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The Mercurio Project and Twins. How to use a Registry

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INTRODUCTION

Characteristics of Twins Registries

An essential element in any investigation of environment-genetics interaction is twin registries. At present there exist two types, one based on the whole population and another relying on voluntary participation. The second type is an important source of epidemiological information regarding the presence or absence of a disease (concordance) between members of monozygotic and dizygotic pairs. However, this type presents two serious drawbacks: the data do not cover all twins, and since the twins enrolled are those who require the greatest assistance, this results in a serious selection bias [2]. On the contrary, the first type has neither of these defects and represents an invaluable source of information for epidemiological studies on the concordance of pysiological features, morbidity and mortality, together with genetic and environmental influences and their interaction [1,3-5].

The "Mercurio Project" and twins: main features

April 1993 saw the first enrolment in a Multiple Pregnancies Registry designed to collect data from 47 Italian hospitals. This registry forms part of a larger survey, the "Mercurio Project", originally set up in April 1992 under the aegis of the Italian Multicentre Birth Defects Registry (IPIMC). The aims of the registry are the following:

(a) to set up longitudinal epidemiological studies on the health, morbidity and mortality of twins
(b) to assess the role of environmental-genetic interaction in determining childhood be-

haviour and disease (using case-control studies where each twin is matched with his or her cotwin)
(c) to draw the attention of socio-sanitary personnel to twins and their special needs.

This paper has a twofold aim: first, to explain enrolment methodology, and second, to present and illustrate some of the data collected.

MATERIALS AND METHODS

Enrolment in this registry is a six stage process:

1. Registration of all multiple pregnancies (identified ecographically), irrespective of the number of children born, and the number of pre- or peri-natal deaths.
2. Completion of an enrolment form by a pediatrician for each delivery, containing information regarding: family and pregnancy history, anthropometric variables, and the health of twins. When a suitable diagnostic methodology has been selected, collection of data on placentation and zygosity then begins.
3. Completion of a second enrolment form with the same information as above, this time for newborns born immediately after the twins (these subjects are also employed as a control-group for comparative studies).
4. Informed enrolment of the parents of twins and singletons.
5. Consignment of enrolment forms to a coordination centre for inclusion in a special data base; these forms are accompanied by a special monthly report from each hospital regarding all newborns, both twins and otherwise.
6. Periodical telephone interviews by specialists, of mothers participating in the follow up (at 3,9 and 18 months). The interviews are designed to gather information on matters such as breast feeding, immunization, accidents at home, and linguistic, psychomotory and mental development, etc.

RESULTS

General features

On 31st January 1994, the registry contained neonatal data regarding 694 babies born between April and November 1993. As not all the pregnancies were included, the multiple pregnancy rate still remains to be calculated. The characteristics of the 332 multiple pregnancies enrolled are illustrated in Table 1. Twin pregnancies amounted to 307 (92.7%) of the total. Twin births were made up of 229/303 (75.6%) like-sex babies and 74/303 (24.4%) unlike-sex babies. By Weinberg's rule, monozygotic twins amounted to 51.3% and dizygotic 48.7%.

Birthweight

Table 2 illustrates the birthweight of multiple births grouped according to sex. As regards low birthweight (<2,500g), this amounted to 60.5% in twins and 97.3% in mul-

Table 1 - Distribution of multiple pregnancies according to number and sex of newborn

Number fetuses	Total pregnancy	Sex of newborn			
		Like sex		Unlike sex	Sex indefinite
		Male	Female		
2	307	124	105	74	4 (1)
3	22	2	7	12	1 (2)
4	2	0	0	2	0
5	0	0	0	0	0
6	1	0	0	1	0
Total	332	126	112	89	5

(1) twin pregn.: Female with VSD and miscarriage 5 wk
Female with kidney hypoplasia and miscarriage
Male with esophageal atresia and miscarriage 6 wk
Male with microcephaly and fetus papyraceus with anencephaly miscarried at 30 wk
(2) triplets (IVF): Male with con hypospadias enoscrotal and 2 fetuses miscarried

Table 2 - Birthweight grouped according to sex and pregnancy

Birthweight (g)	Twins			Total	Multiple
	MM	FF	MF		
<1500	24	25	9	58	38
1500–2499	120	106	75	301	35
2500>	100	74	60	234	2
Total	244	205	144	593	75
Median	2322	2250	2415	2350	1490

Mean birthweight difference in twins p = 0.057

Key: MM = male/male
FF = female/female
MF = male/female

tiple births. Very low birthweight (<1,500g) for these two categories was 9,8% and 50.7%, respectively. Average birthweight was 2,329g in twins, 1,490g in multiple births and 3,225g in the case of singletons. For mixed-sex twins, the average birthweight was highest; in like-sex twins, male/male birthweights were greater than female/female. Intertwin birthweight discordancy was observed to be low (under 15%) in 204 twin pairs (69.1%), mild (over 15 but less than 24.9%) in 61 twin pairs (20.7%) and severe (over 25%) in 30 twin pairs (10.2%).

Mortality

An analysis was made of mortality in the first 3 months on a total of 610 newborns from twin pregnancies and 78 from multiple pregnancies (Table 3). The mortality rate was

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Table 3 - Mortality rate in multiple births for the first 3 months

	Twins				Multiple	Total
	MM	FF	MF	X?		
Miscarriage				4	2	6
Stillborn	4	3	0	0	1	8
Deaths -7d	8	5	1	0	10	24
Deaths 8-29	3	1	4	0	3	11
Deaths 30-89	1	1	1	1	2	6
Total Deaths	16	10	6	1	16	49
[%	6.5	4.8	4.1		20.5	7.1]
Total born	248	210	148	4	78	688

?: Sex indefinite

7.1% (49/688), in like-sex twins 5.7%, in unlike-sex twins 4.1%, and in multiple births 20.5%. Perinatal mortality amounted to 3.4% (21/610) for twins and 14.1% (11/78) in multiple births. The mortality rate within twin pairs showed a concordance of 33.3% (8/24): 33.3% (4/12) male/male, 11.1% (1/9) for female/female and 100% (3/3) for male/female. For triplets, this concordance amounted to 20% (1/5).

Congenital malformations

For this report we considered malformations diagnosed at birth and those identified up to the 3rd month of age. For the first category, we employed data for all newborns born from multiple pregnancies (610 twins and 78 multiples) while the second type consisted only of the interview data gathered at a later stage (352 twins and 46 multiples) (Table 4). The second category of malformations included: in males, pulmonary stenosis, hypospadias and pyloric stenosis; in females, clubfoot and a double-outlet right ventri-

Table 4 - Congenital malformations diagnosed at birth or up to the 3rd month

	Twins				%	*Total*
	MM	FF	MF	X?		
At birth	15	7	3	4	4.7	610
0-3 months	3	2	1	0	1.7	352
	Multiple					
At birth			4		5.1	78
0-3 months			6		13.0	46

?: Sex indefinite

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cle (diagnosed at birth as a ventricular septal defect). An analysis of congenital malformations concordance showed:

(a) Among twins: a pair with hydrocephaly and kidney disease
a pair with atrial septal defect.

(b) Among triplets: a clubfoot, a non-specified heart murmur, no defect
a hydrocephaly, a non-specified lung disease, no defect
a non-specified heart murmur, an inguinal hernia, no defect
a ventricular septal defect, an inguinal hernia, no defect.

Length of hospital stay

We evaluated the length of hospital stay (nursery + ward) for twins or multiples vs singletons up to the 3rd month of age (Table 5). We observed that for twins the average duration was 17 days, for the multiples 34 and for singletons 6.9 days. This difference was due to the greater incidence of low birthweight in multiple births. Since 50% of the triplets were of very low birthweight (< 1,500g), the average stay was considerably higher. After stratification for weight, however, it was observed that the average number of days spent in hospital by twins was not noticeably different from that for singletons.

Table 5 - Length of stay in hospital during the first 3 months, classified according to birthweight

Birthweight	Newborns	Hospital stay (days)	Mean of hospital stay (days)
	Twins		
<2500	205	4864	23.7
2500>	143	1116	7.8
*Total**	*352*	*6000*	*17.0*
	Multiple		
<2500	46	1598	34.7
2500>	0	—	
Total	*46*	*1598*	*34.7*
	Singletons		
<2500	13	348	26.8
2500>	262	1558	5.9
Total	*276*	*1912*	*6.9*

*includes unknown birthweight

Breast-feeding

Information on breast-feeding was obtained from interviews on all babies born alive (334 twins, 34 multiple newborns and 276 singletons) (Table 6). A comparison of twins

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Table 6 - Incidence of breast and bottle feeding, classified according to pregnancy and birthweight

Birthweight	No. babies	Feed		
		breast	breast + bottle	bottle
		Twins		
<2500	191	17.3	44.5	38.2
2500>	143	24.5	54.5	21.0
Total	*334*	*20.3*	*48.8*	*30.9*
		Multiple		
<2500	34	8.8	38.2	53.0
2500>	0	0	0	0
Total	*34*	*8.8*	*38.2*	*53.0*
		Singletons		
<2500	13	30.8	15.4	53.8
2500>	263	75.3	16.7	8.0
Total	*276*	*73.2*	*16.7*	*10.1*

and control group showed a considerably lower incidence of exclusive breast-feeding among the former; this held true for babies both above or below 2,500g in weight. However, it should be noted that a large number of twins received mixed feeding (breast and bottle); when the mixed-fed twins were combined with the breast-fed twins and again compared with the controls, the breast-fed twin rate was still lower than that of the singletons. Babies born from multiple births are clearly at a greater disadvantage, however. As regards twin feeding concordance, only in 8.1% (13/161) of the pairs were the babies given different feeding. The number of exclusively breast-fed twins amounted to 26/161 (16.1%) and those receiving mixed feeding 79/161 (49.1%).

Risk factors associated with twin pregnancies

A case-control study was carried out to examine a possible association between maternal age, level of schooling, parity and like or unlike sex twinning respectively (Table 7). The results failed to show any significant association between these factors and like-sex twins. On the other hand, a significant association was revealed between unlike-sex twins and the maternal level of schooling (under 8 years) both as regards crude OR (1.79; 95% c.i. = 1.0-3.2) and adjusted OR (1.94; 95% c.i. = 1.1-3.4).

Ovulation stimulator drugs and pregnancies

The purpose of this case-control study was to confirm a possible association between multiple pregnancy and ovulation stimultor drugs (Tables 8 and 9). For this study, data were gathered on 191 multiple pregnancies (177 twins and 14 multiples) and 276 single

Table 7 - Crude and adjusted odds ratio (OR) (M-H) for like-sex twinning (LS) vs controls, and unlike-sex twinning (UL) vs controls for maternal age, parity and level of schooling

	LS Crude	LS M-H	UL Crude	UL M-H
Maternal age (>30, 30>)	1.02	0.95	0.87	0.64
Parity (1, 2>)	1.11	1.07	1.03	1.35
Years of school (<8; 8>)	1.31	1.34	1.79 (1.0–3.2)	1.94 (1.1–3.4)

Table 8 - Odds ratio (OR) for twinning vs singletons for use of ovulation stimulator drugs

	Pregnancy			
	Twins	LS	UL	Single
Exposure	11	4	7	3
No exposure	166	129	37	273
Total	177	133	44	276
OR	6.03 1.56–34.01	2.82 0.47–19.49	17.22 3.67–105.85	

Key: LS = like sex
UL = unlike sex

Table 9 - Odds ratio (OR) for multiple births versus singletons for use of ovulation stimulator drugs

	Pregnancy	
	Triplets	Single
Exposure	12	3
No exposure	2	273
Total	14	276
OR	546.00 (lc 95% 119.21–2302.58)	

pregnancies. The results demonstrated an association between ovulation stimulator drugs and twinning (OR = 6.03, c.i. 95% = 1.56-34.01), particularly as regards unlike-sex twins (OR = 17.22, c.i. 95% 3.67-105.85). An even stronger association was identified between ovulation stimulator drugs and multiple pregnancies (OR = 546.00, c.i. 95% = 119.21-2,302.58). Since the 'natural' twinning rate is approximately 1% and

that of triplets around 1/10,000, the risk of a multiple pregnancy amounts to 12%, that of a twin pregnancy 6%, and that of triplets or higher order 6%.

DISCUSSION

The "Mercury Project", comprising the "Twins Registry" was set up in April 1993 and represents the first Italian study on twinning assistance. The project sets out to examine the risk factors of this phenomenon, together with the mortality, morbidity and quality of life of twins and multiple newborns. It is hoped that at a later stage, this registry will also enable operators to set up schemes to meet special needs of twins and their families. It was encouraging to note that participation in the project was excellent, both on the part of hospitals and parents. The enrolment rate amounted to 89.3% (293/332), higher in the south of Italy (91.2%) than the north (84.3%). This indicates a keen interest by both doctors and the families themselves. The findings presented in this paper are only the initial ones, which is why no results' discussion has been developed at this point. The findings are presented merely to illustrate the potential of such a project, which involves 47 hospitals located throughout Italy, which, every year, deal with over 40,000 pregnancies.

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