed using ABI 3130 Genetic Analyser (Applied Biosystems, Foster City, CA) and ABI 3500/ 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA); the alleles were defined using ABI 3130 Data Collection Software and 3500/3500xl Data Collection Software v3.0. The data collected was later analysed using Gene Mapper ID-X Software and Gene Mapper (R) ID Software v3.2 (Applied Biosystems, Foster City, CA).

Results and Discussion:

The DNA Analysis of different conceptus samples produced either mixed male or female DNA profiles on separation through electrophoresis. Repeated sampling and extraction of conceptus tissue samples produced similar results. While analyzing such cases for interpretation, they can be broadly divided into two categories.

(I) The DNA profiles obtained from a conceptus are mixed and of male origin

1. Paternity Conclusion

The mixed DNA profiles obtained from the product of conception were found to contain a complete set of alleles from the mother and one set of alleles from the alleged father at all STR loci. On comparison of the mixed male conceptus DNA profile, with reference DNA profiles of mother and alleged father, one can differentiate and find one set of paternal alleles in the mixed profile. Giving a conclusive proof of paternity.

To further support our findings, Y Chromosome STR profiling was performed using the conceptus sample and blood sample of the alleged father. The Y Chromosome STR profiles were found to be identical, and they belonged to the same patrilineal lineage.

2. Paternity Exclusion

On comparing the mixed male conceptus DNA profile with reference DNA profiles of mother and alleged father, the mixed male DNA profile was found to contain both sets of alleles from the DNA profile of mother at all STR loci, and one set of paternal alleles could be identified; it could be assessed that the alleged father was excluded as the biological father of the conceptus.

Further proof for exclusion was provided by a mismatch of the different Y- Chromosome STR profiles obtained from the mixed male conceptus sample and blood sample of the alleged father.

(ii) The DNA profiles obtained from a conceptus are mixed and female.

1. Paternity Conclusion

On comparison of the mixed female DNA profile obtained from the received conceptus sample, with reference DNA profiles obtained from the blood samples of mother and alleged father, the mixed female DNA profile was found to contain both sets of alleles from mother at all STR loci and on set of allele could be ascertained, which was then matched and compared with the DNA profile of the alleged father/ accused and paternity conclusion could be assessed at all loci.

2. Paternity Exclusion

On comparison of the mixed female DNA profile obtained from the conceptus sample, with the reference DNA profiles obtained from the blood samples of mother and alleged father, the mixed female DNA profile was found to contain both sets of alleles from the mother at all STR loci and on set of allele could be ascertained, which was then matched and compared with the DNA profile of the alleged father/ accused and paternity exclusion could be confirmed.

Conclusion:

Since no other biological evidence was collected in above referenced cases/ crimes, the evidence at hand was of utmost importance. Repeated attempts to obtain any single source DNA profiles from the collected product of conception were to no avail. To avoid further loss of evidence against the perpetrator, the mixed DNA

profiles obtained were used for paternity testing and put to conclusion. In cases were the conceptus samples were of male origin, the use of Y- chromosome STR profiling was added as evidence for proof of paternity. In cases where the collected conceptus samples were of female origin, paternity inclusion and exclusion was successfully reported. Such cases have been reported by our laboratory over a span of 14 years.

Through this paper, the authors propose that the use of mixed STR profiles obtained from pregnant victims of sexual offences can be successfully used for assessing paternity and the involvement of accused/ perpetrator in the crime. It should certainly be used, so as to avoid any loss of evidence, since re-collection of fetal/ abortus samples is impossible.

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Number of STR Peaks	Zygotic Condition	Allele Properties
Four Peaks	Heterozygote	No overlapping alleles (genotypes are unique)
Three Peaks	i. Heterozygote + Heterozygote ii. Heterozygote + Homozygote	i. One overlapping allele ii. No overlapping alleles (genotypes are unique)
Two Peaks	i. Heterozygote + Heterozygote ii. Heterozygote + Homozygote iii. Homozygote + Homozygote	i. Two overlapping alleles (genotypes are identical) ii. One overlapping allele iii. No overlapping alleles (genotypes are unique)
Single Peak	Homozygote + Homozygote	Overlapping allele (genotypes are identical)

Table No. 1 Different Peak Conditions found in a Mixed STR DNA Profile.

STR LOCUS	GENOTYPE		
	Mother	Conceptus (fetal sample)	Alleged Father
D8S1179	10,15	10,**15**	11,**15**
D21S11	30,31.2	**29**,30,31.2	29,32.2
D7S820	9,12	9,**11**,12	10,11
CSF1PO	11,11	**10**,11	10,12
D3S1358	15,16	15,**16**	16,19
THO1	6,9	6,7,**9**	6,7
D13S317	11,12	11,12,**13**	12,13
D16S539	11,13	**9**,11,13	9,9
D2S1338	20,23	20,23,**24**	20,24
D19S433	14,14	**13**,14	13,13
vWA	16,17	**16**,17	16,16
TPOX	8,9	8,9,**11**	11,11
D18S51	15,17	**13**,15,17	13,16
AMELOGENIN	X, X	X, Y	X, Y
D5S818	10,11	10,**11**	11,11
FGA	24,25	24,**25**	25,25

Table 2 : **Case 1- Paternity Conclusion**: the complete genotype of mother, alleged father and the mixed male profile of the conceptus sample. Complete matching of alleles at all 15 STR loci with alleged father can be observed.
Kit Used - ABI AmpFlSTR Identifiler PCR Amplification Kit.

YSTR LOCUS	Conceptus (fetal sample)	Alleged Father
B_DYS456	14	14
B_DYS3891	14	14
B_DYS390	23	23
B_DYS38911	30	30
G_DYS458	17	17
G_DYS19	14	14
G_DYS385	15,19	15,19
Y_DYS393	12	12
Y_DYS391	10	10
Y_DYS439	12	12
Y_DYS635	22	22
Y_DYS392	11	11
R_Y_GATA_H4	11	11
R_DYS437	14	14
R_DYS438	9	9
R_DYS448	21	21

Table 3: **Case 1- Paternity Conclusion** Confirmed using Y- STR DNA Profiling:
Y- Haplotypes match at all loci; confirming that the profiles originate from the same paternal lineage.
Kit Used - ABI YFiler PCR Amplification Kit.

STR LOCUS	GENOTYPE		
	Mother	Conceptus (fetal sample)	Alleged Father
D8S1179	10,15	10,15,17	12,15
D21S11	30.2,32.2	29,30.2,32.2	30,31
D7S820	9,10	9,10,12	11,11
CSF1PO	12,12	12,**12**	9,**12**
D3S1358	15,16	15,16,18	14,17
THO1	9.3,10	6,9.3,10	9,9.3
D13S317	11,12	11,12,13	9,12
D16S539	11,13	9,11,13	11,11
D2S1338	17,25	17,**18**,25	**18**,22
D19S433	12,13	12,12.2,13	12,16
vWA	17,18	15,17,18	14,14
TPOX	8,11	8,11,12	8,11
D18S51	14,16	14,**15**,16	14,**15**
AMELOGENIN	X, X	X, Y	X, Y
D5S818	11,11	11,**12**	**12**,12
FGA	22,25	22,**24**,25	21,**24**

Table 4: **Case 2- Paternity Exclusion**: the complete genotype of mother, alleged father and conceptus sample. Paternity exclusion at 10 out of 15 STR loci can be observed.
Alleles that match with the alleged father are highlighted.
Kit Used - ABI AmpFlSTR Identifiler PCR Amplification Kit.

Y- STR LOCUS	Product Of Conception	Alleged Father
B_DYS456	15	16
B_DYS3891	13	13
B_DYS390	23	24
B_DYS38911	31	31
G_DYS458	17.2	16
G_DYS19	15	16
G_DYS385	13,18	11,15
Y_DYS393	12	14
Y_DYS391	10	10
Y_DYS439	13	10
Y_DYS635	20	23
Y_DYS392	11	11
R_Y_GATA_H4	11	13
R_DYS437	14	14
R_DYS438	10	11
R_DYS448	21	20

Table 5: **Case 2- Paternity Exclusion** Confirmed using Y- STR DNA Profiling:
Y- Haplotypes do not match; confirming different paternal lineage.
Kit Used - ABI YFiler PCR Amplification Kit.

STR LOCUS	**GENOTYPE**		
	Mother	Conceptus (fetal sample)	Alleged Father
AMELOGENIN	X,X	X,X	X,Y
D3S1358	15,15	15,**15**	**15**,15
D1S1656	8,15	8,15,16	12,14
D2S441	11,11	11,**11**	**11**,14
D10S1248	13,16	13,14,16	15,16
D13S317	8,12	8,**12**	8,**12**
Penta E	12,12	**12**,15	11,12
D16S539	10,12	10,11,12	8,12
D18S51	15,16	**12**,15,16	**12**,17
D2S1338	18,23	18,**20**,23	**20**,25
CSF1PO	12,12	11,12	12,12
Penta D	9,13	9,11,13	10,10
TH01	7,8	**6**,7,8	**6**,6
vWA	16,17	16,17,**18**	15,**18**
D21S11	31.2,32.2	**30**,31.2,32.2	**30**,32.2
D7S820	8,12	8,**11**,12	**11**,11
D5S818	11,12	11,**12**	**12**,13
TPOX	9,11	9,**10**,11	9,**10**
D8S1179	13,15	13,**14**,15	10,**14**
D12S391	18,18.3	18,18.3**,23**	18,**23**
D19S433	14,14	14,14	13,16
SE33	25.2,32.2	22.2,25.2,32.2	20,31.2
D22S1045	11,11	11,14	11,16
DYS391	-	-	10
FGA	20,25	20,25,26	24,24.2
DYS576	-	-	--
DYS570	-	-	--

Table 6: Case 3- Paternity Conclusion: the complete genotype of mother, alleged father and female conceptus sample. Paternity conclusion at all 23 STR loci can be observed.
Alleles that match with the alleged father are highlighted.
Kit Used – PowerPlex Fusion 6C System. (PCR Amplification Kit)

STR LOCUS	GENOTYPE		
	Mother	Conceptus (fetal sample)	Alleged Father
AMELOGENIN	X,X	X,X	X,Y
THO1	7,8	**6**,7,8	**6**,6
D3S1358	15,15	15,**15**	**15**,15
vWA	16,17	16,17,**18**	15,**18**
D21S11	31.2,32.2	**30**,31.2,32.2	**30**,32.2
TPOX	9,11	9,**10**,11	9,**10**
DYS391	-	-	
D1S1656	8,15	8,**14**,15	12,**14**
D12S391	18,18.3	18,18.3,**23**	18,**23**
SE33	25.2,32.2	25.2,**31.2**,32.2	20,**31.2**
D10S1248	13,16	13,**16**	15,**16**
D22S1045	11,11	11,**11**	**11**,16
D19S433	14,14	14,	13,16
D8S1179	13,15	13,**14**,15	10,**14**
D2S1338	18,23	18,**20**,23	**20**,25
D2S441	11,11	11,**11**	**11**,14
D18S51	15,16	**12**,15,16	**12**,17
FGA	20,25	20,25,26	24,24.2
D16S539	10,12	10,11,12	8,12
CSF1PO	12,12	12,12	12,12
D13S317	8,12	8,**12**	8,**12**
D5S818	11,12	11,**12**	**12**,13
D7S820	8,12	8,12	11,11

Table 7: **Case 4 - Paternity Conclusion**: the complete genotype of mother, alleged father and female conceptus sample. Paternity conclusion at all 21 STR loci can be observed.
Alleles that match with the alleged father are highlighted.
Kit Used – Investigator 24plex QS Kit