Sri Lanka Journal of Forensic Medicine, Science & Law — 2024; 15(1):11-17
DOI: https://doi.org/10.4038/sljfmsl.v15i1.7956

CASE REPORT

COMPARATIVE DNA PROFILING OF CONCEPTUS PARTS IN PATERNITY DISPUTES AND RAPE INVESTIGATIONS: A CASE STUDY

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ABSTRACT

Forensic laboratories frequently receive cases related to abortion, particularly in the context of paternity disputes and rape crimes. However, the focus has traditionally been on the foetus, while the placenta and its associated components, which start development after one month and mature within three months, are often overlooked. This study represents the comparison of DNA profiling from various parts of the aborted conceptus, including the placenta (foetal side), placenta (maternal side), umbilical cord, and foetus. The results have produced consistent and expected profiles but with meticulous foetal tissue processing. Due to the significant presence of maternal blood in these components, a rigorous washing procedure for the aborted foetal material is imperative before DNA extraction can be carried out. The generated profiles emphasize the equal forensic utility of different conceptus components, challenging the conventional focus on the foetus alone. This study paves the way for collecting and processing of various developed parts of the conceptus for DNA profiling as required by the specific case scenarios.

Keywords: *DNA evidence; foetus; forensic investigation; placenta; umbilical cord.*

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ARTICLE HISTORY
Received: 26.10.2023
Received in revised form: 03.06.2024
Accepted: 21.04.2024
Available online: 25.06.2024

INTRODUCTION

In forensic science, the examination of abortion material holds significant importance in resolving paternity disputes and convicting perpetrators of rape crimes[1,2]. While foetuses are frequently collected from hospitals and sent to forensic laboratories, it is worth noting that the conceptus consists of more than just the foetus[3-5]. The development of the placenta and its associated components, which commences after the first month of pregnancy and is fully developed within three months, is often overlooked. The foetus originates from the zygote, the product of fertilisation of male and female gametes (spermatozoa and ovum), which takes place in the fallopian tube. The zygote progresses into a morula while descending from the fallopian tube to the uterine wall and firmly establishing itself within the uterine wall. This marks the commencement of embryonic development.

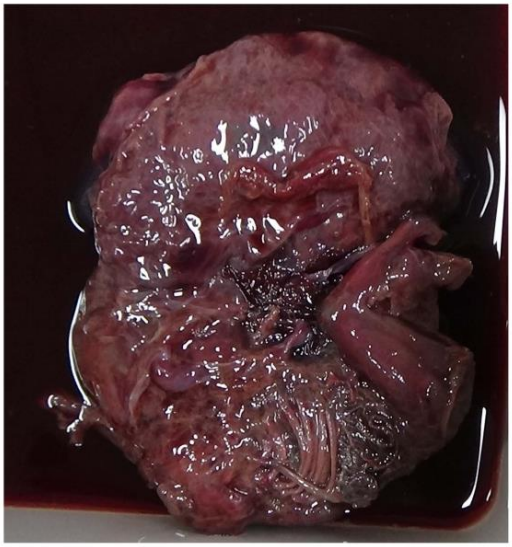
The solid morula consists of 16-32 cells, forming within 1-3 days after fertilization[6]. On the fourth day, the blastula formation initiates, giving rise to the inner cell mass and trophoblast. The inner cell mass further differentiates into the epiblast and hypoblast[7].In the third week, gastrulation transforms the epiblast into three germ layers: ectoderm, mesoderm, and endoderm.

The external coating of the blastocyst, originating from the trophoblast, constitutes the surface layer of the placenta[8]. Consequently, the placenta can be classified as a foetal organ with distinct constituents, including the parenchyma, chorion, amnion, and umbilical cord. This paper delves into DNA profiling using the placenta, umbilical cord and the foetus. This is usually neglected in almost all aborted foetal cases in which the medical officer concentrates on collecting the foetus alone when sending the foetus to establish its paternity. Therefore, the primary focus of this study is to ensure the coherence of DNA profiles, necessitating the collection of various embryonic components under specific circumstances and availability. The ramifications of this experiment will influence the collection, preservation, and DNA profiling of tissues other than the foetus. This has a huge impact on the traditional approach of frequently concentrating on foetus DNA during forensic inquiries linked to abortion cases. The study highlights the forensic importance of different elements of the conceptus, paving the way for a more inclusive strategy towards forensic analysis in such instances.

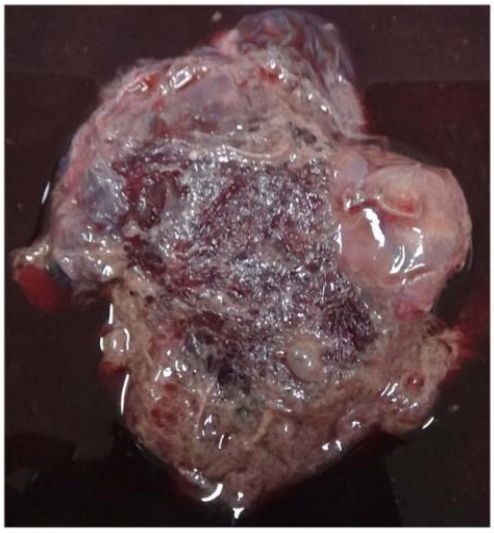
MATERIALS AND METHODS

DNA Extraction

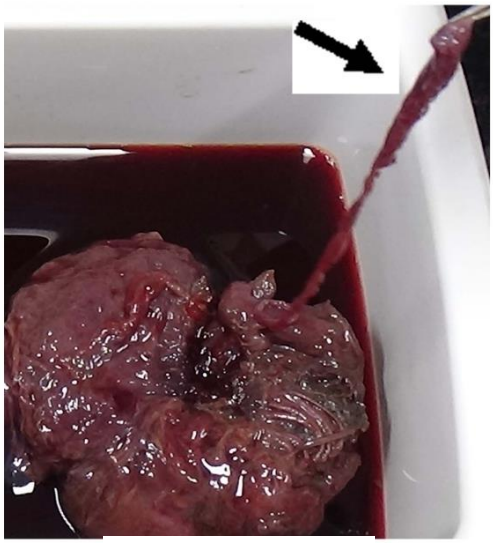
In the laboratory, we received a three-month-old, aborted conceptus, accompanied by a reference blood sample from the accused (alleged father), for forensic profiling and paternity testing. We excised 10 mg of tissues from various parts of the conceptus (maternal side placenta, foetal side placenta, umbilical cord, and foetus) as illustrated in Figure 1. These samples were meticulously collected with sterile scissors and placed in appropriately labelled 1.5 ml microcentrifuge tubes. Before lysing the tissues, they underwent two washes with TE buffer [tris(hydroxymethyl) aminomethane chloride (Tris-Cl) 10 mM, ethylenediaminetetraacetic acid (EDTA) 1 mM]. The lysis process was conducted at 56°C using a lysis buffer consisting of 10 mM Tris-Cl (pH 8.0), 200 mM NaCl, 1% sodium dodecyl sulfate (SDS), 10 mM (EDTA), 1 mM dithiothreitol (DTT), and 20 mg/ml proteinase K. For tissues, the lysis proceeded overnight, while blood sample as such underwent lysis for 2 hours. After lysis, the samples were combined with an equal volume of PCI (Phenol:Chloroform:Isoamyl alcohol in a ratio of 25:24:1) solution, briefly vortexed, and then centrifuged at 10,000 revolution per minute (rpm) for 5 minutes. The aqueous supernatant, containing DNA, was separated, and precipitated at -20°C for at least 30 minutes using 1 volume of 2-propanol ($CH_3CHOHCH_3$) and 0.1 volume of 3M sodium acetate (CH_3COO^- Na^+). Following this, the sample was centrifuged at 14,000 rpm for 15 minutes, and the resulting pellet was washed with 70% ethanol at room temperature (RT) and subjected to another round of pelleting centrifugation. The supernatant was discarded, and the DNA pellet was dried at RT before being resuspended in the final volume of 40 µl TE buffer.



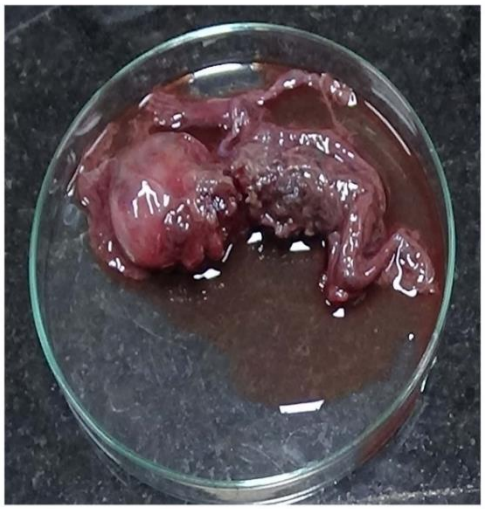
A - Placenta (foetal side)



B - Placenta (maternal side)



C - Umbilical cord



D - Foetus

Figure 1: Abortion materials (conceptus) used in the present case study.

The abortion materials (conceptus) consist of A. Placenta (maternal side), B. Placenta (foetal side) with an intact foetus, C. Umbilical cord, and D. Foetus.

DNA Quantification

The eluted DNA underwent quantification using real-time polymerase chain reaction (RT-PCR) with the Quantifiler® Trio DNA Quantification kit from Applied Biosystems[9]. This process involved a DNA standard solution (stock = 100 ng/µl), Quantifiler PCR Reaction Mix, and Quantifiler Human Primer Mix.

The Human Primer Mix (8.0 µl/sample) and PCR Reaction Mix (10.0 µl/sample) were combined and dispensed into reaction wells (18 µl each). Then, 2.0 µl of the sample or standard human DNA of known concentration was added to create a 20 µl PCR reaction system. The quantity of DNA was determined using the real-time PCR machine Software v2.3 (7500 Real-Time PCR System, Applied Biosystems). The concentrated DNA was diluted in TE buffer to achieve a concentration of 0.1ng/µl which was subsequently used for DNA amplification.

Short Tandem Repeat (STR) amplifications and Capillary Electrophoresis

For multiplex PCR reactions, we utilised the PowerPlex Fusion 6C® Kit from Promega Inc[10]. This kit enabled the co-amplification of 23 autosomal STR loci, three Y-STR loci, and a gender locus (Table 1). The PCR amplification mixture, composed of 25 µl in total (10 µl of PCR reaction mix, 5 µl of PowerPlex® Primer Set, and 10 µl of DNA template), was subjected to the following conditions: initial denaturation at 95°C for 5 minutes, followed by 28 cycles of denaturation at 94°C for 1 minute, annealing at 59°C for 1 minute, and extension at 72°C for 1 minute. The process concluded with a final extension step at 60°C for 10 minutes. The resulting PCR products were subsequently analysed using an 11 µl electrophoresis system that included 0.4 µl of WEN Size Standard dye, 9.6 µl of Hi-Di™ (highly deionized) formamide, and 1.0 µl of PCR product. For allele call determination, the allelic ladder of the PowerPlex Fusion 6C® Kit was used. Capillary electrophoresis was performed on an ABI-3500 Genetic Analyzer with a 36 cm 8-capillary array (Applied Biosystems) at 60°C oven temperature. The raw data files (.hid files) generated were imported to the GeneMapper® ID-X-v1.6 software and were analysed using its features. The electropherograms of the different samples used in this study are separately available as supplementary material (see Supplementary Materials).

RESULTS

Autosomal DNA profiling from various parts of the aborted conceptus, including the foetal side of the placenta, maternal side of the placenta, umbilical cord, and foetus, has produced consistent and expected profiles. The allelic called data and its comparative analysis are presented in Table 1, which shows the generation of the same human female DNA profile from the different parts of the conceptus, where at least one of the alleles at all the autosomal loci is shared with the corresponding locus of the male DNA profile generated from the alleged father's sample. The consistently generated profiles highlight the equal forensic utility of different conceptus components, challenging the conventional focus on the foetus alone. These DNA profiles were further subjected to manual statistical analysis for paternity testing, as elucidated below.

Establishment of Paternity through Statistical Analysis

The paternity index (PI) was calculated as the ratio of A to B, where A represents the likelihood ratio of the alleged father being the biological father (null hypothesis), and B is the probability (population allele frequency) of a random man from the population (alternative hypothesis for all other possible outcomes)[11].

The value of B (autosomal STR frequency) was obtained from the population STR frequency data of random unrelated individuals from the State of Madhya Pradesh, India[12].The combined paternity index (CPI) was obtained by multiplying all individual paternity indices (PI) calculated for each locus. CPI serves as a measure of the strength of genetic evidence supporting the hypothesis that the alleged man is the father of the child, as opposed to someone else from the population. In this case, the calculated CPI (2.88×10^{11}) (Table 1) was significantly greater than the critical value of 1. To further assess the strength of the hypothesis that the tested man is the biological father, as opposed to a random man from the population, based on non-genetic evidence, the Prior Probability of Paternity (POP) was computed as POP = CPI / (CPI + 1) = 0.99999. This result closely approaches the certainty value of 1. In the court system, a value of 0.5 is often considered a blind assumption based on the testimony of the father, mother, and other witnesses, indicating equal chances for both hypotheses to occur.

Table 1: Autosomal-STR profiles of Samples and Paternity Establishment

S. No.	Name of locus	Placenta (Maternal Side)	Placenta (Foetal Side)	Umbilical cord	Foetus	Alleged Father	Paternity Index (Duo Case) #
1	Amelogenin	X, X	X, X	X, X	X, X	X, Y	-
2	D3S1358	16, 16	16, 16	16, 16	16, 16	16, 16	4.05
3	D1S1656	15, 16.3	15, 16.3	15, 16.3	15, 16.3	15, 17	1.46
4	D2S441	11.3, 12	11.3, 12	11.3, 12	11.3, 12	12, 14	3.97
5	D10S1248	15, 17	15, 17	15, 17	15, 17	14, 17	4.24
6	D13S317	8, 10	8, 10	8, 10	8, 10	8, 13	1.20
7	Penta E	16, 16	16, 16	16, 16	16, 16	16, 22	5.50
8	D16S539	10, 11	10, 11	10, 11	10, 11	8, 11	0.76
9	D18S51	12, 15	12, 15	12, 15	12, 15	12, 13	2.66
10	D2S1338	20, 25	20, 25	20, 25	20, 25	19, 25	4.24
11	CSF1PO	10, 11	10, 11	10, 11	10, 11	10, 12	1.32
12	Penta D	9, 9	9, 9	9, 9	9, 9	9, 11	2.56
13	TH01	9, 9	9, 9	9, 9	9, 9	9, 9	2.72
14	vWA	16, 17	16, 17	16, 17	16, 17	14, 16	1.20
15	D21S11	29, 30	29, 30	29, 30	29, 30	28, 30	1.23
16	D7S820	8, 11	8, 11	8, 11	8, 11	8, 11	1.86
17	D5S818	12, 12	12, 12	12, 12	12, 12	12, 12	3.14
18	TPOX	8, 8	8, 8	8, 8	8, 8	8, 9	1.37
19	D8S1179	11, 11	11, 11	11, 11	11, 11	11, 15	6.33
20	D12S391	16, 18	16, 18	16, 18	16, 18	16, 22	125.0
21	D19S433	13, 14	13, 14	13, 14	13, 14	13, 14	19.1
22	SE33	17, 23.2	17, 23.2	17, 23.2	17, 23.2	23.2, 26.2	10.42
23	D22S1045	11, 11	11, 11	11, 11	11, 11	11, 16	1.52
24	DYS391	-	-	-	-	11	-
25	FGA	22, 24	22, 24	22, 24	22, 24	22, 23	1.97
26	DYS576	-	-	-	-	19	
27	DYS570	-	-	-	-	18	
							Combined Paternity Index= 2.88×10^{11}

#Paternity indices for each locus were computed based on random unrelated individuals from Madhya Pradesh, India[12]. The combined paternity index (for the foetus and alleged father) suggests that the alleged father is 2.88×10^{11} times more likely to be the father of the female child, compared to a random individual from the same population.

DISCUSSION

In the present forensic case study, we have introduced the DNA profiles obtained from different components of the conceptus, including the placenta and umbilical cord, which match the DNA profile of the female foetus. The tissues obtained from different sides of the placenta (maternal and foetal sides) yielded a consistent foetal DNA profile. This study thus holds significant implications for the retrieval of abortus material by medical professionals for forensic examination, as substantial amounts of placenta with umbilical cord start to develop roughly one month after conception. It has been observed that DNA profiling of only foetuses less than 1.5 months of gestation resulted in predominantly the mother's profile being generated. Between approximately 1.5 to 3 months, a mixture profile of mother and the foetus is commonly obtained. Therefore, during this gestational period (1-3 months), DNA analysis of the placenta should be considered for examination besides giving priority to the foetus, as it originates from the developing foetus itself. The reproduction of results from other parts of the conceptus also fosters the reliability of the DNA results. However, measures such as thorough washing (2-3 times) of these underdeveloped conceptus tissues must be undertaken, as they are often inundated with maternal blood during the abortion process. However if a mixture profile is generated, the mixture analysis may be performed manually or with an available mixture analysis software in the laboratory.

This case study emphasizes the importance of DNA profiling of the placenta and umbilical cord alongside examination of the aborted foetus during the 1-3 month gestational period, as they originate from the developing foetus itself. Reproducing results from these components enhances the reliability of DNA analysis, but it's vital to thoroughly wash these tissues to prevent maternal blood contamination. Therefore, the preservation of both the placenta and umbilical cord becomes crucial, providing additional evidence for intricate paternity and sexual assault cases in forensic inquiries.

CONCLUSION

This forensic case study ushers in DNA profiles from different components of the conceptus, including the placenta and umbilical cord, exactly matching with the female foetus' DNA profile. Tissues from different sides of the placenta consistently yielded the foetal DNA profile, suggesting implications for forensic examination of all these abortus material. Consequently, this experimental forensic research emphasizes the necessity of preserving the placenta and umbilical cord as crucial and invaluable supplemental evidence alongside the foetus. This practice will effectively address the problem, where there is a challenge of obtaining DNA profiles from less mature foetuses (which are 1.5 to 3 months old) or the profile has not been generated from the foetus alone. By integrating these frequently neglected elements of the conceptus into routine forensic practice, there is high potential to enhance the capacity to solve several paternity cases/disputes and effectively handle such sexual assault crime cases.

ACKNOWLEDGEMENTS

None.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

ETHICAL ISSUES

This article does not include any studies involving human participants or animals conducted by any of the authors. The “certificate of authority” to examine/use the samples forwarded (placenta with intact foetus) was given by the Superintendent of Police, South Andaman District, in accordance with a criminal case under the relevant sections of the Protection of Children from Sexual Offenses (POCSO) Act.

SOURCES OF SUPPORT

None.

AUTHOR CONTRIBUTIONS

NPN: Conducting PCR reactions, capillary electrophoresis and DNA analysis; final approval of the version to be published. **AB:** Foetal tissue dissection and DNA isolation; final approval of the version to be published. **DAK:** Foetal tissue dissection and DNA isolation; final approval of the version to be published. **AJK:** Conception of the

work; drafting the work and revising it critically for important intellectual content; final approval of the version to be published.

SUPPLEMENTARY MATERIALS

The electropherograms of the different samples used in this study are available at https://drive.google.com/file/d/12S-8gYwWL7xK4fjbAfsbhocVYKCv2eOi/view?usp=drive_link.

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