Time trends in the natural dizygotic twinning rate

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BACKGROUND: The natural dizygotic (DZ) twinning rate has been proposed as a reliable and useful measure of human fecundity, if adjusted for maternal age at twin birth. The aim of this study was to analyze age-adjusted trends in natural DZ twinning rates over the past 40 years using data from the ‘East Flanders Prospective Twin Survey (EFPTS)’.

METHODS: This study involved 4835 naturally conceived twin pregnancies between 1969 and 2009 from the population-based Belgian ‘EFPTS’. Age-adjusted trends in the incidence of natural DZ twin pregnancies were calculated using a generalized linear model with Poisson distribution.

RESULTS: Both the natural DZ twinning rates and maternal age at twin birth increased in a linear fashion from 1969 to 2009. When age-adjusted, we found that the trend in the natural DZ twinning rate was stable during the whole time period.

CONCLUSIONS: According to our population-based data and after age-adjustment, a stable natural DZ twinning rate could be observed over the last four decades. Under the assumption that the spontaneous DZ twinning rate is a sensor of fecundity, this indicates a stable ‘high’ fecundity for this population.

Key words: twins / dizygotic / fecundity / time trend

Introduction

There has been growing concern about the decline in male fertility over the past few decades since Carlson et al. (1992) reported that mean sperm counts decreased by 50% during the second half of the last century. In addition, with today’s couples generally wanting fewer or no children, there has been a steep decline in birth rates in Western societies since the 1960s (Lutz W et al., 2003). It has been argued that this downward trend might also be related to a general loss of fecundity, which is the capacity of couples to conceive and have children.

In a recent report, te Velde et al. claimed that whether or not population fecundity is declining can neither be confirmed nor rejected (te Velde et al., 2010). The authors argue that the fundamental reason we still do not know whether population fecundity is declining is the lack of an appropriate surveillance system. In their opinion, determining total sperm counts (as a measure of male reproductive health) in combination with time to pregnancy (as a measure of couple fecundity) in carefully selected populations is a feasible option for such a monitoring system.

Apart from those indicators, the incidence of naturally conceived dizygotic (DZ) twinning has also been proposed as a potential marker for fecundity (Tong et al., 1997; Ferrari et al., 2007). Mothers of DZ twins are alleged to be more fecund than mothers of singletons, and the association between high fecundity and natural DZ twinning has been tested in several studies. Some of these studies found an association between a long waiting time to pregnancy and a reduced probability of DZ twinning (Basso et al., 2004; Ferrari et al., 2007; Zhu et al., 2007; Hoekstra et al., 2008). Others showed a reduced DZ twinning rate in subfertile men, suggesting that decreasing trends in semen quality could contribute to lower twinning rates (Richiardi et al., 2004). Fathers of DZ twins were shown to have a better semen quality than normal fertile men, which further supports the assumption that spontaneous DZ twinning rate can be used as a sensor of male fecundity (Asklund et al., 2007).

The incidence of multiple pregnancy rates in Western Europe fell in the 1950s until the mid-1960s to the mid-1970s, but was followed by a continuing rise (Macfarlane and Blondel, 2005). This steep increase in the incidence of multiple births is mainly due to the advancing maternal age at twin birth and the use of fertility drugs with or without IVF and related assisted reproductive technologies (ARTs). At present, almost half of all twin pairs in Belgium and the Netherlands are born after infertility treatment, while Dutch mothers are among the oldest at...
birth of their first child (Derom et al., 2002; Gielen et al., 2010). Therefore, recent trends in DZ twinning rates cannot be used as such to study fertility measures; one is completely confounded about what has been happening to naturally conceived DZ twinning rates over the last decades.

Very few data are available concerning natural twinning rates in Western countries. It has been suggested that (i) there continues to be a decline in the incidence of natural twinning but this trend cannot be demonstrated because of the advances in assisted reproduction (James, 1995; Tong et al., 1997), and (ii) the total natural twinning rate could be used as a potential parameter to monitor population fecundity because the monozygotic (MZ) twinning rate is assumed to be remarkably stable over time (Bulmer, 1970; James, 1982; Tong et al., 1997). Total natural twinning rates are therefore thought to reflect changes in natural DZ twinning rates.

Faisal et al. (2008) assessed trends in twinning for two decades (from 1980 until 2004) using the Cardiff Birth Survey, a population-based registry in South Wales, UK. After age-adjustment and exclusion of the medically conceived twins, their data showed a gradual and continuous increase in natural twinning rates. Two other studies, one in Italy (Astolfi et al., 2003) and one in Denmark (Herskind et al., 2005), also found evidence for a rather stable and even an unexpected increase in the natural twinning rates. None of these studies however had data on the zygosity of the twins.

The purpose of this study was 2-fold: first, to analyze trends in natural DZ twinning rates over the past 40 years and determine whether the increasing maternal age influences these trends; and second, to analyze the same trends in total natural twinning rates to see whether the changes in age-adjusted total natural rates reflect the changes in natural DZ twinning rates. The data come from the East Flanders Prospective Twin Survey (EFPTS), a population-based register of multiple births in the province of East Flanders (Belgium), with known zygosity data.

## Materials and Methods

### East Flanders Prospective Twin Survey

The EFPTS, started in 1964, is a population-based register of multiple births in the Belgian province of East Flanders. The twins (and higher order births) are ascertained at birth, basic perinatal data are recorded, and choriontype and zygosity are determined. Between July 1964 and the end of December 2009, 8048 twin pairs, who met the World Health Organization criteria for live born infants (birthweight ≥ 500 g or gestational age ≥ 22 weeks, if birthweight unknown), were registered (Loos et al., 1998; Derom et al., 2002). Because of small numbers, triplets and higher-order multiple births were excluded.

Data recorded by the obstetrician at birth included gestational age, birthweight, maternal age, parity, mode of delivery, mode of conception, i.e. naturally conceived versus ART (including ovulation stimulation and IVF with or without ICSI) and live born (including early neonatal death) versus stillborn. Zygosity was determined by sequential analysis based on sex, fetal membranes (chiorionicity), umbilical cord blood groups, placental alkaline phosphatase and, since 1982, DNA fingerprints. Unilexed twins and same-sexed twins with at least one different genetic marker were classified as DZ; monochorionic twins were classified as MZ. For all same-sexed dichorionic twins with the same markers, a probability of monozygosity was calculated using a lodscore method (Vlietinck, 1986). After DNA-fingerprinting, a probability of monozygosity of 0.999 is reached. However due to financial stringency, DNA fingerprints could not be determined on all twin pairs. Therefore, in this study only same-sexed dichorionic twins with the same markers, reaching a probability of 0.95 or more, were considered MZ. The remaining dichorionic twins were classified as unknown.

The total numbers of twin and singleton pregnancies for the province of East Flanders were obtained from the National Institute of Statistics, Belgium, for the period between 1964 and 1988 and from the Flemish Study Center of Perinatal Epidemiology (SPE), a comprehensive clinical birth database of Flanders (Cammu et al., 2007) for the period from 1989 to 2009. No data on maternal age were available for the singleton births. Since 1991 the SPE has collected data on the mode of conception (natural versus ART) so that ART pregnancies were excluded from the total number of pregnancies in East Flanders during that period. Between 1980 (the start of ART treatments) and 1991 (when ART births were 1.66%), the data were interpolated to exclude the ART pregnancies: a total of 1340 births were excluded increasing gradually from 0% in 1980 to 1.66% in 1991. In the end, between 1969 and 2009, there were 720 722 pregnancies in the area, of whom 707 150 were naturally conceived.

The total twin, DZ and MZ twin frequencies were calculated on the basis of total number of yearly pregnancies in East Flanders and corrected according to EFPTS registration percentages. The registration rate in EFPTS gradually increased from 65% in 1964 to more than 95% of all twin pregnancies in East Flanders since the 1990s. Only years where the registration rate was more than 70% were included in the analysis: from 1969 onwards until 2009, with the exception of 1970. In these years, 7448 twin pairs were recorded. Twin pairs born after fertility treatment without further intervention (ovulation induction only; n = 1031) or after IVF/ICSI or related techniques (n = 1218) or with unknown mode of conception (n = 109), unknown zygosity (n = 193) or unknown maternal age (n = 62) were excluded for the final analysis of all natural twin pregnancies. In total, data from 4835 naturally conceived twin pairs were analyzed, of which 2685 were DZ twin pairs, including 1322 opposite-sex twin pairs. The percentage of boys in all natural twin pairs was 50.24%.

### Statistical analyses

The annual total, DZ and MZ spontaneous twinning rates, were calculated as the annual number of total, DZ and MZ spontaneous twin pregnancies per 1000 spontaneous pregnancies. Since annual twinning rates are frequencies (count data), a Poisson regression analysis was performed (generalized linear model with Poisson distribution) with birth as the unit. The annual total, DZ and MZ spontaneous twinning rates were estimated with and without adjustment for the annual mean maternal age of respectively all the twins, the DZ twins and the MZ twins. Only when the influence of the maternal age was significant, the age-adjusted rates were used.

Second-order polynomials were also tested. A maximum likelihood approach with accompanying Akaike’s information criterion was used to compare nested models. In addition, the significance of an estimate (B) was evaluated with the Wald χ² test. To compare the estimates of different models, a t-test was performed. Note that the presented estimates are log transformed and would then represent a percent change per year.

### Results

Figure 1 illustrates the trends in naturally conceived twin pregnancies over the 40-year study period in East Flanders. The natural DZ twinning rate showed an increase over the years from 4.3 to 6.1 per thousand pregnancies. The same gradual increase was observed in the total natural twin population, while the natural MZ twinning rate remained constant.
Analysis of the maternal age at twin birth also revealed a progressive increase over the years (see Fig. 2). This was true for all the mothers of both naturally conceived MZ and DZ twins. Mothers of DZ twins are on average 1 year older than mothers of MZ twins.

When adjusted for the age of the mother at twin birth, analysis of a time trend did not show any significant change over the years for the natural DZ twin frequencies ($\beta = 0.0033$, SE 0.0027, $P = 0.23$) (Fig. 3). The time trend of all natural twin pregnancies continued to reveal an increase over the years even after age-adjustment ($\beta = 0.0046$, SE 0.0020, $P = 0.02$). These estimates were however not significantly different ($t$-test 0.21, $P = 0.84$). There was no influence of the age of the mother at twin birth on the natural MZ twin frequencies.

**Discussion**

Suggestions have been made for monitoring natural DZ twinning rates for assessing temporal changes in a population’s reproductive health. The aim of present study was therefore to analyze time trends in the natural DZ twinning rate using data from the EFPTS, a population-based register of multiple births in the province of East Flanders (Belgium), and as result to detect increasing or declining temporal trends in fecundity in the past 40 years.

After exclusion of all ART births, the overall trends in natural DZ twinning rates in our data set showed a gradual increase over the years. The MZ twinning rates remained constant representing 45% of all natural twin pregnancies in 1980 and 36% in more recent years. Very few data are available concerning natural twinning rates in European countries and one has to rely on data before the use of fertility drugs to make a valid comparison. In Sweden, 46% of twin pairs were estimated to be MZ in 1980 (Kaprio and Marttila, 2005), which is in agreement with our data. In England and Wales, Scotland, Germany, the Netherlands, Switzerland and Italy, the estimated percentage of DZ twins declined from 70% in 1950 to 55–60% between 1980 and 1985 (Astolfi et al., 2003; Macfarlane and Blondel, 2005). After 1985 the proportion of DZ twins increased drastically in all these countries mainly due to the use of fertility drugs.

Maternal age at twin birth also increased gradually for both MZ and DZ twins. It is long known that one of the main factors affecting natural DZ twinning rates is maternal age: DZ twinning rate increases four-fold from age 15 to 35. (Bulmer, 1970; Hall, 2003; Hoekstra et al., 2008). Therefore, in all studies of twinning rates, maternal age must always be taken into account by direct or indirect standardization (Fellman and Eriksson, 1990). When adjusted for the maternal age at twin birth, the observed increase in the natural DZ twinning rate disappeared and a constant time trend over the 40 years remained. If monitoring natural DZ twinning rate is a good marker of fecundity in the general population, this result is rather reassuring as it indicates a stable population fecundity. It also proves that it is possible to measure age-adjusted and ART-adjusted natural twinning rate in a population.

The strength of the data presented here stems from several important factors: the fact that EFPTS is a population-based register at birth, which has been shown to be well representative of the whole population (Vlietinck, 1986); that the data cover 40 years with all main

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**Figure 1** Unadjusted natural twinning rates in East Flanders (1969–2009). Annual frequencies (per thousand) of all naturally conceived twin pregnancies (upper curve), all naturally conceived DZ twin pregnancies (dotted curve) and all naturally conceived MZ twin pregnancies (lower curve).

**Figure 2** Age of the mother at twin birth in East Flanders (1969–2009). Yearly mean maternal age at birth of all natural DZ twin pregnancies (small dots) and all natural MZ twin pregnancies (large dots).
variables coming from the same database, such as age of the mother at
twin birth, zygosity of the twins, sex of the infants, mode of conception
and infertility treatment; that all these variables were collected at birth
by medical records; and that all ART cases, even those that were
the result of treatment with ovulation-inducing agents alone without IVF,
were excluded.

If true incidence of natural DZ twinning rates cannot be calculated
because no zygosity data are available, age-adjusted total natural twin-
ing rates can be analyzed. After age-adjustment and exclusion of the
medically conceived twins, our data showed also a stable and even
increasing trend in total natural twinning rates. Albeit not statistically
different from the natural DZ twinning rate, the slope of the increase
of the age-adjusted total natural twinning rate was slightly greater.
These results are in agreement with three other studies in Italy,
Denmark and South Wales which also observed a slight increase in
natural twinning. In our data set, this slight increase cannot be attrib-
uted to the MZ twinning rate, which is stable over time during that
period and not influenced by the rising age of the mother at twin
birth. Mothers of MZ twins are younger at twin birth than mothers
of DZ twins and the different interaction between mother age at
twin birth and zygosity could explain these small differences in the
slopes of the time trends over the years.

One limitation of this study is a possible immigration effect that may
have contributed to the incidence of twinning presented here. However, we do not think this would affect our results as the prevalence
of Caucasians in the province of East-Flanders is over 99%, even with an
increased prevalence of foreign descent since 1964 (Cammu et al.,
2007).

Another limitation of this report is the assumption of an association
between natural DZ twinning and fecundity. Are parents of DZ twins
more fertile or more fecund than other parents on the average? There
are several lines of evidence for the affirmative answer and at least one
piece of evidence for the negative. The high proportion of twins
among prompt postmarital conceptions and postwar conceptions
and illegitimate conceptions has long been used as evidence for the
fecundity hypothesis (Eriksson and Fellman, 1967; Bulmer, 1970;
James, 1986). DZ twins are the result of multiple ovulations followed
by successful fertilization of at least two ova, multiple implantations
and maintenance of a multiple pregnancy to term. The more recent
findings of an association between DZ twinning and a shorter time
to pregnancy (a marker for couple fecundity) on the one hand and
a better semen quality (a marker for male reproductive health) on
the other hand is further in line with the general belief of spontaneous
DZ twinning as a marker of high fecundity.

Basso et al. (2004) found that women conceiving within 2
months of trying had a much higher risk of giving birth to twins
compared with women taking longer than 1 year. They corrobo-
rated their findings using a larger data set with known zygosity
and further found that the reduced frequency of natural twinning
among infertile couples was due to a low DZ twinning prevalence
and that increasing time to pregnancy was associated with decreas-
ing DZ twinning prevalence (Zhu et al., 2007). Using a nested
case–control design Ferrari et al. (2007) reported independently
that women with a time to pregnancy of less than 6 months
were more likely to have a multiple birth than women reporting
a time to pregnancy of more than 6 months.

Richardi et al. (2004) found a reduced chance of DZ twinning in
subfertile men, suggesting that the decreasing trends in semen
quality could contribute to the low twinning rate. After adjustment
for maternal age, they reported fewer DZ twins fathered by men
who subsequently developed testicular cancer, which is associated
with subfertility. Asklund et al. (2007) studied the semen quality in a
group of fathers of naturally conceived twins. They found that
fathers of DZ twins had more normal and more motile sperm than
the reference group of fathers of singletons. Their sperm count was
also higher, although not significantly. These results lend further
support to the assumption that spontaneous DZ twinning rate can
be used as a sensor of male fecundity in a population.

On the other hand, DZ twinning rate increases with maternal age,
which is well known to result in decreased fecundity. In line with this
fertility paradox is also the fact that mothers of spontaneous DZ twins
seem to be more resistant to the negative effects of BMI and smoking

Figure 3 Models for the natural twinning rates in East Flanders (1969–2009). Model for the age-adjusted twinning frequencies (per thousand) of all
natural twins (upper curve) and all natural DZ twins (dotted curve). Model for the twinning frequencies (per thousand) of all natural MZ twins (lower
curve). The presented curves are for a mean maternal age of 28 years.
Natural DZ twinning rate

compared with mothers of MZ twins and singletons (Hoekstra et al., 2008). Surprisingly, the same factors (maternal age, smoking and obesity) that are associated with natural DZ twinning are also associated with infertility in singleton mothers and mothers of MZ twins. Apart from these, genetic factors also play a role in increasing the risk of having DZ twins but so far studies on the genetic cause of natural DZ twinning have raised more questions than answers. The chances that more than one cause for this intriguing human phenomenon will surface are real (Derom et al., 2006; Painter et al., 2010).

In conclusion, after ART and age-adjustment, our population-based data showed a stable natural DZ twinning rate over the last four decades. Under the assumption that spontaneous DZ twinning rate is a sensor of fecundity, these data indicate a stable high fecundity for this population. With the continuing debate about whether there has been a decline in human fertility, and its possible causes, we believe that it is important to have a global monitoring of the DZ twinning rates in different populations and to stimulate renewed interest in the determinants of twinning in particular with regard to zygosity and the absence of fertility-related treatment.

Authors’ roles

All authors of this research paper have directly participated in the planning and execution, have approved the final version submitted and have no conflict of interest in connection with this paper. C.D. and M.G. took part in study concept and design. C.D., M.G., H.P., J.-P.F. and M.P.A.Z. played a role in analysis interpretation of data. C.D., M.G. and M.P.A.Z. were involved in drafting the manuscript. C.D., M.G., H.P., J.-P.F. and M.P.A.Z. critically revised the manuscript for important intellectual content. M.G. and M.P.A.Z. did statistical analysis. J.-P.F. and M.P.A.Z. took part in study supervision.

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