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Maternal and Neonatal Variables in Twins: an Epidemiological Approach*

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Abstract. Population studies on human twinning are scarce in Argentina. In order to analyze frequencies and certain maternal and neonatal variables related to twin births, we studied a series of 69.678 consecutive newborns with 500 g of weight and over, which occurred at a public hospital in the province Buenos Aires, during 14 years (1982-1995). The frequency of twin births (10 per 1000 deliveries) and sex ratio were similar to other studies reported in Caucasian population. Maternal age and order of gravity/parity were positively correlated with twinning rates, more markedly so in dissimilar sex-pairs. Stillbirths and neonatal deaths were more frequent in twins than in singletons, but less frequent when comparing groups of same weight. Congenital malformations were not found to be significantly more frequent in twins than in the total newborn population. However, their occurrence, predominantly in like-sexed pairs and the concordance for defect type in doubly affected same-sex pairs, suggests that monozygotic twinning carries an increased risk for malformation.

Key words: Twinning, Epidemiology and Malformations

INTRODUCTION

Twins occupy a special place in human genetics, since Galton proposed them as a model for analysis of hereditary and environmental components in human traits. Twinning occurs often enough – approximately 1:80 pregnancies – to constitute an important biologic event. Because of the frequency with which obstetric and neonatal hazards accompany multiple pregnancy, it is recognized as having medical and epidemiological relevance [6].

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The incidence of twinning varies considerably in human populations, the major changes relating to the dizygotic component. Among the biological factors affecting twinning, the influence of maternal age, parity and ethnic composition is well known, while the role played by other factors is less clear [7, 21]. Population studies on human twinning are scarce in Latin-American countries [2, 5] and particularly in Argentina [10, 11, 17]. Data refer in great part to a northern hemisphere populations and may differ from southern, because of ethnics and sociocultural differences. Genetic and environmental factors are probably also different. Thus, biological factors associated with human twinning in our population may differ from those usually accepted.

MATERIAL AND METHODS

The present study involves all twin births, over 500 g of weight, delivered at the Hospital Maternoinfantil de Mar del Plata, a state-owned institution, during the fourteen-year period, from January 1982 to December 1995. The hospital population is composed predominantly of Caucasoids and belong to the lower socioeconomic group. Data were collected from obstetric ward records and hospital newborn discharge diagnoses. Birth data were registered according to the protocol rules established by the ECLAMC (Congenital Malformations Latin-American Collaborative Study), with which our hospital has collaborated since 1982. The nature of this study permits this recording of observations with high accuracy. In the present study the following variables were considered for analysis (but could not always be assigned to all individuals): maternal age, order of pregnancy, parity, gestational age, newborn sex, birth weight, Apgar score at 1 and 5 minutes, birth order, fetal and neonatal deaths, and presence of malformations diagnosed before discharge from hospital.

Twinning incidence was defined as the number of twin pair deliveries per 1.000 births, taking into account both live or stillborn childbirths. Zygosity of twins was estimated by Weinberg's differential method [24] and James's method [14]. The theoretical rate of triplets was estimated according to Hellin's law [24].

The statistical treatment of the data included Wilks test to corroborate normality of the variables, Student's t test and Mann-Whitney test for differences between means, chi-square for comparison of frequencies. A multiple regression analysis was used in order to examine the variables best correlated with birth weight.

RESULTS

Demographic data and incidence of twinning

During the study period there were 69.678 consecutive newborns with 500 g of weight and over. A total of 68.624 liveborns and 1.054 stillborns occurred in 68.268 single deliveries, 690 twin and 10 triplet deliveries (Table 1). There were two deliveries of conjoined twins, 1 in 1983 with stillborn male thoracopagus and 1 in 1994 with liveborn female thoracoischiopagus babies. The conjoined twins and the triplets were excluded from the following analyses.

Table 1 - Summary of data (1982-1995)

Year	Total births	Fetal deaths	Neonatal deaths	Twin pairs	Triplet births	Total deliveries	Twinning incidence ‰
1982	4.072	66	77	32		4.040	7.9
1983	3.877	71	85	41		3.836	10.6
1984	3.811	69	72	40	1	3.769	10.6
1985	3.994	85	58	30		3.964	7.5
1986	4.481	88	66	38	1	4.441	8.5
1987	4.509	66	76	39	1	4.468	8.7
1988	4.993	101	55	61		4.932	12.4
1989	5.229	77	46	61	1	5.166	11.8
1990	6.058	100	90	53		6.005	8.8
1991	5.996	88	82	66		5.930	11.1
1992	5.880	98	85	65	1	5.813	11.1
1993	5.591	50	71	52	3	5.533	9.4
1994	5.377	52	68	59	1	5.316	11.1
1995	5.810	43	72	53	1	5.755	9.2
Total	69.678	1.054	1.003	690	10	68.968	10.0

The frequency of twin birth was 10,01 per 1000 deliveries. A total of 10 sets of triplets were born (0,14 per 1000 or 1:6.898 deliveries). According to Hellin's law the number expected would be 1:9.967 deliveries (the difference was not significant; $p > 0,5$).

Sex-ratio

The sex ratio of twins was 0,49 as compared with 0,51 for singletons ($p > 0,2$; n.s). In seven pairs, the sex of one or both twins could not be determined.

Zygosity

Zygosity determined by Weinberg's method showed 38,19 % MZ and 61,80% DZ pairs. By James's method zygosity estimation was 33,78% MZ and 66,22% DZ. The number and percentage of estimated MZ and DZ pairs are shown in Table 2. Data about placenta and fetal membranes were available only in 30 twin pairs and could not be considered for the analysis.

Table 2 - Zygosity estimation

Method	MZ		DZ	
	N° of pairs	%	N° of pairs	%
Weinberg	262	38.2	424	61.8
James	232	33.8	454	66.2

Maternal age, parity and order of pregnancy

Data for maternal age, parity and order of pregnancy were compared between twins and singletons. Means of maternal age (27,39 ± 6,42) and order of pregnancy (3,46) were statistically significantly higher in twins than in singletons (25,25 ± 6,4 and 2,96 respectively) ($p < 0{,}001$; $p < 0{,}05$). The parity was higher in twins (1,85) compared to singletons (1,73), but the difference was not significant (Table 3).

It is well established that DZ twinning rates increase with maternal age and parity. In order to confirm this correlation, means of maternal age, order of pregnancy and parity were compared between same sex pairs and opposite sex pairs (Table 3). Maternal age was higher in opposite sex-pairs ($p < 0{,}001$). Order of pregnancy and parity were higher too, but the difference was not significant.

Birth weight and gestational age

Mean birth weight in all twins was 2226,89g ± 635,36g and 63% (877 babies) weighed less than 2500g. Considering only liveborns, birth weight was 2262,5 ± 602,3, and 66% weighed less than 2500g.

The variables best correlated with birth weight were gestational age ($r = 0{,}76$) for the firstborn twin, and for the second, gestational age ($r = 0{,}7$), Apgar score at 1' ($r = 0{,}42$) and Apgar score at 5' ($r = 0{,}40$).

The mean length of gestation of twin pregnancies was 36,13 weeks.

Differences in birth order

Differences between twin 1 and twin 2 were analyzed according to weight and Apgar score, at 1 and 5 minutes (Table 4).

Congenital malformations

The frequency of malformed twins was 2,53% (35 in 1380 twin babies). The frequency of malformed in the total population for the same period was 2,33%. (the difference was not significant). All malformed but 7 occured in 26 pairs of same sex. Of the 5 pairs with both twins malformed (3 of same sex), four were concordant for the type of defect (Table 5).

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Table 3 - Maternal variables

	Singletons (1982-88) (mean ± sd)	Twins (mean ± sd)	p-value	Twins (1982-1995) Same-sex	Opposite-sex	p-value
Maternal age	25,25 ± 6,4	27,39 ± 6,4	< 0,001	26,76 ± 7,14	28,90 ± 7,05	< 0,001
Order of pregnancy	2,96	3,46	<0,05	3,25	3,96	n.s.
Parity	1,73	1,85	n.s.	1,65	2,32	n.s.

Table 4 - Birth weight and vitality by twin order in liveborn twins

	Weight (g)	Apgar Score 1 min.	5 min.
Twin 1	2283,1 ± 613,7	8,31	9,31
Twin 2	2241,6 ± 590,3	7,79	8,89
difference	n.s.	p < 0,001	p < 0,001

Table 5 - Congenital malformations in twins

Year	Sex	Sex of co-twin	Type of defect	Weight	Maternal age
1982	M	M	Club foot	2000	30
1982	F	F	Amniotic bands	1600	18
1982	M	pair both malf	Single umbilical artery	2500	31
1982	M		Single umbilical artery	2250	31
1982	F	F	Hip dislocation	2200	35
1983	F	F	Thyroglossal cyst	1750	26
1983	M	M	Moebius syndrome	1800	32
1983	M	F	Pre-auricular tag	1900	36
1983	M	M	Caudal appendix	2250	39
1983	M	M	Multiple malformed infant	3200	36
1984	I†	pair both malf.	Omphalocele, ambiguous genitalia, limb reduction defect	1000	20
1984	F†		Hydrocephaly, congenital heart disease, club foot	1600	20
1986	M	pair both malf.	Multicystic kidneys	3500	27
1986	M		Multicystic kidneys	3470	27
1986	M*	M	Papyraceous fetus	300	25
1987	M†	pair both malf.	Incompletely formed prepuce	1900	21

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Year	Sex	Sex of co-twin	Type of defect	Weight	Maternal age
1987	M		Incompletely formed prepuce	1830	21
1987	M	M	Incomplete prepuce and chordee	3050	19
1988	M†	M	Macroglossia	2320	34
1988	M†	M	Congenital heart disease	2200	24
1989	F	F	Hydrops fetalis	1480	26
1989	M†	M	Ileal atresia	2150	28
1989	F	M	Mosaic trisomy 21	2830	24
1990	M	F	Scaphocephaly	1400	34
1990	M	M	Interventricular septal defect	1840	41
1991	M*	M	Spina bifida, dorsal+omphalocele	1000	17
1991	F	F	Spina bifida, sacral	2200	33
1991	M	M	Hudrocephaly	1180	39
1991	M	pair both malf.	Club foot	3100	24
1991	F		Club foot	3050	24
1992	M*	F	Macerated fetus with maxilary hypoplasia and cleft palate	3000	38
1992	F	F	Angioma tuberosum of nose and upper lip	1670	17
1992	M	M	Club foot	1720	27
1993	M	M	Bronchial cyst, congenital	1780	27
1995	F	M	Exstrophy of bladder	3150	24
Conjoined Twins					
1983	M *	M *	Thoracopagus		
1994	F	F	Thoracoischiopagus	2700	20

M: male; F: female; I: undetermined sex
*: Stillborn; †neonatal death

Table 6 - Stillbirths rates in singletons and twins according to birth weight (1982-1988)

Weight (g)	Twin births	Stillbirth rates		Singleton births	Sillbirth rates	
		n	‰		n	‰
500-1000	30	11	366.6	313	171	546.3
1001-1500	35	2	57.0	299	71	237.4
1501-2000	91	4	44.0	520	44	84.6
2001-2500	160	1	6.2	1454	47	32.3
> 2500	190	–	–	24077	143	5.9
Total	506	18	35.6	26.663	476	17.8

Fetal and neonatal mortality

There were 50 fetal deaths in twins (35,92‰), as compared to 14,7‰ in singleton births. Stillbirth rate in twins was double as compared to singletons. Stillbirth rate, taking into account weight groups (1982-1988), was lower in twins than in singletons (Table 6).

Of the 1334 liveborn twin babies, 88 died in the neonatal period (65,96‰). Neonatal mortality in singletons was 14,6‰ (1003 in 68.624 liveborns). Thus, neonatal mortality was 4,5 times higher in twins than singletons. Twin babies who died in the neonatal period had a mean birth weight of 1156,82 g ± 485,23. There were 44 neonatal deaths [1] for the first twin and the same number for the second.

DISCUSSION

Incidence

The incidence of twinning in this series, 10 per 1000 deliveries, was similar or slightly lower than that formerly reported for Caucasians. This seems to be declining according to recent studies [1, 5].

The analysis of twinning rate in the province of Neuquén, Argentina, has reported an incidence of 9,5 per 1000 deliveries, which is close to our results [10].

It should be noted that several epidemiological studies exclude pairs with no liveborn members, thus underestimating to a varying degree the incidence of twinning.

The rate of triplets (1:6.898) was not significantly higher than expected by Hellin's law. This is in agreement with the results of a study [18] which found no difference between observed and expected triplet sets.

Sex-ratio

The sex-ratio at birth shows variability in human populations, but always deviates in favor of males, with values ranging from 101 to about 113 males per 100 females [3]. According to a study [4] sex ratio in twin births is lower than in singletons. Our data supports this finding since the sex ratio of twins (0,49) was lower than that of singletons (0,51), but the difference was not found to be significant.

Zygosity

Diagnosis of zygosity of twins was not available. However, according to Weinberg's rule, 262 pairs of twins (38,19%) would be expected to be MZ and 424 pairs (61,80%) would be expected to be DZ. According to James's method there were 33,78 % MZ and 66,22% DZ. The accuracy of Weinberg's rule has been well substantiated by Potter's study [23]. However, a review of studies of a total of 1334 DZ twin pairs identified and blood-typed at birth, suggests that same-sex pairs outnumber opposite-sex pairs in a ratio of about 8:7. The difference between this ratio and that 1:1 postulated by Weinberg is significant ($p < 0{,}01$) [14]. A study [14] claims that (1) where DZ twinning rates are moderate, as in white populations, the Weinberg estimate of MZ rates may be acceptable, if slightly high, and (2) where DZ rates are high (as in Nigeria), the Weinberg estimate of MZ rates may be almost double the true rates [14]. The calculated incidence of MZ twinning in our population would be 3,8 per 1000 deliveries, which is in agreement to the reported incidence, about 3 to 4 per 1000 [22]. This series support the idea that MZ twinning is constant throughout the world.

Maternal variables

It is well established that the rate of twinning is positively correlated with maternal age as well as parity independent of maternal age. Most authors have documented that this increment is due to an increase in DZ twinning rate only [5, 21]. In like manner, order of pregnancy is another factor that predisposes women to produce DZ twins. This finding may be related to an enlargement of the anterior pituitary with succesive pregnancies, in addition to this gland's natural enlargement with age [9]. The increased order of pregnancy observed in this series is coincident with the information of the literature.

Mortality

Stillbirth rate in twins was double compared to singletons. Except Potter's study which reported a lower stillbirth rate in twins (31‰), several authors have documented higher rates: 42‰, 81‰, [8, 13]. According to birth weight groups, stillbirth rates were lower in twins than in singletons, suggesting that twins are more likely to be growth retarded and thus more mature than singletons of equivalent weight. Neonatal death of twins (65,9‰) were 4,5 times higher than in singletons (14,6‰). Neonatal death rate in the present study was one of the lowest compared to the existing literature 75‰, 139‰, 92‰, 65‰ [23, 19, 18, 13, 20].

Our results are, therefore, in agreement with those of others authors [23, 13] that the greatly increased risk of morbidity and mortality in twins may be attributed to their lower mean birthweight and prematurity, rather than to twinning per se.

Congenital malformations

The problem of malformations in twins has been the object of many studies [8, 12, 15-17]. Data from the population-based Metropolitan Atlanta Congenital Defects Program (MACDP) have shown that the over-all rate of malformed infants is higher for twins than for singletons. This elevated risk appears limited to same sex twins and, hence, related to monozygosity [16]. A study [17] has reported a 4,4% of malformed twins and 2,2% of malformed singletons, with the registers of the ECLAMC.

Our results showed that congenital malformations were not found to be significantly more frequent in twins than in the total newborn population. Despite the lack of zygosity determination, some interesting inferences can be made regarding congenital malformation in same sex and opposite sex twins in the present series. The occurrence of malformations mostly in like-sexed pairs suggests, as found by others author [16, 17], that MZ twinning carries an increased risk of malformations. Moreover, concordance rates for defects in like-sexed pairs offers another indirect evidence for the previous consideration.

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