

# The East Flanders Prospective Twin Survey (EFPTS)

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The East Flanders Prospective Twin Survey (EFPTS) is a registry of multiple births in the province of East-Flanders, Belgium. It has several unique features: it is population-based and prospective, with the possibility of long-term follow-up; the twins (and higher order multiple births) are ascertained at birth; basic perinatal data are recorded; chorion type and zygosity are established; and since 1969 placental biopsies have been taken and frozen at  $-20^{\circ}\text{C}$  for later determination of genetic markers. The EFPTS is the only large register that includes placental data and allows differentiation of three subtypes of monozygotic (MZ) twins based on the time of the initial zygotic division: the dichorionic-diamniotic pairs (early, before the fourth day after fertilisation), the monochorionic-diamniotic pairs (intermediate, between the fourth and the seventh day post fertilisation), and the monochorionic-monoamniotic pairs (late, after the eighth day post fertilisation). This added a new dimension to didymology (the science of twins;  $\delta\delta\mu\omicron\varsigma$  = twins): the timing of twinning. Studies can be initiated on primary biases, those originating "in utero". Such studies may throw new light on the controversy over the validity of the classic twin method, the consequences of early embryological events (before and just after implantation of the embryo), the origin of congenital malformations, the sex proportion of multiples, the gene-environment interactions as far as intrauterine environment is concerned, to name but a few.

The East Flanders Prospective Twin Survey (EFPTS) was started in July 1964 at the University of Gent, Department of Obstetrics, by Robert Derom and Michel Thiery, a twin himself. The founders became interested in twin surveys when one of them (RD), studying fetal oxygenation during labour, unexpectedly discovered that, as a rule, the second-born twin suffered from a low degree of intra-uterine hypoxia (Derom, 1965). The survey was built according to the models of the Aberdeen twin studies (MacGillivray et al., 1988) and The Birmingham Twin Survey (Cameron, 1968). Doris Campbell, Ian MacGillivray, Hugh Cameron and John Edwards were at its font. In 1989, EFPTS moved to the Center of Human Genetics of the University of Leuven (Belgium). At present it is hosted in "Twins", a non-profit Association for Scientific Research in Multiple Births. It is now partly funded by the Center of Human Genetics of the University of Leuven, "Twins" and, since 2001, the division of Population Genetics of the University of Maastricht (Netherlands).

The main aims of EFPTS are:

1. The determination of:
  - the prevalence of multiple births in a well defined geographic area and the number of mono-, di-, tri... zygotic multiple births;

- the number, nature and concordance of congenital malformations;
  - the obstetrical (duration of pregnancy, pregnancy and birth complications, birth weight, induction of ovulation, placental weight, insertion of the cord, etc ... ) and obstetrico-neonatal outcomes (intra-uterine growth, congenital malformations, perinatal and infant morbidity and mortality, etc...); and
  - other phenotypes on later age such as behavior, learning and school problems, intelligence, postnatal somatic growth, cranio-facial growth, skeletal and dental maturation, sexual maturation, blood pressure, and so forth.
2. The investigation of the causes of the multiple pregnancy and the influence of zygosity and time of zygotic division on the investigated traits.
  3. The use of improved methods of multiple birth studies for the determination of:
    - the genetic predisposition of normal traits, diseases and malformations; and
    - the role of the environment (with special emphasis on the prenatal environment), both individual-specific as well as common environmental influences, through the non-genetic, social and cultural transmission from the parents.

## Subjects and Methods

The inclusion criteria are the following: all the multiple maternities where one of the children weights more than 500g or, if birth weight is unknown, the gestational age is at least 22 weeks, born in the Province of East Flanders (1,336,000 inhabitants, 16,880 births per year).

The specific methodology of this survey includes:

- the determination of the zygosity of each multiple birth with near certainty to certainty through examination of the placental membranes and vascular anastomoses, the blood groups and the DNA-fingerprints (if necessary);
- the collection of medical data from the gynaecologists and the neonatologist; and

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- the follow-up of the multiple through one of the studies and/or questionnaires and the establishment of a three-generation pedigree through civil birth registers.

Contact with the families of the multiples is kept by:

- a visit by volunteer mothers of twins to the mothers expecting twins or multiples, at home or in the obstetrical ward if they already delivered;
- a bi-annual newsletter and a TWIN hotline for the public and the parents of twins with psychological, educational, medical or practical problems. One psychologist backed by a team of physicians and specialists man the telephone;
- the organization of three general meetings per year and several specific meetings per year on subjects which most concern the parents such as language development, behavior and the development of individuality, schooling, educational problems; and
- the organization of evening meetings where the parents of multiples just meet with each other and exchange experiences.

These services to families with multiple births is a joint venture of EFPTS and the Association for Scientific Research in Multiple Births (Twins) and is mainly based on the work of volunteers.

Between 1964 and 2001 6050 twin pairs, 208 triplets, 15 quadruplets, 5 quintuplets, and 1 octuplet were registered and investigated. Presently more than 2000 twins have been enrolled in up to seven different studies. In some of these studies the parents and sibs of the twins were also examined.

## Results

Some of the most important results are:

- Twin surveys are a valuable method in the study of malformations; in monozygotic (MZ) pairs most malformations are discordant (Cameron et al., 1983).
- The development of a method to calculate the probability of monozygosity in the same sexed dichorionic (DC) twin pairs with the same blood groups (Meulepas et al., 1998).
- The development of a method to determine the zygosity of twins, even macerated, through DNA fingerprinting (Derom et al., 1985; Derom et al., 1991).
- The MZ monochorionic monoamnionic (MC-MA) twin pairs, which split after the 8th day after fertilisation, are predominantly girls (Derom et al., 1988).
- The MZ monochorionic-diamnionic (MC-DA) twins, which split after the 4th day after fertilization, have a higher mortality rate than dichorionic MZ (DC-MZ) and dizygotic (DZ) twins. If the division takes place after the 8th day, the mortality risk increases even more dramatically, but is lower than reported hitherto (Derom et al., 1991; Loos et al, 1998).
- After standardization for gestational age, the birth weight of twins is mainly determined by maternal factors especially the mother's genetic make-up, and to

a lesser extent by the chorion type and the genes of the foetuses and their common environment (Vlietinck et al., 1989).

- Since the mid-1980s there is a real explosion in the number of multiple births, primarily caused by the increasing administration of ovulation stimulating agents and the use of other assisted reproduction techniques. We were the first to draw attention to the extent of this epidemic. This increase in the rate of multiple pregnancies represents an important public health problem because, if this trend continues, the rate of very preterm births and very-low-birth weight infants in the population will undoubtedly continue to rise (figure 1) (Derom et al., 1993). Recently there has been a steep decrease of the number of higher order multiple births in favour of singletons and twins, because of a selective feticide (selective abortion of one or more embryos or fetuses) policy. The frequency of zygotic splitting after artificial induction of ovulation is higher than after naturally occurring ovulation, which is of fundamental biological importance (Derom et al., 1987).
- The inheritance of spontaneous dizygotic twinning has been studied in collaboration with the Netherlands Twin Register (NTR) by segregation analysis of 1422 three-generation pedigrees; the parity-independent phenotype of "having DZ twins" was consistent with an autosomal monogenic dominant model with a gene frequency of 0.035 and a female-specific life-time penetrance of 0.10 (Meulemans et al., 1996).
- At prepubertal age, genetic factors have the predominant effect on physical fitness (Maes et al., 1996) and muscle circumferences are some of the most heritable characteristics in humans (Loos et al., 1997).
- The assumption that the division of the zygote occurs stepwise later in respectively DC-MZ, MC-DA and MC-MA pairs, has been recently demonstrated to be highly probable by studying X-inactivation within MZ female pairs : X-inactivation patterns are totally symmetrical in MC-MA pairs, almost symmetrical in MC-DA pairs and asymmetrical in DC-MZ pairs (Chitnis et al., 1999; Monteiro et al., 1998; Puck, 1998).
- A significant effect of chorion type on the heritability of two IQ-subtests was found: the MC twins resembled each other more than the DC-MZ twins on the subscales

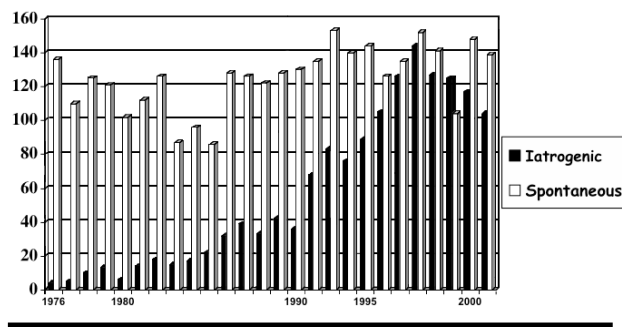


Figure 1 Yearly numbers of spontaneous and iatrogenic twin maternities: EFPTS 1976–2001.

- Arithmetic and Vocabulary. The effect accounts for respectively 14% and 10% of the total variance (Jacobs et al., 2001).
- Lower birth weight is a causal risk factor for child problem behavior, the effects of which may well extend into adulthood (van Os et al., 2001; Wichers et al., 2001).
  - In unlike-sexed twins the length of gestation and the birth weight of the male co-twin is influenced by his female co-twin and not the other way around (Loos et al., 2001).
  - An adverse prenatal environment during twin pregnancies has small, but permanent effects on health in adult life: adult body composition, blood pressure, and glucose-metabolism have part of their origins in utero, but they are programmed through different prenatal environmental influences. Furthermore, the prenatal environment seems to program men and women in a different way (Loos et al., 2001; Loos et al., in press).

## Discussion

Criticism has been addressed to the classical twin studies with regard to the intra-uterine environment of MZ twins. In an often quoted paper, Price reviewed the antenatal and natal difference-producing factors in MZ pairs. The most important of these is undoubtedly chorionicity. Other factors, however, must also be considered: gestational age, birth sequence, birth weight and, in those twins born vaginally, the presentation of both twins (cephalic, breech, transverse). Price concludes:

It would seem to follow that part of the time and effort which will doubtless be expended on research with twins in the next decade or two could well be spent on identifying twin pregnancies two months or more before term, and obtaining much more complete information than we now possess as to the effects of prenatal and natal factors in the two types of MZ pairs. The results of such a study might show that the twin method, as ordinarily applied, is too crude for purposes of modern nature-nurture studies. At the same time, pairs of the monozygotic type might prove to be of more interest and value for theoretical problems of developmental genetics than is commonly supposed (Price, 1950).

Regrettably, Price's expectation has not materialized.

With a sizeable group of more than 600 dichorionic MZ and 1380 monozygotic (MZ) twin pairs the EFPTS can perform classic twin studies with no biases, i.e. studies that are based on the crucial assumption that MZ and DZ twins have a similar intrauterine environment. Previous results have indicated that this may not be the case (Phillips, 1993; Price, 1950). Furthermore, comparison of dichorionic MZ, monozygotic (MZ) and DZ twins will allow to evaluate the degree of gene-environment interactions as the prenatal environment is concerned. In contrast to all dichorionic MZ and DZ twins, monozygotic (MZ) twins share their chorion, their blood supply and therefore their immune system during intrauterine life. This could have long term effects on phenotypes in later life as postulated by Phillips

(1993), and Martin et al. (1997). As retrospective analysis of the placentation is still impossible to carry out, the effect of chorion type on phenotypes in later life will be one of the primary EFPTS research goals. Finally, with more than 1400 twins and triplets born after various techniques of assisted reproduction (ART), EFPTS is the ideal resource for the long-term follow-up study of these multiples on a population-based manner. As with recent reports suggesting increased rates of cerebral palsy among children born after IVF (Strömberg et al., 2002), these findings need to be validated in other population registers.

## Future plans

- Follow-up study of population-based cohorts (1975–2001) of iatrogenic multiple maternities as compared to spontaneous ones; iatrogenic maternities will be divided according to the type of ART: ovulation induction — IVF with and without intracytoplasmic sperm injection (ICSI); besides general health, special attention will be given to congenital anomalies and IQ.
- The expansion of the “prenatal programming” study in twins by adding to the collected data more DZ twin pairs, examining more quantitative phenotypes influencing the carbohydrate and lipid metabolism, the pituitary-adrenal axis hormones, the growth factors and the body composition, and determining the genetic make-up of the twins and their parents by examining well-established candidate genes and “positional candidate genes”.
- Establishment of a European multicentric, prospective and population-based registry of multiple births with accurate recording of placentation (chorionicity) and determination of zygosity.
- Relationship between drugs used for ART and type of twinning; not all ART multiple births are polyzygotic; the prevalence of monozygotic multiple births seems to be elevated in these births as compared to controls; preliminary results point out to clomiphene as the possible drug responsible for the increase.
- Validation of the Weinberg method to calculate the prevalence of MZ twinning in a population; doubts have been raised recently, some authors suggesting that the sex proportion in DZ twinning shows a secular trend to the male side.
- Use of spontaneous DZ/MZ twinning rate as a measure of human fertility in East Flanders; according to Tong et al. (1997) this ratio could be reliable as an epidemiological parameter to estimate fertility.
- Biological (mental and physical) between- and within-pair differences in MZ twins according to the time of splitting of the zygote: early (DC), intermediate (MC-DA) and late (MC-MA).

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