

Naif Arab University for Security Sciences Arab Journal of Forensic Sciences & Forensic Medicine

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# Paternity Cases within a Medicolegal Context: A Case Study of Heteropaternal Superfecundation in Iraq Hannan K. Mahmood <sup>1,\*</sup>, Ammirah J. Omar <sup>2</sup>, Khalifa M. Salih <sup>1</sup>

 <sup>1,\*</sup> Paternity and Kinship Division, Living Investigation Department, Medico-Legal Directorate, Ministry of Health, Baghdad, Iraq
 <sup>2</sup> Training and Human Resources Division, Medico-Legal Directorate, Ministry of Health, Baghdad, Iraq

Received 12 Oct. 2017; Accepted 14 Dec. 2017; Available Online 31 Dec. 2017

### Abstract

Establishing paternity through genetic testing is an essential aspect of the justice system in many countries. This may be complicated by the presence of dizygotic twins born due to heteropaternal superfecundation, which results in the birth of non-identical twins with different fathers. These cases are rare. This paper presents information on previous studies of this phenomenon along with a case study from Iraq which involved a paternity issue referred to the Medical-Legal Directorate (MLD) in Baghdad in August 2011.

Results revealed that the two non-identical twins were from different fathers. A maternity test was performed to test the possibility of switching at birth. Results negated this possibility, and maternity was later confirmed using DNA typing.

**Keywords:** Forensic Sciences, Heteropaternal Superfecundation, Non-identical twin, DNA Typing, Y Chromosome STR, Polymorphism

\* Corresponding Author: Hannan Kh. Mahmood Email: MLI\_Bag41@yahoo.com

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doi: 10.26735/16586794.2017.012





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Due to the rarity of heteropaternal superfecundation, this case study serves to highlight the importance of being aware of its occurrence, especially within legal and judicial contexts.

# قضايا الأبوة في إطار سياق طبي؛ دراسة حالة أبوة متباينة لحالة تلقيح مُضَاعَف (مفرط) في العراق.

#### المستخلص

يُعد إثبات الأبوة من خلال الاختبارات الوراثية جانباً أساسياً من جوانب نظام العدالة في دول عدة. وقد يكون هذا الاختبار معقداً بسبب وجود التوائم متباينة اللاقحة (الزايجوت) والتي ولدت بسبب وجود أبوين مختلفين أحدثا تلقيح مضاعف، مما يؤدي إلى ولادة التوائم الغير متطابقة من آباء مختلفين، وهذه الحالات نادرة.

تقدم هذه الورقة معلومات عن الدراسات السابقة المتعلقة بهذه الطاهرة جنباً إلى جنب مع دراسة حالة من العراق والتي تنطوي على قضية أبوة تابعة لإدارة الطب الشرعي في بغداد في آب/ أغسطس 2011.

أظهرت النتائج أن التوأمين غير المتطابقين كانا من أبوين مختلفين. وكان اختبار الأمومة قد أجري لاختبار إمكانية حدوث التبديل عند الولادة. وقد نفت النتائج هذه الإمكانية، وحددت الأم في

CrossMark

وقت لاحق باستخدام تحليل السمات الوراثية للحمض النووي. نظرا لندرة ظاهرة الأبوة المتباينة لحالات التلقيح المضاعف، فإن دراسة الحالة هذه تسَلط الضوء على أهمية أن نكون على بينة عند حدوثها ولا سيما في السياقات القانونية والقضائية.

الكلمات المفتاحية: علوم الأدلة الجنائية، تباين الأب في حالات التلقيح المضاعف، التوأم غير المتطابق، تحديد سمات الحمض النووي، تعدد أشكال التتابعات القصيرة المتكررة في الكرومسوم واي (Y).

# 1. Introduction

In numerous countries, extramarital intercourse is a crime punishable by law, whether consensual or otherwise. Furthermore, false accusations of intercourse or rape are punishable by law in various legal systems. Therefore, establishing paternity through genetic testing is an essential aspect of the justice system in those countries.

The Iraqi legal system makes extramarital intercourse a crime [3], and, in addition, there are religious and cultural elements of Iraqi society prohibiting it. This affirms the necessity of the aforementioned genetic testing within the Iraqi judicial system.

Typically, paternity tests involve the mother, the child, and one or more alleged fathers [4]. These cases may also involve identical (monozygotic) or non-identical (dizygotic) twins. In the case of dizygotic twins, establishing paternity may be complicated due to hetero paternal super fecundation.

Heteropaternal superfecundation is the fertilization of two or more oocytes during the same ovulatory cycle by separate coital acts [5]. These cases may be further complicated when a women has sexual intercourse with two different men during one poly-ovulatory menstrual cycle, resulting in the birth of twins with different fathers. The frequency of twins with different fathers is very low. In a study conducted by Girela et al., it represented 1:13,000 reported paternity cases involving twins in America [6]. Results of a study by Wenk et al. found that the frequency of heteropaternal superfecundation was 2.4% among dizygotic twins in their study sample [7]. Research into this is rare; however, there are studies from Denmark, Turkey, and China [8-10].

Omar et al. conducted analytical and statistical studies on various paternity test cases in Iraq that fell within a medicolegal context [1-2]; however, there is no previous study into heteropaternal superfecundation in Iraq.

This paper presents a legal case study of heteropaternal superfecundation in Baghdad.

## 2. Case Report

In August 2011, a man and two non-identical twins were officially referred to the Iraqi Medico-Legal Department (MLD) by the Court of Law in Baghdad for paternity investigations. The man claimed that the twins' ID cards presented him as the biological father of both; however, he contested this because of a temporary physical separation between his wife at the time she conceived them.

# 3. Materials and Methods

Blood samples were obtained on FTA cards from the father, mother and both twins. Direct extraction and DNA typing was made using PowerPlex<sup>®</sup> 18D PCR amplification kit (Promega corporation).



Simultaneous amplification of 18 STR loci (D3S1358, THO1, D21S11, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, VWA, D8S1179, TPOX, FGA, D19S433, D2S1338 & amelogenin ) was done in one run, while simultaneous amplification of 23 Y Chromosome STR loci (DYS576, DYS389I, DYS448, DY-S389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385, DYS456 and YGATAH4 ) was made using PowerPlex23<sup>®</sup> Y system Kit (Promega) in another run for the man and the twins. All the procedures were done according to the manual recommendation on 9700 PCR instrument (Applied Biosystem).

Capillary electrophoresis, run, and detection of amplified products was conducted with ABI prism 3130xI Genetic analyzer (16 capillaries array system-Applied Biosystem) following the manufacturer's protocol. Data collection was performed with data collection V.3.0 software (Applied Biosystem). Samples were analyzed by Genemapper® V.3.2 software (Applied Biosystem). In addition, blood samples were collected in separate tubes for conventional blood group analysis.

## 4. Results and Discussion

The results of Y Chromosome STR profiles for the father and twins are listed in Table-1. The analysis of the Y chromosome STR profile of the alleged father mismatched with the Y chromosome STR profile of the 1st and 2nd twin in 16 out of 23 genetics loci; therefore, this test excluded the alleged father from being the biological father of twins. Unexpectedly, the Y chromosome STR profile of the non-identical twins were mismatched from each other in many loci. This raised the question whether they were real twins related to the same mother.

Seemingly, from the same table, it is clear that there were mismatches in YSTR profiles between the non-identical twins in 14 out of 23 loci. This shows that both twins descended from different fathers.

The MLD biological staff initiated more and further investigations to confirm the twins had the same mother. This was done to exclude the possibility of switching at birth.

Blood group and Rh subgroups were investigated and listed in Table-2. The ABO blood group system and Rh subgroup E, exclude the link of the 1st twin to both parents as mother and father beyond any doubt; however, these markers could not exclude the link with the 2nd twin.

The results of the DNA typing for the alleged father, mother, and non-identical twins are listed in Table-3.

Focusing on the allele numbers of the twins' profiles, the analysis of DNA typing of the alleged father mismatched with the DNA typing of the 1st and 2nd twin in 6 and 8 genetic markers, respectively, out of 17 total autosomal genetic markers (Table-3). Therefore, this test excludes the alleged father from being the biological father for both children. Moreover, the match of DNA profiles between the twins and their mother did not exclude a maternal relationship.

Combined Maternity Indices were calculated and corresponded to 753769665 for the 1st twin and 195702872 for the 2nd twin; thus, the probability of maternity was 99.98%

Y-STR Loci	Alleged Father	1st twin	2nd twin
DYS 576	19	21	17
DYS 389I	14	12	13
DYS 448	20	20	20
DYS 389II	31	28	30
DYS 19	14	14	15
DYS 391	11	10	10
DYS 481	22	23	23
DYS 549	13	12	12
DYS 533	11	11	12
DYS 438	9	11	11
DYS 437	14	15	14
DYS 570	19	18	18
DYS 635	22	20	23
DYS 390	23	24	25
DYS 439	13	12	10
DYS 392	11	14	11
DYS 643	10	11	10
DYS 393	12	12	13
DYS 458	16	18	16
DYS 385	15/18	12/18	11/14
DYS 456	15	15	15
Y-GATA-H4	11	13	13

 Table 1- The results of 23 Y chromosome -23 STR Profiles of father and non-identical twins.

 Table 2- ABO and Rh subgroup systems of the father, mother and twins.

Name	ABO System	Rh	Ε	е	С	с
Father	0	+	Neg	+	+	+
Mother	В	+	Neg	+	+	Neg
1st twin	А	+	+	+	+	+
2nd twin	В	+	Neg	+	+	+



Name	Alleged Father	1st non-identical twin	2nd non-identical twin	Mother
D3S1385	17	15	15	15
	17	17	15	17
THO1	6	7	7	6
	9.3	7	10	7
D21S11	27	28	28	28
D18S51	28	31	30	29
	13	12	15	12
	13	15	17	17
PentaE	14	10	10	17
	14	18	18	18
D5 S818	8	12	13	11
	12	13	13	13
D13S317	8	12	8	11
	13	12	12	12
D7S820	9	10	8	10
	10	10	10	11
D16S539	11	9	9	11
	12	11	11	11
CSF1PO	10	9	10	10
	13	10	12	10

16.2

XY

XY

XY



PENTA-D

VWA

D8S1179

TPOX

FGA

D19S433

D2S1338

Amelogenin



16.2

XY

and 99.95%, respectively.

Upon questioning, the mother admitted that she was engaged in prostitution for financial purposes, but she refused to name nor recognize the involved men in the pregnancy. This scenario was found as a cause of dizygotic twin maternities in a study by James [12]. He found that the incidence of paternal superfecundation may be substantially higher in small selected groups of dizygotic twin maternities involving women engaged in prostitution.

Based on the above results, a report was sent to the court with the following statement: According to the mismatch of DNA and Y Chromosome profiles between the alleged father and twins, these investigations exclude the paternity of the assumed legal father to the named 1st and 2nd child; however, according to the match between the DNA profile of the assumed mother with that of the two named children, a maternal relationship could not be excluded and is thus confirmed for both children.

#### 5. Conclusion

The results of this case study show that dizygotic twin maternities can occur, especially in cases involving prostitution. The case study has also revealed that knowledge of this phenomenon is essential in genetic testing. This is especially the case in countries where extramarital intercourse is a crime, such as in Iraq.

#### Acknowledgment

We would like to thank the director of the MLD in Baghdad, Dr. Zaid A. Abbas and the head of Training and Human Resources Division Dr. Momina H. Hassan for supporting this report. Thanks and regards to all technical staff in the Paternity and Kinship Division of Living Investigation Department.

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