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How Frequent Is Heteropaternal Superfecundation?

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Abstract. A newly discovered case of heteropaternal superfecundation (HS) is reported. Three HS cases were found in a parentage test database of 39,000 records. The frequency of HS among dizygotic twins whose parents were involved in paternity suits is 2.4%. Although the study population appears similar to the general population with respect to twinning data, inferences about the frequency of HS in other populations should be drawn with caution.

Key words: Heteropaternity, Parentage, Superfecundation

INTRODUCTION

In nature, superfecundation involves separate copulatory events, fertilization and implantation of two or more released ova during one menstrual cycle, and simultaneous delivery of two or more siblings following a normal gestational interval [6]. When there is a single male partner, the newborns are dizygotic (DZ) full sibs. The frequency of superfecundation is unknown, but may be expected to increase, possibly because of human behavioral changes and partly as the result of the use of fertility drugs and other medical interventions.

Heteropaternal superfecundation (HS) involves the multiple birth of infants sired by different men. HS is rare, but was initially evident because of racial differences between or among offspring [1,7]. More recently, it has been identified by genetic marker analyses in studies of genetic disease or in parentage disputes [5,8,9,11,12,13]. We report a case of HS and comment on its frequency.

METHODS

Case History and Tests

A twenty-year-old mother of twenty-month-old twins accused two half brothers (RB and JC) of paternity. All five individuals appeared to be white and the adults declared themselves caucasian in court. The clinically healthy twins were dizygotic; one was male and the other female. Initial genetic tests of parentage were carried out in response to court orders. Tests included hemagglutination studies of erythrocyte marker systems ABO, RH, MNS, KEL, FY, and JK; and cytotoxicity studies of HLA markers of subloci A, B and C. The combined probability of exclusion of these tests is 97.3%.

One of the men (RB) was excluded from paternity of the girl on the basis of the KEL and HLA systems (both "direct exclusions"), but he remained unexcluded from paternity of the boy. The second man (JC) was excluded from paternity of the boy by the MNS system alone (also a "direct exclusion"), but not from paternity of the girl. The mother stated that there were no other putative fathers. Neither man denied fertility or access to the mother.

Additional tests were undertaken because the findings suggested that each of the two accused men was a father of only one of the two children. Furthermore, laboratory policy requires two exclusionary findings at different loci to assure nonparentage. Tests included isofocusing of alleles of loci PGM1, ACP1, ESD, TF, GC; agglutination inhibition for IGH and IGK; and use of single locus DNA probes following digestion with Hae III and Southern analysis for loci YNH24,D2S44; TBQ7,D10S28; and 3'HVR,Chr16. Additional electrophoretic tests that were carried out at reference laboratories included those for loci GLO, HP, and PLG. Methods and calculation of paternity indices (PIs) were performed in customary ways [10].

Frequency Estimates

The total number of completed paternity cases was counted by computer. From these, the numbers of monozygotic (MZ) and dizygotic (DZ) twin cases were determined from the records in blacks, whites and mixed racial couples. Zygosity was determined from all the genetic information available and notations, if recorded, of whether the mother considered the twins identical. It was not possible to determine zygosity from Weinberg's rule because the sexes of the twins were not usually recorded [4]. The frequency of heteropaternity was calculated among DZ twins.

RESULTS

The phenotypes of the mother, her two children, and the two men are shown in Table 1. Combining the genetic tests, the odds favoring RB as the biologic father of the male child were 1.7×10^6:1; the odds favoring JC as the father of the female child were 5.5×10^5:1. The relevant findings from the two cases we reported previously are included in Table 2.

In 39,000 complete paternity case records, there were 115 cases of MZ twins and 124

Table 1 - Phenotypes of mother, her twins and two partners

Marker system	Mother's phenotype	Male child's phenotype	Female child's phenotype	Phenotype of men	
				(JC)	(RB)
ABO	O	O	O	O	O
MNS	MNs	MSs	NS	MNs	MNSs
RH	cde	cDEe	CcDe	CcDEe	CcDEe
KEL	K-k+	K-k+	K+k+	K+k+	K-k+
FY	a+b+	a+b+	a-b+	a-b+	a+b+
JK	a+	a+	a+	a+	a+
HLA-A	A1, A23	A1, A23	A1, A2	A1, A2	A1, A29
B	B21, B44	B17, B21	B18, B44	B17, B18	B8, B17
C	Blank	Blank	Blank	Blank	Blank
PGM	1+2-	1+	1+	1+	1+
ACP	B	B	BA	BA	BA
ESD	1	1	1	1	1
TF	C2	C1C2	C1C2	C1	C1
GC	1s	1s2	1s2	1s2	1s2
IGH	A,FNB,G 1	FNB 1	AN'G 1	AN'G 1	A,FNB 1,2
IGK	3	3	1,3	1,3	3
PLG	23	12	12	1	2
BF	S	FS	FS	F	FS
HP	12	2	2	12	12
GLO	2	12	12	12	12
YNH24 *	1.99/1.59	3.45 /1.99	1.99/2.85	2.85/1.35	3.45/3.19
TBQ7*	5.20/1.03	5.20/0.90	Not done	Not done	2.36/0.90
3'HVR*	2.51/0.88	0.99/0.88	Not done	Not done	0.99/0.75

Evidence of exclusion of each man for a given child is underlined.
* VNTRs of DNA:YNH24, D2S44, Hae III; TBQ7, D 10S28, Hae III; 3'HVR, Chr 16, Hae III.

Table 2 - Summary of three cases of superfecundation

	Case 1	Case 2	Current case
Maternal age (yrs)	19	24	20
Race of individuals	Caucasian	Black	Caucasian
Fathers available	1	2	2
Loci tested	ABO, RH, MNS, KEL, FY, JK, LE, LU, P, HLA-ABC, ADA, AK, PGM, 6PGD, TF, HP, GC, IGH, IGK, UMPK, HB	ABO, RH, MNS, KEL, FY, JK, HLA-ABC, PGM, ACP, ESD, TF, GC, D2S44, D10S28, 3'HVRChr16	ABO, RH, MNS, KEL, FY, JK, HLA-ABC, TF, PGM, ACP, GC, ESD, IGH, BF, IGK, PLG, HP, GLO, D2S44, D10S28, 3'HVRChr16
Loci excluding paternity	MNS, ACP, ESD, IGH	HLA-ABC, ACP, ESD, GC, 3'HVRChr16, D2S44, D10S28	KEL, IGH, PLG, IGK, HLA-A, D2S44
Odds of paternity			
First man	79:1	4.2×10^3:1	1.7×10^6:1
Second man	Not available	4.0×10^8:1	5.5×10^5:1

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Table 3 - Distribution by race and maternal age of MZ and DZ twins encountered in parentage analyses

Maternal age	Black children		White children		Children mixed racially	
(yr)	MZ	DZ	MZ	DZ	MZ	DZ
14-19	15	9	24	5	2	2
20-24	9	21	20	26	4	5
25-29	5	10	16	26	6	1
30-34	3	7	4	6	1	1
35-42	3	3	2	2	1	0
Totals (= 239)	35	50	66	65	14	9
Mean maternal age (23.09 for all)	23.1	24.2	22.9	24.1	24.6	22.6

cases of DZ twins. (There were the additional three cases of twins with evidence of heteropaternity reported here). The racial and age distributions of MZ and DZ twins are shown in Table 3.

DISCUSSION

Delivery of multiple offspring sired by different males is a rarity in man. Two dozen cases have been reported worldwide, but the number of heteropaternal twins may increase in the future for several reasons. First, the frequencies of multiple concurrent partners and the number of coital events (which are believed to induce secondary ovulations) have increased [2,3]. Second, use of fertility drugs has increased the frequency with which multiple ova are released. Third, gamete donation can increase the number of multiple, heteropaternal gestations. To date, our laboratory has encountered three cases of heteropaternal twins in 39,000 cases of disputed paternity.

The frequencies of MZ and DZ twins were estimated in order to demonstrate that observations in cases of disputed parentage are not unlike data from other investigations of multiple births. The frequency of MZ twins is considered to be almost constant in the populations studied: about one per 300 births (0.0033). The frequency of DZ twins, however, varies by age and parity of the mother; and by population and factors that are maternally inherited [4]. In the population of disputed parentage cases, the frequency of MZ and DZ twins were 2.9×10^{-3} and 4.2×10^{-3} respectively.

Among twin-bearing primiparous women of ages 15-19 years, a majority of the twins are expected to be MZ and a minority are DZ. In older and multiparous women, there is an expected greater proportion of DZ twins. In the present study, the relative proportions of MZ and DZ twins among all cases involving twins were 0.475 and 0.525 respectively and the average age of mothers delivering twins in the parentage test groups were

similar (Table 3). Young mothers (14-19 years) showed a majority of MZ twins (0.72) as compared to mothers in older age groups.

The estimated frequency of heteropaternal DZ twins among all disputed parentage cases is 1 per 13,000. This figure is probably a low estimate for the general population because of the youth of mothers in the parentage study group, and the likelihood that many of the young women are primiparous. The frequency of three cases per 127 DZ twins (2.36%) in the study group may be more valid. Whether this frequency applies to the general population remains to be observed.

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