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Heteropaternal Superfecundation in Dizygotic Twins: Biological Mechanism, Genetic Identification and Forensic Implications

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Abstract

Heteropaternal superfecundation (HS) is a form of dizygotic twinning in which two oocytes released during the same ovulatory cycle are fertilized by sperm from different biological fathers. Although the phenomenon is uncommon and is most often recognized during disputed paternity investigations, published reports suggest that its true frequency is difficult to determine because many cases are never investigated or reported. This review summarizes the biological basis of HS, the genetic relationship between heteropaternal twins, approaches used for forensic identification, reported frequency estimates, and the implications for paternity testing and twin research. Early investigations relied on blood-group, HLA and protein markers, whereas modern forensic investigations use autosomal short tandem repeats (STRs), Y-chromosome STRs and amelogenin analysis. A 1992 database study identified three HS cases among 39,000 parentage records and reported a frequency of approximately 2.4% among dizygotic twin cases involved in paternity disputes, while emphasizing that this estimate should not be generalized to the population. A 2020 Colombian case demonstrated exclusion of the alleged father for one twin while supporting paternity for the other. A 2021 report further demonstrated the use of microhap sequencing of maternal plasma cell-free DNA with conventional STR confirmation. A 2025 case report and literature review examined 2,679 paternity tests over ten years and identified one HS case among 31 twin-related exclusions. Overall, HS illustrates why both dizygotic twins should be tested individually in paternity disputes and why continued development of sensitive molecular methods may increase recognition of previously undetected cases.

Keywords: Heteropaternal superfecundation, dizygotic twins, superfecundation, paternity testing, STR, Y-STR, forensic genetics, microhaplotype.

Introduction

Twin pregnancies are commonly divided into monozygotic (MZ) and dizygotic (DZ) categories. MZ twins originate from the same fertilized oocyte and are therefore genetically very similar, whereas DZ twins result from the fertilization of two separate oocytes and are genetically comparable to ordinary full siblings.

Heteropaternal superfecundation represents an unusual subtype of DZ twinning in which the two oocytes are fertilized by sperm from different men during the same ovulatory cycle.

The term superfecundation refers to fertilization of two or more oocytes during the same cycle following separate reproductive events. When the sperm originate from one father, the resulting twins are dizygotic full siblings. When sperm from different fathers fertilize the oocytes, the resulting twins are heteropaternal. The 1992 study by Wenk and colleagues described three HS cases in a database of 39,000 parentage records and emphasized that frequency estimates from disputed-paternity populations cannot automatically be applied to the general population.

Interest in HS is not limited to paternity disputes. Because heteropaternal twins share, on average, about one-quarter of their inherited genetic material, they resemble half-siblings genetically rather than ordinary full siblings. This distinction can be relevant to forensic identification, kinship analysis, organ-donation considerations and the interpretation of twin research.

Aim and Objectives:

The aim of this review is to summarize the available literature on heteropaternal superfecundation in dizygotic twins. The objectives are to:

- Define heteropaternal superfecundation and distinguish it from ordinary dizygotic twinning;
- Describe the biological and genetic basis of the phenomenon;
- Summarize important reported cases and frequency estimates;
- Review DNA-based approaches used to identify different

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biological fathers;
- Discuss forensic, legal and research implications; and
- Identify limitations in the current literature and areas for future investigation.

Biological Mechanism

During a normal ovulatory cycle, one or more oocytes may become available for fertilization. Superfecundation occurs when more than one oocyte is fertilized during the same cycle. When the fertilizing sperm originate from different men, the event is termed heteropaternal superfecundation. The resulting pregnancy is therefore dizygotic because two separate oocytes are involved.

The 2019 review and case report notes that, after ovulation, the opportunity for fertilization of an oocyte is limited, while sperm can remain viable for several days. This biological timing provides a mechanism by which sperm from different sources can fertilize separate oocytes released during the same cycle.

The phenomenon should be distinguished from superfetation, in which conception occurs at a different time after an established pregnancy. HS concerns fertilization of multiple oocytes within the same ovulatory cycle and does not require separate pregnancies.

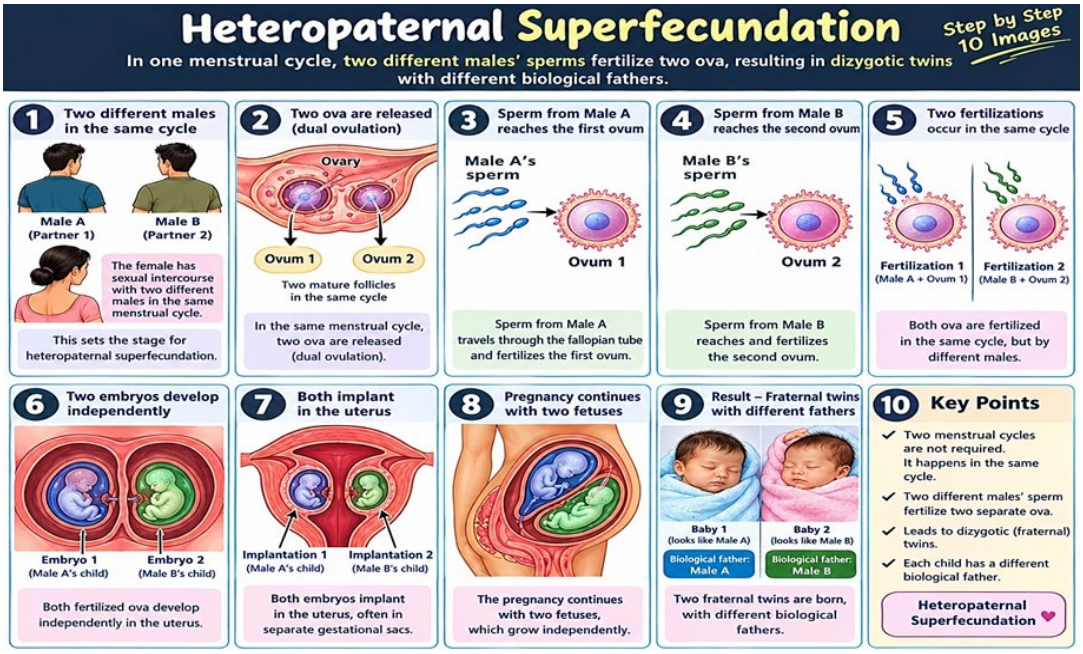


Fig 1: Process of heteropaternal superfecundation: two ova released in the same cycle are fertilized by sperm from two different males, resulting in dizygotic twins with different biological fathers.

Genetic Relationship of Heteropaternal Twins

The genetic relationship between heteropaternal twins differs from that of ordinary DZ twins. Ordinary DZ twins share the same mother and father and are expected to share approximately half of their segregating genetic variants on average. In contrast, heteropaternal twins share their maternal genetic contribution but have different paternal genetic contributions. Consequently, their average genetic relationship is similar to that between half-siblings, approximately 25% by descent.

This relationship was demonstrated particularly clearly in the 2019 case reported by Segal, Craig and Umstad. The twins shared 1,793 centimorgans (cM), a value within the reported half-sibling range and close to the expected mean. The alleged father showed genetic relatedness to only one of the twins, supporting heteropaternal twinning.

Comparison of Twin Types

Feature	Monozygotic Twins	Dizygotic Twins	Heteropaternal DZ Twins
Number of oocytes	One	Two	Two
Biological fathers	One	One	Two
Genetic relationship	Very high genetic similarity	Full-sibling relationship	Half-sibling-like relationship
Typical forensic issue	Usually not paternity-related	Paternity may be disputed	Important in disputed paternity
DNA testing	May establish zygosity	Can establish relatedness	Can identify different paternal relationships

Historical Development and Reported Cases

Historical descriptions of heteropaternal twinning preceded modern DNA testing. The 1992 Wenk *et al*. paper cites an early report from 1810 and describes later use of genetic markers to investigate suspected cases. The same paper reported three cases from a 39,000-record parentage database. The 2019 Segal *et al*. report described adult female twins whose genetic data showed a half-sibling-like relationship. The authors emphasized that some HS cases may remain undetected because twins can appear sufficiently similar to be accepted as ordinary DZ twins.

In a 2020 Colombian case, a pair of male twins was examined after a paternity request. Autosomal STR, Y-STR and amelogenin testing was performed. The alleged father was supported as the biological father of one twin, whereas multiple Y-STR and autosomal STR incompatibilities

excluded him as the father of the other twin. The paternity index for the supported twin was 2.5134 × 10^7 with a reported probability of paternity of 99.9999%.

A 2021 report demonstrated a newer approach: microhap sequencing of maternal plasma cell-free DNA during the tenth week of pregnancy. The result indicated different fathers for the twins and was confirmed using standard STR analysis, illustrating the potential of non-invasive molecular approaches in early pregnancy.

A 2025 case report and literature review described a court-ordered paternity investigation using 19 STR markers and amelogenin. The study also examined 2,679 paternity tests collected over ten years. The alleged father was confirmed for twin 1 but not twin 2; 14 of 19 STR loci showed absent alleles in twin 2. The dataset contained 553 paternity exclusions, including 31 twin-related cases, of which one was identified as HS.

Frequency and Epidemiology

The frequency of HS remains uncertain. The most frequently cited numerical estimate comes from Wenk *et al*. (1992), who identified three cases among 39,000 complete paternity records. Among dizygotic twins whose parents were involved in paternity disputes, the reported frequency was 2.4%. The authors specifically cautioned that the study population was not necessarily representative of the general population.

The same study estimated a frequency of approximately one heteropaternal DZ twin pair per 13,000 disputed-parentage cases, but again described this as potentially a low estimate for the general population. The authors considered the 3 cases among 127 DZ twin pairs (approximately 2.36%) a potentially more informative figure for their study group, while stating that its applicability to the general population remained uncertain.

The 2019 review similarly concluded that the baseline frequency is unknown and may be underestimated because many twin pairs are never subjected to paternity testing or molecular investigation.

Therefore, reported percentages should be interpreted as frequencies within particular forensic or disputed-parentage populations rather than as population prevalence. Differences in study design, paternity-testing practices, access to molecular diagnostics and ascertainment of twin zygosity can all influence the observed frequency.

Forensic Genetic Identification

Autosomal STR Analysis

Autosomal short tandem repeats are highly polymorphic DNA markers widely used in human identification and paternity testing. In an individual, each autosomal locus contains alleles inherited from the mother and biological father. In a paternity investigation involving twins, each twin must be evaluated separately. If an alleged father is compatible with one twin but incompatible with the other at multiple informative loci, different paternal relationships may be suspected.

Y-chromosome STR Analysis

Y-STR markers can provide additional paternal-line information for male individuals because the Y chromosome is transmitted through the paternal line. The 2020 Colombian case used 17 Y-STR markers and observed 14 exclusions for the twin who was not biologically related to the alleged father.

Amelogenin

The amelogenin locus is commonly included in forensic genetic kits to assist with sex-chromosome typing. In the Colombian investigation it was included together with autosomal STR and Y-STR analyses. It contributes to the overall genetic profile but is not, by itself, sufficient to establish heteropaternal superfecundation.

Paternity Index and Probability of Paternity

When genetic evidence supports paternity, statistical measures such as the paternity index and probability of paternity can summarize the strength of the evidence under the assumptions used in the analysis. The Colombian case reported a paternity index of 2.5134 × 10^7 and a probability of paternity of 99.9999% for one twin, while the other twin was excluded.

Microhaplotypes and Cell-free DNA

The 2021 microhap study demonstrates an emerging approach using highly polymorphic microhaplotypes and maternal plasma cell-free DNA. The study reported identification of HS at the tenth week of pregnancy and used conventional STR analysis for confirmation. This approach is notable because it suggests a potential non-invasive route to resolving complex prenatal paternity questions earlier than traditional invasive sampling.

Summary of Selected Published Evidence

Study	Design / Sample	Main Genetic Method	Key Finding	Importance
Wenk *et al*., 1992	39,000 parentage records	Blood groups, HLA, protein and DNA markers	3 HS cases; ~2.4% among DZ twin paternity cases	Important frequency estimate; not general-population prevalence
Segal *et al*., 2019	Adult female twin case	DNA matching / shared cM	1,793 cM between twins; father related to only one	Shows half-sibling-like relationship and under-detection issue
Mogollón *et al*., 2020	Male DZ twins in Colombia	Autosomal STR, Y-STR, amelogenin	Father supported for one twin and excluded for the other	Demonstrates need to test both twins
Ou & Bai, 2021	Female twins at 10 weeks	Microhap sequencing + cfDNA; STR confirmation	Different biological fathers identified	Shows potential for early non-invasive testing
Timasheva & Tuktarova, 2025	Court-ordered case + 2,679 tests	19 STR markers + amelogenin	1 HS case among 31 twin-related exclusions	Recent forensic dataset and literature review

Forensic, Legal and Social Significance

HS has direct relevance to disputed paternity because testing only one twin can produce an incomplete or misleading conclusion about the other twin. The Colombian case specifically emphasized inclusion of both twins in paternity testing.

The phenomenon also demonstrates the importance of complete family sampling. Including the mother helps

determine the maternal contribution and reduces the possibility that apparent paternal incompatibilities are interpreted without adequate context. Where appropriate, additional alleged fathers and relevant relatives may be considered according to forensic laboratory procedures.

Beyond paternity, the 2019 review discussed implications for organ donation and twin research. Because HP twins have a half-sibling-like genetic relationship, assuming that all DZ twins share the same father can introduce errors in specialized kinship interpretation.

Importance in Twin Research

Twin studies often compare MZ and DZ twins to estimate the relative contribution of genetic and environmental factors to traits. If an HP pair is classified as an ordinary DZ pair, the pair may be less genetically similar than expected for full siblings. Segal and colleagues noted that this could influence estimates of genetic influence in relatively small twin samples. Molecular zygosity testing can distinguish HP pairs from ordinary DZ pairs more reliably than appearance-based questionnaires alone.

Limitations of the Current Literature

- The number of published HS cases is small, so estimates are sensitive to ascertainment and publication bias.
- Many available studies are case reports or retrospective laboratory reviews rather than population-based investigations.
- The frequently cited 2.4% estimate comes from a disputed-parentage population and should not be interpreted as population prevalence.
- Older studies used genetic systems that are less informative than current high-resolution DNA methods, making direct comparison across decades difficult.
- The social circumstances leading to paternity testing can influence which cases are detected, so published cases may not represent all HS conceptions.
- Shared-DNA ranges between different biological relationships can overlap; therefore, interpretation should rely on a complete genetic analysis rather than a single numerical value.

Future Directions

Future research should focus on larger, standardized datasets that record the number of DZ twin paternity investigations, the number of pairs tested, the number of confirmed HS cases, and the genetic methods used. Such datasets would allow clearer separation between the frequency of HS among disputed-paternity cases and its occurrence in the wider population.

Advances in massively parallel sequencing, microhaplotypes and cell-free DNA analysis may improve the ability to resolve complex kinship relationships. The 2021 study provides an example of how microhap sequencing and computational analysis can identify HS during pregnancy while using conventional STR analysis for confirmation. Another important direction is the development of clear laboratory protocols for twin paternity testing.

The evidence reviewed here supports testing each twin individually rather than assuming that an alleged father's relationship with one twin automatically establishes paternity for the co-twin.

Conclusion

Heteropaternal superfecundation is an unusual form of dizygotic twinning in which twins share a mother but have different biological fathers. Although its true frequency remains uncertain, multiple documented cases demonstrate that it is a real and diagnostically important phenomenon. The available literature shows a transition from older blood-group and HLA-based investigations to modern STR, Y-STR and sequencing-based approaches. The 1992 frequency study provides an important historical estimate but also highlights the limitations of extrapolating forensic case-series data to the general population. More recent reports demonstrate that molecular testing can resolve apparently complex paternity relationships and that advanced methods may permit earlier or more sensitive detection. For forensic practice, the central lesson is that both dizygotic twins should be evaluated separately in a paternity dispute. Continued accumulation of standardized genetic data will be important for determining how frequently HS occurs and for understanding its broader forensic and research implications.

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