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Epidemiological and birth weight characteristics of triplets: a study from the Dutch twin register

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Summary

From 112 triplet sets, born in The Netherlands from the end of 1986 to the beginning of 1991 and registered in the Dutch Twin Register, several details such as birth weight, gestational age, zygosity, and etiology were assessed by questionnaire, which was filled out by the mother. For 33 triplet sets, zygosity was also assessed by blood typing. Maternal smoking during pregnancy was also noted. Results show a very strong increase in number of triplets caused by artificial fertility enhancing techniques and consequently a shift in the relative contribution of zygosity types to the total number of triplets. Birth weight is predominantly influenced by gestational age. Other effects on birth weight are controlled for possible confounding with gestational age. First born triplets weigh more than later born triplets; boys weigh more than girls; nearly 25% of all individual triplets weigh less than 1500 g, i.e. belong to the category very low birth weight (VLBW); regular maternal smoking produces a 14% birth weight reduction; ovulation induction seems to decrease the sex ratio, i.e. hormonal treatment with ovulation inducing substances increases the probability of female offspring.

Triplet; Birth weight; Multiple birth epidemiology; In vitro fertilization; Ovulation-induction; Sex; Maternal smoking

Introduction

Because the frequency of triplet births is rather low, knowledge about this category of multiples is rather scarce. Generally, a triplet birth is an event that occurs once in every 10 000 maternities. An individual obstetrician's experience with the delivery of a triplet or higher order multiple birth is therefore, by definition, rare.

During the last couple of years there is a rather drastic increase in multiple birth rates because of the growing influence of modern medical techniques: IVF and ovulation induction. In the Netherlands and in Belgium for example, triplet rate (for decades about 1 in every 10 000 maternities) has increased by 4–6 times during the last 5 years. In England and Wales the triplet rate was 26.8 per 100 000 maternities in 1989, whereas it was cons-

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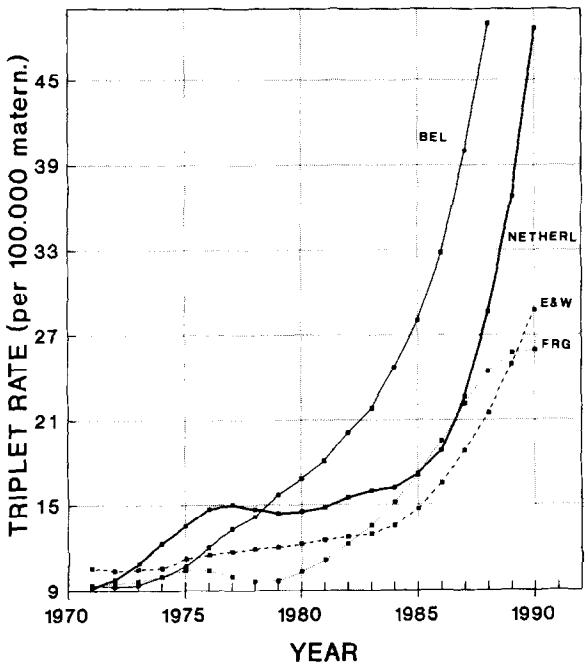

Fig. 1. Triplet rate since 1971 in 4 European countries. BEL = Belgium; NETHERL = The Netherlands; E & W = England and Wales; FRG = West Germany.

tant 10–11 between 1940 and 1980 [1]. This striking growth in triplet rate is visualized in Fig. 1 for some European countries.

It should be noted that the proportional increase in the number of triplets during the last 10 years is far greater than that of twins: several hundreds of percents increase in triplet rate and only about 30% in twinning rate.

It is not unlikely that human interventions in biological processes — IVF and ovulation induction may be considered as such — have changed not only the triplet rate in general and the proportions of zygosity types in particular, but also the usefulness of methods to estimate these numbers.

The present report will describe several (birth) characteristics of 112 triplets, born in The Netherlands between 1986 and 1991 and registrated in the Dutch Twin Register [2]. More specifically, statistical information with regard to gestational age, maternal age, maternal smoking, birth order, sex, etiology and zygosity are provided, separately and with regard to their effect on birth weight. In other recent studies [3,4] there was no appropriate control for the possible confounding effect of gestational age.

The validity of methods for the estimation of proportions of zygosity types among triplets, as proposed by Bulmer [5,6] and by Allen [7,8] will be assessed.

For the estimation of the number of *triplet* zygosity types among a total number of triplets born during a certain period, Allen has proposed a method that is analogous to the classic so called differential rule of Weinberg [9,10] for the estimation of the numbers of MZ and DZ *twins, given the total number of twins and the number of unlike sex twin pairs during a particular period.* Here is Allen's method:

Number of MZ triplet sets = L − 1/2D − 1/4T

Number of DZ triplet sets = D = 2(MZ *twinning rate* × DZ *twinning* rate) N

Number of TZ triplet sets = T = (U − 1/2D) 11/3

In these formulas, L = number of like sex triplet sets, U = number of unlike sex triplet sets and N = total number of maternities. Whereas Allen's method gives *numbers* of triplet zygosity types, given total number of triplet sets, Bulmer [5,6] proposes a method for the estimation of triplet zygosity numbers, given the MZ and DZ *twinning* rates and the number of maternities (the actual number of triplet sets may thus deviate from the estimated number). Here is Bulmer's method:

MZ triplet rate = 1.36 m^2

DZ triplet rate = 2 md

TZ triplet rate = 0.47 d^2

where: m = MZ twinning rate, d = DZ twinning rate. The usefulness of both methods will be tested against the empirical zygosity figures in the triplet sample in the Dutch Twin Register.

Method

Subjects

About 40% of all triplets born in The Netherlands since the end of 1986, is registered in the

Dutch Twin Register. Parents have given their written permission to list their family in the Register and their willingness to cooperate in future research. Once a year a questionnaire is mailed to them, the first one shortly after birth of the triplets. This questionnaire asks about several behavioral and health characteristics, zygosity, smoking and drinking habits, birth weight, etc. The total sample comprises 112 triplet sets (336 individuals).

Data

All information in the present report was acquired with questionnaires, filled out by the mother of the triplets. Data were collected on: birth dates of triplets, mother and father; birth weight; birth order; sex; zygosity; etiology (spontaneous, induced or IVF); number of days in incubator; gestational age; maternal smoking ('not', 'sometimes', 'regular') and maternal drinking ('not', '1 glass per week', 'more than 1 glass per week'); drugs used by the mother. Zygosity was assessed by asking the mother's opinion and the arguments for that opinion. In most cases both the judgement by the obstetrician and the parent's ideas about resemblances and differences in appearance between members of the triplet set were the basic information for the zygosity determination. For a subsample of 33 triplets zygosity was in addition assessed by blood typing. (This was performed by Dr B.A. van Dijk from the 'Universitaire Transfusiedienst' University Hospital Nijmegen, The Netherlands). Assuming that blood typing is the superior method of zygosity assessment, it appeared that there were three misclassifications if only the mother's opinion was taken as the classification criterion: three supposed TZ triplets appeared to be DZ. For these 33 triplets this correction has been worked out in the final presented figures. Assuming that the proportion of misclassifications among the remaining 79 triplet sets is the same as that among the 33 blood typed, the total number of misclassified sets is about 7, which is 6.25%.

In the rest of this report the following abbreviations will be used. M for male, F for female (MFM for example designates a triplet from which the oldest child is a boy, the second a girl and the youngest a boy, etc.), MZ for monozygotic, DZ for dizygotic and TZ for trizygotic.

Results

Types of triplets

Table I gives a listing of relevant triplet figures for both the total number of triplets born in the Netherlands in the years 1987 up to and including 1990 and the comparable figures for the sample of 112 triplets in the Dutch Twin Register, from

TABLE I

Triplet figures in the netherlands (1987–1990) and in the group of triplets registered in the Dutch twin register

Total numbers (1987–1990)			Dutch twin register (Born end 1986 — beginning 1991)	
Number of triplets: 286			112	
Number of maternities: 754 532				
Rate per 100 000 maternities: 37.9				
Number of:				
3 boys	39 =	13.7%	16 =	14.3%
3 girls	57 =	19.9%	22 =	19.6%
2 boys + 1 girl	93 =	32.5%	35 =	32.3%
2 girls + 1 boy	97 =	33.9%	39 =	34.8%
	286	100.0%	112	100.0%

Calculated numbers and proportions of triplet zygosity types according to Allen's method:				Empirical numbers and proportions of triplet zygosity types among registrated triplets:	
MZ =	13 =	4.5% =	1.72[a]	7 =	6.3%
DZ =	58 =	20.3% =	7.69	24 =	21.4%
TZ =	215 =	75.2% =	28.49	81 =	72.3%
	286	100.0%	37.90	112	100.0%

Calculated numbers and proportions of triplet zygosity types according to Bulmer's method:

MZ = 23 = 21.9% = 2.98[a]
DZ = 58 = 55.2% = 7.73
TZ = 24 = 22.9% = 3.20

MZ twinning rate = 4.68[b]
DZ twinning rate = 8.26

[a]Rate per 100 000 pregnancies.
[b]Rate per 1000 pregnancies.

which several characteristics are presented in this paper. Comparison of the population data with the sample data gives an impression about the representativeness of the sample.
The figures in Table I clearly show that the 112 triplets from the Dutch Twin Register can be considered as a representative sample of all twins born during about the same period of time: it can be seen that with regard to the number of same sex and different sex triplets, the composition of the population and that of the sample are nearly identical. The same holds for the calculated numbers in the population of MZ, DZ and TZ triplets according to Allen's method at the one hand and the empirical found zygosity categories in the sample at the other. The latter fact can thus be considered as support of the correctness of Allen's proposed method. For the estimation of the nowadays population triplet rates, the method as proposed by Bulmer seems less appropriate: that method underestimates the present high TZ rate. This would even hold if the empirical zygosity figures (right lower panel in Table I) are corrected under the assumption that among the non-blood typed

TABLE II

Birth weight in triplets

Independent factor	Category and number of subjects	Birth weight in grams (S.D.)	Significance[a]
Overall mean	112 × 3	1832 (500)	
% VLBW (≤ 1500)[b]	82 individuals = 24.4%		
Birth order	oldest (112)	1903 (500)	
	middle (112)	1857 (508)	F (2, 36) =
	youngest (112)	1743 (472)	6.29 $P < 0.002$
Gestational age	< 32 weeks ($N = 37$)	4293 (1034)	F (2, 108) =
	32–34 weeks ($N = 36$)	5692 (905)	48.96
	> 34 weeks ($N = 39$)	6530 (985)	$P < 0.000$
Maternal age	< 29.4 y ($N = 36$)	5413 (1497)	F (2, 108) =
	29.4–32.4 y ($N = 39$)	5541 (1421)	0.22 $P < 0.80$
	> 32.4 y ($N = 37$)	5505 (1052)	$P < 0.80$ NS
Sex	boys ($N = 157$)	1913 (456)	F (1, 36) =
	girls ($N = 179$)	1756 (501)	16.7 $P < 0.001$
Zygosity	MZ ($N = 7$)	5324 (952)	F (2, 108) =
	DZ ($N = 20$)	5279 (1046)	0.98
	TZ ($N = 85$)	5550 (1418)	$P < 0.39$ NS
Etiology	Spont. ($N = 30$)	5607 (1316)	F (2, 108) =
	Induced ($N = 39$)	5299 (1300)	1.55 $P < 0.22$
	IVF ($N = 39$)	5597 (1349)	NS
Maternal smoking	Not ($N = 86$)	5558 (1264)	
	Sometimes ($N = 14$)	5656 (1212)	F (2, 108) =
	Regular ($N = 12$)	4787 (1659)	5.05 $P < 0.008$

[a]All tests have been carried out with statistical control for potential confounding covariance with gestational age.
[b]VLBW, very low birth weight; this category of children is by convention defined as: birth weight below 1500 grams.

triplets the type and proportion of misclassifications is the same as in the group of 33 triplets whose zygosity was assessed by blood typing.

Gestational age

Mean gestational age is 32.2 weeks (S.D. = 2.75) with a minimum of 24 and a maximum of 36.5 weeks. To compare: the mean gestational age of mothers from singletons is 38 weeks and of mothers from twins in the Dutch Twin Register 37 weeks.

Maternal age

Mean maternal age is 30.98 years; S.D. = 3.72 years. The youngest mother is 21.75 years, the oldest 39.35 years. In 1990 mean maternal age of mothers from singletons was 29.0 years and from twins (registered in the Dutch Twin Register) 29.4 years.

Maternal smoking

From all 112 mothers, 86 said they had not smoked at all during the total period of pregnancy, 14 smoked now and then and 12 smoked regularly during the whole period. Thus more than 23% of all mothers smoked.

Sex

Of the triplet babies, 157 were boys and 179 were girls, which is opposite to the sex-ratio generally found in singleton births. More precisely, in singletons the number of boys born per 1000 girls was 1045 during the 1987–1990 period in the Netherlands, i.e. a sex-ratio of 1.045. Among the 112 triplets this ratio is 0.88. The Dutch population value for triplets during the measurement period is 0.87.

Etiology

From the 112 triplet sets, the etiology of 4 sets was unknown. From the remaining 108 triplet sets 30 (28%) were spontaneous, 39 induced (36%) and 39 IVF (36%).

Birth weight

Information on birth weight is presented in conjunction with the parameters listed above and is summarized in Table II. The effects of maternal

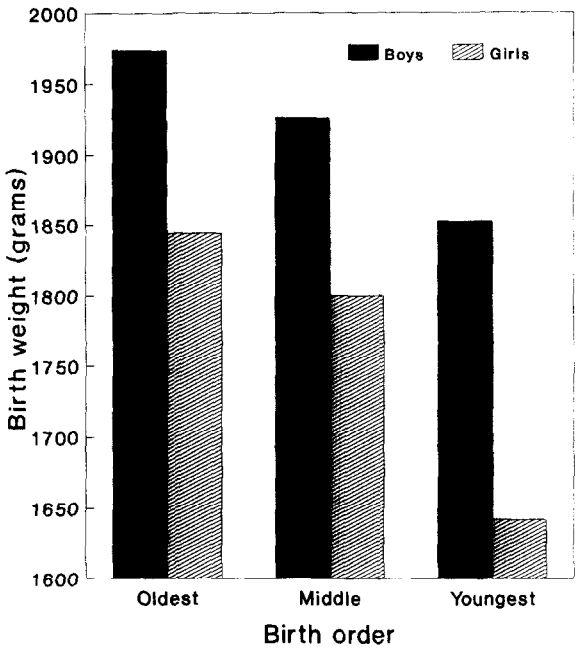

Fig. 2. Significant effects of birth order and sex on birth weight in triplets in a sample of 112 triplets.

smoking, etiology, zygosity and maternal age were carried out in one-way ANCOVA's on the summed weight of the triplet sets, with gestational age as a covariate. The effects of birth order and sex on birth weight were tested in one ANOVA on the individual birth weights. The effect of gestational age is presented and has been tested (one-way ANOVA) on the summed weights of the triplet sets by categorizing this variable in three ranges (see Table II). Figure 2 shows the effects of both sex and birth order on birth weight.

Discussion

The present report shows that the group of triplets, registered in the Dutch Twin Register can be considered as a representative sample of all triplets born in The Netherlands during the years 1987 to 1990. Both number of boys and girls, number of like sex and of unlike sex triplets and the proportions of MZ, DZ and TZ triplets fit the empirical

data reasonably well. At the same time the latter finding (i.e. zygosity categories) validates Allen's proposed rule to calculate them (given the total number of triplets as well as the MZ and DZ twinning rates). On the other hand, Bulmer's suggestion for the calculation of the expected absolute number of MZ, DZ and TZ triplets (given the total number of maternities and MZ and DZ twinning rates) appears to be far from accurate. This could be an indication that IVF and ovulation induction not only have propelled total triplet rate but have also changed considerably the relative contributions of the different zygosity categories to the total number. This is not surprising, since IVF nearly only produce TZ triplets (apart from very rare exceptions). About 36% of the triplets in the sample was born after IVF. None of the 7 MZ triplets were IVF or induced and from all induced triplets only 5 were DZ and the rest TZ. Thus, modern obstetrical technology has mainly increased TZ rate. The figures further demonstrate that the growth in multiple birth rates seems to be much stronger in triplets than in twins: twinning rate in The Netherlands during the years 1990 and 1991 was 14 per 1000 pregnancies and about 11/1000 maternities during the decade before 1985. This is an increase of about 30%, a substantial part of which has to be ascribed to a parallel increase of maternal age [11]. Triplet rate, on the other hand, increased from about 15 per 100 000 pregnancies before 1985 to about 60/100 000 in 1990 and 1991, which is a 400% growth! These differences in growth rate strongly violates Hellin's century old law on the quantitative relationship between twinning and higher multiple birth rates [12] substantially more than was already signalled by Nylander [13]. And this may be the core reason that Bulmer's rule is not satisfactory with regard to the present figures.

The observed low sex ratio of 0.88 is considerably lower than was already observed by Eriksson [14]. He found a sex ratio of 0.992 in 2823 triplets and higher multiples and concluded that the sex ratio decreases with increasing number of fetuses per maternity among both livebirths and stillbirths. The risk for a stillbirth is higher in multiple pregnancies and more so for boys than for girls. The even much lower sex ratio in the present study may have been caused (in addition) by the relative high number of hormonal-induced maternities. James [15,16] has furnished evidence that the maternal gonadotrophin level at the time of conception is causally related to the sex of the resultant zygote, high levels of the hormone being associated with the production of female offspring. In the present study, the sex ratio (number of boys/number of girls) among spontaneous triplet maternities is 44/46, among induced triplets 50/67 and among IVF triplets 55/62. Only the sex ratio for the induced triplets deviates significantly from an expected 0.5/0.5 ratio (binomial test: $z = 1.56$; $P = 0.05$). This is an indication that the overall low sex ratio (in both the population and the present sample) can predominantly be ascribed to the effect of hormonal treatment for the induction of ovulation (in most cases clomiphene).

Birth weight varies with several conditions. The far most important factor is gestational age, accounting for more than 70% of the variance. In testing the effect of other factors influencing birth weight, covariance with gestational age has been eliminated first and average birth weight values have been adjusted accordingly. Birth order and sex (tested in one ANCOVA on the individual triplet weights) had significant effects on birth weight: boys weigh more than girls and first borns weigh more than later born members of a triplet set. The discrepancies between our outcomes and those of other investigators (e.g. Refs. 3,4) could be caused by differences in control for the confounding effect of gestational age. Maternal age does not influence birth weight. This may be attributed to the relative high maternal age in the present sample (nearly 31 years!); young mothers (< 20 years) are known to deliver babies with a somewhat lower birth weight.

Regular smoking by the mother leads to a birth weight reduction of nearly 14% relative to the birth weight of triplets from non-smoking mothers. In a parallel twin study a birth weight reduction produced by maternal smoking of 8% was found. It seems as if the absolute birth weight reduction is the same in singletons, twins and triplets which leads to a stronger proportional effect in triplets and higher multiples.

References

1 Price F. Extraordinary circumstances: Coping with triplets, quads and more. In: Harvey D, Bryan E, eds. The Stress of Multiple Births. London: Multiple Birth Foundation, 1990;119–126.

2 Boomsma DI, Orlebeke JF, Van Baal GCM. The Dutch Twin Register: Growth data on weight and height. Behav Genet 1992;22:247–251.

3 Newman RB, Jones JS, Miller MC. Influence of clinical variables on triplet birth weight. Acta Genet Med Gemellolog 1991;40:173–179.

4 Holczberg G, Biale Y, Lewenthal H, Insler V. Outcome of pregnancy in 31 triplet gestations. Obstet Gynecol 1982;59:472–476.

5 Bulmer MG. The numbers of human multiple births. Ann Hum Genet 1958;22:158–164.

6 Bulmer MG. The Biology of Twinning in Man. Oxford: Clarendon Press, 1970.

7 Allen G. A differential method for estimation of type frequencies in triplets and quadruplets. Am J Hum Genet 1960;12:210–224.

8 Allen G. Frequency of triplets and triplet zygosity types among US births, 1964. Acta Genet Med Gemellolog 1988;37:299–306.

9 Weinberg W. Beitrage zur Physiologie und Pathologie der Mehrlinggeburten beim Menschen. Archiv für die Gesamte Physiologie 1901;88:346–430.

10 Weinberg W. Differenzmethode und Geburtenfolge bei Zwillingen. Genetica 1934;16:282–288.

11 Orlebeke JF, Boomsma DI, Van Baal CCM, et al. Groei van het aantal meerlinggeboorten in Nederland. Medisch Contact 1991;46:1345–1347.

12 Hellin D. Die Ursache der Multiparität der uniparen Tiere überhaupt und der Zwillingsschwangerschaft beim Menschen insbesondere. München: Seitz & Schauer, 1895.

13 Nylander PPS. Frequency of multiple births. In: MacGillivray I, Nylander PPS, Corney G, eds. Human Multiple Reproduction. London: Saunders, 1975.

14 Eriksson AW. Human Twinning in and around the Åland Islands. Helsinki: Societas Scientiarum Fennica, 1973;159 pp. Dissertation.

15 James W. The sex ratio of infants born after hormonal induction of ovulation. Br J Obstet Gynaecol 1985;92:299–301.

16 James W. Hormonal control of sex ratio. J Theor Biol 1986;118:427–441.