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The Validity of Weinberg's Rule in the East Flanders Prospective Twin Survey (EFPTS)

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Abstract. Most population studies of twins estimate the number of monozygotic (MZ) and dizygotic (DZ) pairs by Weinberg's differential rule. This rule assumes that within the DZ twins the numbers of unlike-sexed (U) and like-sexed (L) twins are equal. The literature on the validity of Weinberg's rule is still controversial. In this prospective population-based study (EFPTS) of 2,589 twin pairs, of whom 2,577 were of known zygosity and placentation, the estimates of Weinberg's rule agree well with the results of direct zygosity determination.

Key words: Twins, Weinberg's rule, Zygosity determination, Ovulation induction, Population study

INTRODUCTION

Most large studies using twins and the twin method lack information about exact zygosity. The incidence of monozygotic (MZ) and dizygotic (DZ) twins must therefore be estimated. Weinberg's differential rule [9] estimates the number of DZ and MZ twins as DZ = 2U and MZ = L − U, where L stands for the number of like-sexed and U for the number of unlike-sexed twin pairs. This method assumes that (1) the sex proportion (proportion of males among all births) of DZ twins is 0.5, and that (2) the sex of both members of a DZ pair is determined independently and with the same probability. Therefore, the number of unlike-sexed pairs and like-sexed pairs among DZ twin pairs must be equal.

Even if the first assumption were not exactly true, then Bulmer and Allen showed that the correction to this rule would have a negligible effect [1,2]. The second assumption is invalidated if, as suggested by James [5,6], the sex of the

offspring is related to the day of conception within the menstrual cycle. In this hypothesis more like-sexed than unlike-sexed DZ twins are produced and Weinberg's rule should underestimate the number of DZ twins and overestimate the number of MZ twins.

We report on the validity of Weinberg's rule in a consecutive series of 2,589 twin pairs.

MATERIAL AND METHODS

The East Flanders Prospective Twin Survey (EFPTS), sponsored by the Flemish Society of General Practitioners and the Flemish Society of Obstetrics and Gynaecology in the province of East Flanders (Belgium), registered 2,589 twin pairs between July 1964 and December 1985. The diagnosis of zygosity was based on sex, fetal membranes, umbilical-cord blood groups (AB0, Rhesus, MNSs, Kell and Duffy), placental alkaline phosphatase, and in some cases RFLP's [3] (Table 1). The placental membranes were examined macroscopically on the fresh specimen and,

Table 1 - Number of twin pairs according to zygosity and placentation (EFPTS 1964-1985)

	Number of twin pairs	%
Monozygotic: monochorionic	746	28.8
Dizygotic: different sex	714	27.6
Dizygotic: same sex, different markers	654	25.3
Dichorionic: same sex and markers	463	17.9
Unknown markers and/or sex	12	0.5
Total twins	2589	100.0

when present, a piece of the dividing septum was subjected to microscopic examination. Same-sexed dichorionic pairs with at least one different genetic marker and unlike-sexed pairs were classified as DZ. Monochorionic twins were classified as MZ. On the basis of the genetic markers the probability of monozygosity was calculated for all dichorionic twins with identical sex and genetic markers [8]. Of the 2,589 twin pairs 12 dichorionic pairs (11 of the same sex and 1 of unknown sex) had no genetic markers performed and no zygosity could thus be determined. In a total of 2,577 twin pairs there was sufficient information to determine zygosity. Of this total, 714 were of different sex (U) and 1,863 of the same sex (L). There were 22 pairs without information on the placenta but in whom the genetic markers were examined. So their zygosity could be assigned and they were classified as DZ. The goodness of fit test was performed by a χ^2 test.

RESULTS AND DISCUSSION

The representativity and possible sampling biases were evaluated by comparing the data of the EFPTS with those from the population of East Flanders. The overall and yearly number of single and multiple births in East-Flanders were obtained from the National Institute of Statistics. The EFPTS covered an average of 68% of all twins born during that period in East Flanders. The coverage rate gradually increased over years up to 91% recently. The number of unlike-sexed twin pairs and of stillbirths was not different between the EFPTS and the total Flemish population ($P > 0.05$).

Table 2 - Probability of zygosity of twins

Zygosity	Certainty	Source	Number	Cumulative number	
				MZ	DZ
MZ	proven	MC	746	746	
	$P_{MZ} \geq 0.99$	DC SS SM	62	808	
	$P_{MZ} = 0.95\text{-}0.98$	DC SS SM	219	1027	
	$P_{MZ} = 0.90\text{-}0.94$	DC SS SM	73	1110	
	$P_{MZ} = 0.80\text{-}0.89$	DC SS SM	45	1145	
	$P_{MZ} = 0.50\text{-}0.79$	DC SS SM	38	1183	
					1394
DZ	$P_{MZ} < 0.50$	DC SS SM	26		1368
	proven	US+ 0 dif	110		1258
	proven	SS+ $\geq$ 1 dif	654		
	proven	US+ $\geq$ 1 dif	604		

MZ: monozygotic, DZ: dizygotic, MC: monochorionic;
DC SS SM: dichorionic like-sexed with same markers;
SS: same sex, US: different sex;
Cumulative number: for MZ the numbers were cumulated from top to bottom; for DZ the numbers were cumulated from bottom to top.

The combined information on sex, fetal membranes and genetic markers proved zygosity in 2,114 twin pairs (81.6%): 746 monochorionic and thus MZ pairs, and 1,368 dichorionic pairs of different sex or with one (457) or more (801) differences in genetic markers and thus DZ pairs. None of the 740 monochorionic pairs in whom the genetic markers were performed, showed a confirmed difference in any marker. To check the first assumption of Weinberg's rule, the sex proportion of the proven DZ twins was calculated. In this study there were 1,410 male out of 2,736 DZ twin

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children, or a sex proportion at birth of 0.515, which is not significantly different from the assumed 0.50 ($P > 0.10$). The second assumption, that the number of unlike-sexed pairs among DZ twins equals that of the like-sexed, proved also not to deviate significantly (654 like-sexed, 714 unlike-sexed DZ pairs, $P > 0.10$).

Table 3 - Zygosity of the spontaneous twins (1978-1985)

Zygosity	Certainty	Source	Number	Cumulative number	
				MZ	DZ
MZ	proven	MC	284	284	
	$P_{MZ} \geq 0.99$	DC SS SM	30	314	
	$P_{MZ} = 0.95\text{-}0.98$	DC SS SM	90	404	
	$P_{MZ} = 0.90\text{-}0.94$	DC SS SM	18	422	
	$P_{MZ} = 0.80\text{-}0.89$	DC SS SM	16	438	
	$P_{MZ} = 0.50\text{-}0.79$	DC SS SM	6	444	
DZ	$P_{MZ} < 0.50$	DC SS SM	1		430
	proven	US+ 0 dif	26		429
	proven	SS+ $\geq$ 1 dif	203		403
	proven	US+ $\geq$ 1 dif	200		200

MZ: monozygotic, DZ: dizygotic, MC: monochorionic;
DC SS SM: dichorionic like-sexed with same markers;
SS: same sex, US: different sex;
Cumulative number: for MZ the numbers were cumulated from top to bottom;
for DZ the numbers were cumulated from bottom to top.

As all like-sexed dichorionic pairs with identical markers are likely to be MZ, their genetic markers were used to calculate the probability of monozygosity by the lod-score method [8] (Table 2). If $P_{MZ} > 0.90$ is taken as the threshold for monozygosity, there were 1,110 (45%) MZ and 1,368 (55%) DZ pairs out of 2,478 twin pairs with sufficient information to be classified. In the remaining 109 pairs (4,2%) more markers should be examined to improve the probability of their zygosity diagnosis. However, if they are classified according to their most likely diagnosis, one ends up with 1,183 (45.9%) MZ and 1,394 (54.0%) DZ twin pairs.

According to Weinberg's rule one expected 1,428 DZ ($2 \times U = 2 \times 714$) and 1,149 MZ pairs ($L - U = 1{,}863 - 714$). These figures did not differ significantly from the 1,394 DZ and 1,183 MZ twin pairs observed, when, as before, each twin pair was allocated to its most likely zygosity ($P > 0.10$).

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Since its introduction in the sixties, the artificial induction of ovulation (AIO) is known to increase the number of multiple pregnancies. Because of its mode of action, the artificial ovulation induction is generally believed to produce multiple births by the fertilization of several eggs [7] and therefore to result in DZ twinning. This could be a possible source of error in Weinberg's rule.

Artificial induction of ovulation in the EFPTS was only ascertained from 1978 on. The zygosity of the spontaneous twins can thus only be investigated during the period from 1978 to 1985. During this period an average of 11% of all twins was the product of an artificial induction of ovulation [4]. The zygosity of the spontaneous twins is listed in Table 3. There were 226 twin pairs of different sex (U) and 648 of the same sex (L). According to Weinberg's rule one expected 422 MZ and 452 DZ twin pairs. Again, this did not differ significantly from the 444 observed MZ and 430 DZ pairs, when as before each pair was allocated to its most likely zygosity ($P > 0.10$). Even within the 102 twin pairs born after ovulation induction, we can demonstrate that the observed numbers of MZ and DZ pairs do agree with the expected numbers according to Weinberg's rule. The numbers, however, are small.

Taken together our data fit with Weinberg's rule.

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