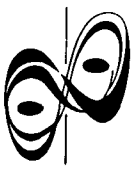

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Zygosity, Placental Membranes and Weinberg's Rule in a Danish Consecutive Twin Series

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Abstract. The usual assumption that monochorionic twins are monozygotic has recently been questioned, based on blood group discordance in 3 of 12 monochorionic pairs. Therefore, this study evaluates the validity of zygosity diagnosis based on examination of placental membranes, and at the same time evaluates Weinberg's differential rule in a Danish consecutive twin series. All twin pairs, 265 like-sexed and 87 unlike-sexed pairs, born at Odense University Hospital, Denmark, from 1 January 1980 through 31 August 1988, were ascertained. In 229 like-sexed pairs, zygosity was determined by serological methods, and in 190 of these, the fetal membranes could be reliably classified. Fiftynine monochorionic twin pairs were identified and none of those pairs were discordant for any genetic markers. Therefore, we conclude that monochorionic twins can be considered monozygotic. The distribution of monozygotic and dizygotic pairs was in accordance with Weinberg's rule.

Key words: Zygosity diagnosis, Chorionicity, Weinberg's rule

INTRODUCTION

Two types of twins, monozygotic (MZ) and dizygotic (DZ), are usually described. DZ twins, like ordinary sibs, have on average 50% of their genes in common [12] and dichorionic (DC) as well as diamniotic (DA) fetal membranes. MZ twins have identical genes but the placentation and development of the membranes can take place in three different ways depending upon the embryonic stage at the time of splitting [2,5]. At the morula stage (day 0,3), splitting leads to double placentation and DC/DA membranes as in DZ twinning. If implantation of the two embryos is next to each other, coalescence of the two placentae will often result; otherwise, two separate placentae are seen. If splitting occurs at the early blastocyst stage (day 3-8), one single placenta is formed and the

membranes will be monochorionic (MC). However, as the amniotic cavity has not yet developed, the twins will become DA. In contrast, monoamniotic (MA) membranes are found if splitting takes place at late balstocyst stage (day 8-13).

Twin zygosity can be reliably diagnosed by the use of polymorphic genetic markers, such as blood group antigens, enzymes, and serum types [6].

Examination of the fetal membranes is an established method of zygosity diagnosis of twins [5]. If MC membranes are demonstrated, the twin pair is considered to be MZ, whereas DC twins can be either MZ or DZ. However, in a few case reports [1,11], DZ pairs with MC membranes have been described and, recently, a series of 12 MC twin pairs were presented, 3 of which differed by some of the blood groups [10], implying that some MC twins may be DZ.

Whereas investigation of the fetal membranes and polymorphic genetic markers is used in zygosity diagnosis in individual twin pairs, Weinberg's method is used to estimate the mumber of DZ and MZ pairs in a population [2]. The rule has been questioned in several studies [3,4] where the number of MZ twins seems to be overestimated.

The purpose of this study is to examine the reliability of zygosity diagnosis based on fetal membranes in a consecutive Danish twin series. Furthermore, the validity of Weinberg's method will be tested.

MATERIALS AND METHODS

All twins born in the Department of Gynaecology and Obstetrics, Odense University Hospital, were ascertained through the birth records for the period of 1 January 1980 through 31 August 1988.

Since 1 January 1980, the majority of like-sexed twins have had a blood sample taken from the umbilical cord for zygosity diagnosis. The samples have been analysed at the Institute of Forensic Genetics, University of Copenhagen, for a panel including 15 to 17 of the following blood, serum, and enzyme types: AB0, MNS, Rhesus, K, Fy^a, Hp, Gc, Tf, PGM-1, AcP, GPT, EsD, GLO, AK, PGD, ADA, GLT/Gt. Twins were classified as DZ if they were discordant for one or more of those types, and MZ when all types were concordant. With these markers it can be estimated that about 99.5% of all DZ pairs may be expected to show some serological difference proving their dizygosity.

Placentae were formalin-fixed and submitted for routine histopathological examination at the Institute of Pathology, Odense University Hospital. A macroscopic description was made, noting at which sites sections were taken. Sections were routinely taken from the cords, membranes, septum, and placental parenchyma.

The haematoxylin and eosin stained sections have been revised by two pathologists (AG and KK) without knowledge of the serological results. Included in the study were placentae where either a section from the T-zone was present or when it was specifically noted that a section was from the dividing septum. Doubtful cases were excluded. The T-zone is defined as the point at which the septum joins the surface of the placentae.

Chi-square test has been used for contingency table analysis and for comparison of observed vs expected number of twin pairs. A p-value less than 0.05 has been considered to be statistically significant.

The study has been approved by the regional ethical committe.

RESULTS

During the study period, 22,947 births were recorded; 352 were twin deliveries, giving a twinning rate of 15.3 per 1,000 births. Table 1 shows the sex combinations of the twin pairs; about 1/4 were of unlike sex and 3/4 of like sex.

Table 1 - Twin births at Odense University Hospital 1 (January 1980 through 31 August 1988) by sex combination

	MF twins	MM twins	FF twins	All twins
No.	87	136	129[a]	352
%	24.7	38.6	36.6	100.0

[a] Includes 1 conjoined pair.

Zygosity

The information on genetic markers and fetal membranes in like-sexed pairs is given in Table 2. As seen, 190 had both investigations performed. In 39 pairs, only genetic markers were determined, either because the placentae were unfit for evaluation (35 cases) or because the placentae were not submitted for examination for unknown reasons (4 cases). As judged from the serological results, 33 were MZ and 6 DZ. In 36 pairs no investigations were carried out (24 because of birth complications, including stillbirth, 1 because of conjoined twinning, and 11 for unknown reasons).

The serological zygosity diagnoses in those pairs where the placentae and membranes could be evaluated are presented in Table 3. In all 59 MC pairs the genetic markers were concordant, whereas one or more genetic markers were discordant among 34 of the 56 monoplacental DC twin pairs. This difference is statistically highly significant ($\chi^2_{(1)} = 50.9$, $p << 0.001$)

Table 2 - Genetic markers and placental membranes in like-sexed twin pairs according to sex

Genetic markers[a]	Placental membranes[a]	MM twins	FF twins	All twins
+	+	95	95	190
+	−	20	19	39
−	−	21	15[b]	36
Total		136	129	265

[a] + = available; − = nonavailable.
[b] Includes 1 conjoined pair.

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Table 3 - Zygosity based on genetic markers related to number of placentae and chorionic membranes

Zygosity	1 placenta		2 placentae	Total
	Monochorionic	Dichorionic		
MZ	59	22	30	111
DZ	0	34	45	79
Total	59	56	75	190

Weinberg's Rule

Of the 352 twin pairs, 87 were of unlike sex. According to Weinberg's rule, 87 of the 265 like-sexed pairs should be DZ and 178 MZ. Based on the serological findings, 85 have been classified as DZ, 144 as MZ, and 36 have not been classified. If the conjoined pair is included in the MZ group, a total of 145 may be considered to be MZ. The zygosity of the remaining 35 like-sexed pairs is unknown. Supposing the MZ/DZ ratio to be identical with the one among pairs in which only serological examinations were performed, a total of $35 \times (33/39) = 30$ pairs should be MZ. If instead the MZ/DZ ratio equals the ratio among pairs with both serological and membrane findings, the estimated number of MZ pairs would amount to $35 \times (111/190) = 20$. The total number of MZ pairs may then be estimated to be included in the interval 165–175 which is not significantly different from the expected number of 178 ($p > 0.05$).

DISCUSSION

Diagnosis of twin zygosity of like-sexed twins is often made by evaluation of the fetal membranes, monochorionic placentation being regarded as proof of monozygosity. In case reports, genetically different MC twins have been described. In one case, unlike-sexed twins were found to be MC, but examination of the placenta was interpreted as disintegration of an originally DC placenta [11]. In another report of genetically different MC twins, fertilisation of the first polar body was proposed [1].

The common assumption of MC placentation being proof of monozygosity has been questioned by Mortimer [10]. Since many studies have based their zygosity diagnosis on placental membranes, the issue has wide implications. Mortimer identified 12 MC pairs and found discordance in one minor blood group in 3 pairs which were then classified as DZ. It is interesting that only one single type was discordant among 16 red cell antigens, as most DZ twins usually are discordant for several types. In Mortimer's study the chorionicity was based on a combined macroscopic and microscopic inspection of the membranes. However, no specific information was given of the T-zone, which in cases of distorted membranes may be imperative [5].

In the present material, all 59 MC twin pairs were identified as MZ. The zygosity

diagnosis based on the serological findings has a 99.5% probability of identifying DZ twins, as discordance in only one marker is considered proof of dizygosity. Membrane diagnosis was made either by microscopic evaluation of the dividing septum when it was specifically described, or the T-zone [5]. Therefore, we conclude that, for all practical purposes, MC twins can be considered as MZ. This is in accordance with the study by Vlietinck et al [13] who found no genetic marker discordance among 740 MC pairs.

A high twinning rate (15.3 per 1000 deliveries) was observed. This may be due to referral of relatively more twin pregnancies from district hospitals in the region. The twinning rate in Denmark as a whole, for the period of 1980 through 1985, was 10.4 per 1000 deliveries [4]. In most Caucasian populations, the MZ rate is approximately 3.4 per 1000 [2] but in this series it was 7.3 per 1000. The high MZ rate, as well as the high percentage of MA placentae (3.2%), may be the result of a higher frequency of obstetric complications leading to referral of relatively more of these twins.

Among 75 pairs, no reliable information concerning placentae and membranes were available, but in 39 of these, zygosity could be established by the serological analyses (33 MZ and 6 DZ pairs). As approximately half of the MZ pairs in this series are MC, a bias towards underestimating the number of MC twins might have occurred. The majority (24/36) of nondefined zygosities were due to birth complications, which is seen more often among MC twins [2,9].

Weinberg's method has been questioned by James [7,8], claiming that the rule underestimates the number of DZ twins. By pooling results from several studies, Bulmer [3] found no such underestimate, and in a study of 2,589 pairs Vlietinck [13] found his results in accordance with Weinberg's rule. In our study, Weinberg's rule would predict 178 MZ pairs. Assuming the MZ/DZ ratio among the 35 unclassified pairs to fall between the corresponding ratios in the group completely evaluated and the group only serologically studied, the number of MZ pairs lies between 165 and 175, which is not significantly different from 178. Furthermore, 73% of the unclassified group had single placentae, as compared to 60% of the defined groups, and a major part of that group was not examined due to birth complications, which is more often seen among MC twins, suggesting that the majority of the unclassified twins were MZ. Applying James' modification of Weinberg's rule, the expected number of MZ pairs should be $352-(87+8/7\times 87)=166$, which gives no better fit than Weinberg's unadjusted rule.

In conclusion, our study confirms that, for all practical purposes, monochorionic placentation can be considered to be proof of monozygosity and that Weinberg's rule is valid.

REFERENCES

1. Bieber FR, Nance JM, Morton CC, Brown JA, Redwine FO, Jordan RL, Mohanakumar T (1981): Genetic studies of an acardiac monster: Evidence of polar body twinning in man. Science 213:775-777.
2. Bulmer MG (1970): The Biology of Twinning in Man. Oxford: Clarendon Press.
3. Bulmer MG (1976): Is Weinberg's method valid? Acta Genet Med Gemellol 25:25-28.
4. Vital Statistics 1985. København 1987: Danmarks statistik.
5. Fox H (1978): The placenta in multiple pregnancy. In Bennington JH (ed): Pathology of the Placenta. London: WB Saunders.

6. Hrubec Z, Robinette D (1984): The study of twins in medical research. N Engl J Med 310:435-441.
7. James WH (1979): Is Weinberg's differential rule valid? Acta Genet Med Gemellol 28:69-71
8. James WH (1984): Twins. N Engl J Med 311:58.
9. Johnson SF, Driscoll SG (1986): Twin placentation and its complications. Semin Perinatol 10:9-13.
10. Mortimer G (1987): Zygosity and placental structure in monochorionic twins. Acta Genet Med Gemellol 36:417-420.
11. Nylander PPS, Osunkoya BO (1970): Unusual monochorionic placentation with heterosexual twins. Obstet Gynecol 36:621-625.
12. Stern C (1973): Principles of Human Genetics. San Francisco: WH Freeman and Company.
13. Vlietinck R, Derom C, Derom R, Van den Berghe H, Thiery M (1988): The validity of Weinberg's rule in the East Flanders Prospective Twin Survey (EFPTS). Acta Genet Med Gemellol 37:137-141.

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