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# Infertility, infertility treatment and twinning: the Danish National Birth Cohort

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**BACKGROUND: We have previously observed that an increasing time to pregnancy (TTP) is associated with a reduced frequency of twin deliveries in couples not receiving infertility treatment. By using updated information, we assessed the frequencies of dizygotic (DZ) and monozygotic (MZ) twin deliveries as a function of infertility (TTP > 12 months), as well as infertility treatment. METHODS: From the Danish National Birth Cohort (1997–2003), we identified 51 730 fertile couples with TTP ≤ 12 months, 5838 infertile couples who conceived naturally with TTP > 12 months and 5163 infertile couples who conceived after treatment. Information on zygosity, available for part of the cohort (1997–2000), was based on standardized questions on the similarities between the twins at the age of 3–5 years. RESULTS: Compared with fertile couples, the frequency of DZ twin deliveries was lower for infertile couples conceiving naturally (odds ratio 0.4, 95% confidence interval 0.2–0.7) and was much higher for infertile couples conceiving after treatment (17.3, 14.4–20.7). The frequency of DZ twin deliveries decreased with TTP in untreated couples, whereas the frequency of MZ twin deliveries remained constant. CONCLUSIONS: The frequency of DZ twin deliveries decreased with TTP and substantially increased with infertility treatment, whereas MZ twin deliveries remained substantially unchanged.**

*Key words*: infertility/infertility treatment/time to pregnancy/twinning/zygosity

## Introduction

Spontaneous dizygotic (DZ) twinning has long been considered a marker of high fecundity (Tong and Short, 1998), and the decline in the frequency of twin deliveries in many industrialized countries in the mid-20th century raised concern about potential adverse environmental effects on reproductive health (James, 1986). Since then, the frequency of twin deliveries has been steadily increasing worldwide, primarily due to treatment for infertility (Westergaard *et al.*, 1997; Imaizumi, 1998; Herskind *et al.*, 2005; Wright *et al.*, 2005).

Using a segment of the Danish National Birth Cohort (DNBC), we previously observed that increasing time to pregnancy (TTP) correlated with lower twinning prevalence (Basso *et al.*, 2004), but we had no information on zygosity. In this article, we updated the data from the same cohort to examine DZ and monozygotic (MZ) twinning as a function of infertility (measured by a TTP of >12 months). We also explored the effect of different types of infertility treatment on the frequency of twin deliveries.

## Methods

### Study population

The DNBC has been described in detail elsewhere (Olsen *et al.*, 2001). From June 1997 to February 2003, 85 381 women (92 892 pregnancies) participated in the first of four scheduled interviews during the first or second trimester of pregnancy. The women were asked whether their pregnancy was planned, and if so, how long they had tried to become pregnant before succeeding. If they reported a TTP of 6 months or longer, they were also asked whether they or their male partners had received any infertility treatment prior to this pregnancy. If a woman reported that she or her male partner received infertility treatment, she was further asked ‘what kind of infertility treatment did you or your male partner receive?' Fixed response categories included six specified types of treatment and allowed for a verbal description (questionnaire is available at www.bsmb.dk). The treatments included intracytoplasmic sperm injection (ICSI), *in vitro* fertilization (IVF), intrauterine insemination (IUI), hormonal treatment (HT), surgery and hysterosalpingography. If a woman reported more than one procedure, she was classified according to the above priority sequence.

We identified three groups of planned pregnancies: 52 380 conceived by fertile couples with TTP $\leq$ 12 months, 5910 conceived naturally by infertile couples with TTP $>$ 12 months and 5564 conceived by infertile couples after infertility treatment. If a woman contributed more than one pregnancy, we included only the first one. We included four common treatment procedures: ICSI, IVF, IUI and HT. Other procedures were excluded ($n = 299$), leaving 5265 pairs of couples defined as treated.

Pregnancy outcomes were identified by linkage with the cohort to the National Hospital Register and the Medical Birth Register by means of the unique Danish civil registration number. We excluded pregnancies ending in spontaneous abortion or induced abortion, as well as hydatidiform moles or ectopic pregnancies ($n = 752$). We further excluded 44 pregnancies with unknown pregnancy outcome due to emigration or other reasons and 28 sets of triplets. Our analysis sample thus consisted of 61 145 singleton deliveries (including 189 stillbirths) and 1586 twin deliveries (including 2 deliveries with both twins stillborn, and 16 deliveries with one twin stillborn). Stillbirth was defined as fetal death occurring after 28 weeks of gestation, the official definition in Denmark during the study period.

### *Zygosity information*

Information on zygosity was obtained by linkage to the Twin Register (Skytthe *et al.*, 2002), where zygosity had been updated for births occurring through 2000. Zygosity assessment was based on the established similarity questionnaire (Skytthe *et al.*, 2002), administrated in 2003 when the twins were between 3 and 5 years of age. The questions were: (i) Are/were you and your twin as a like as two peas in a pod or as ordinary sibling? (ii) Are/were you mistaken by family and friends? (iii) Are/were you mistaken by kindergarden teachers and mates? (iv) Do/did you have the same eye and hair colour? The questions were answered by the twins' parents. Twins described as being as alike as two peas in a pod, with the same eye and hair colour, were classified as MZ if at least one of the questions about being mistaken was answered 'yes'. Twins described as alike as ordinary siblings and answering 'yes' to all the other questions were also classified as MZ. The rest were classified as DZ. All inconsistent answers were classified as unknown zygosity.

During the period of 1997–2000, information on zygosity was obtained from the Twin Register for 545 pairs of same-sex twins. All the opposite-sex twins (353 pairs) were classified as DZ twins.

### *Statistical analysis*

The frequency of twin deliveries (twinning prevalence) was defined as the number of twin deliveries per 100 total deliveries including live- as well as stillbirths. Odds ratios (ORs), with 95% confidence interval (CI), of twinning were calculated by means of logistic regression. When analysing overall twinning, we included all live births and stillbirths (1997–2003). When we took zygosity into account, we only included twins born between 1997 and 2000. In each analysis we included only the twin type under study and used singletons as the reference category. A test for trend was performed for twinning as a function of TTP by treating TTP categories as ordinal numbers in the model. Potential confounders included maternal age at conception (<25, 25–29, 30–34, 35+ years), parity (1, 2+), smoking (yes, no) and pre-pregnancy body mass index (BMI) (<25, 25+ kg/m$^2$), similar to our previous study (Basso *et al.*, 2004).

## Results

The characteristics of the study population are shown in Table I. When compared with fertile couples, infertile couples conceiving naturally had a lower twinning prevalence (OR 0.6, 0.5–0.8), whereas infertile couples conceiving after treatment had a much higher twinning prevalence (OR 14.1,

**Table I.** Characteristics of study population according to time to pregnancy (TTP) and infertility treatment, 1997–2003

| | Fertile couples (TTP $\leq$ 12) | | Infertile couples conceiving naturally (TTP $>$ 12) | | Infertile couples conceiving after treatment | |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % |
| Maternal age, years | | | | | | |
| < 25 | 6 364 | 12.3 | 501 | 8.6 | 190 | 3.7 |
| 25–29 | 23 504 | 45.4 | 2 185 | 37.4 | 1 597 | 30.9 |
| 30–34 | 17 296 | 33.4 | 2 203 | 37.7 | 2 225 | 43.1 |
| 35 + | 4 566 | 8.8 | 949 | 16.3 | 1 151 | 22.3 |
| Parity | | | | | | |
| 1 | 24 549 | 47.5 | 3 139 | 53.8 | 3 826 | 74.1 |
| 2 + | 27 180 | 52.5 | 2 699 | 46.2 | 1 337 | 25.9 |
| Missing | 1 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Smoking | | | | | | |
| No | 39 865 | 77.1 | 4 040 | 69.2 | 4 091 | 79.2 |
| Yes | 11 843 | 22.9 | 1 795 | 30.7 | 1 071 | 20.7 |
| Missing | 22 | 0.0 | 3 | 0.1 | 1 | 0.0 |
| Pre-pregnancy body mass index (BMI), kg/m$^2$ | | | | | | |
| < 25 | 37 180 | 71.9 | 3 759 | 64.4 | 3 414 | 66.1 |
| 25 + | 13 764 | 26.6 | 1 958 | 33.5 | 1 665 | 32.2 |
| Missing | 786 | 1.5 | 121 | 2.1 | 84 | 1.6 |
| Offspring | | | | | | |
| Singletons | 51 041 | 98.7 | 5 787 | 99.1 | 4 317 | 83.6 |
| Pairs of twins | 689 | 1.3 | 51 | 0.9 | 846 | 16.4 |

Table II. Odds ratios (ORs) with 95% confidence intervals (CIs) of dizygotic (DZ) and monozygotic (MZ) twinning according to TTP and infertility treatment, based on zygotic information from the Danish Twin Register, 1997–2000 (reference group: fertile couples)

| | Fertile couples (TTP ≤ 12) | Infertile couples conceiving naturally (TTP > 12) | | | Infertile couples conceiving after treatment | | |
|---|---|---|---|---|---|---|---|
| | *n* (%) | *n* (%) | Crude OR | Adjusted OR (95% CI) | *n* (%) | Crude OR | Adjusted OR (95% CI) |
| Singletons | 30 071 (98.6) | 3 320 (99.0) | | | 2 340 (83.6) | | |
| Pairs of twins | 421 (1.4) | 34 (1.0) | | | 458 (16.4) | | |
| DZ | 265 (0.9) | 12 (0.4) | 0.4 | 0.4 (0.2–0.7) | 383 (13.7) | 18.6 | 17.3 (14.4–20.7) |
| MZ | 78 (0.3) | 11 (0.3) | 1.3 | 1.3 (0.7–2.5) | 8 (0.3) | 1.3 | 1.5 (0.7–3.2) |
| Unknown | 78 (0.3) | 11 (0.3) | | | 67 (2.4) | | |

Logistic regression; analysis unit is pregnancy; adjusted for maternal age, parity, smoking and pre-pregnancy BMI. In analysis on DZ twinning, MZ twins together with twins of unknown zygosity were excluded, and vice versa.

12.5–15.8). When we restricted our analysis to infertile couples, couples conceiving after treatment had an OR of twinning of 21.1 (15.8–28.2) compared with couples conceiving naturally.

We had no information on zygosity for 156 pairs (including 15 pairs of twins with one or both twins stillborn) out of 913 pairs of twins born between 1997 and 2000. Among all same-sex live-born twins (545 pairs), there was no significant difference in identification of zygosity among the study groups: 75.4% for fertile couples, 62.5% for infertile couples conceiving naturally and 73.8% for infertile couples conceiving after treatment ($P = 0.38$). According to our analyses (including 35 731 singletons, 97 pairs of MZ twins and 660 pairs of DZ twins), the frequency of DZ twin deliveries was lower for infertile couples conceiving naturally, but substantially higher for infertile couples conceiving after treatment (Table II). The frequency of MZ twin deliveries was not significantly different among the three groups.

Analysis restricted to untreated couples showed that an increasing TTP was associated with a reduced frequency of DZ twin deliveries, but not of MZ twinning (Table III).

All types of treatment (ICSI, IVF, IUI and HT) were associated with a higher prevalence of DZ twinning (Table IV). Compared with fertile couples, we found an OR of 30.0 (24.2–37.1) for combined ICSI and IVF and an OR of 10.6 (8.4–13.3) for combined IUI and HT. We had very few MZ twins (eight pairs) to meaningfully examine the association between different types of treatment and MZ twinning.

Introduction of maternal age and parity in the models resulted in >5% change in some estimates, whereas smoking and pre-pregnancy BMI had little effect. The frequency of DZ twinning, but not of MZ twinning, increased with increasing maternal age (data not shown).

## Discussion

Using a larger data set, we corroborated our previous finding that a TTP of longer than 12 months was associated with a reduced frequency of natural twinning (Basso *et al.*, 2004). We further found that the reduced frequency of natural twinning among infertile couples was due to a low DZ twinning prevalence and that increasing TTP was associated with decreasing DZ twinning prevalence. Infertility treatment dramatically increased the frequency of DZ twin deliveries.

The finding of an association between TTP and overall twinning prevalence is also in line with two recent studies that showed that multiple pregnancies tended to have shorter TTP than singleton pregnancies (Axmon and Hagmar, 2005; Ferrari *et al.*, 2006). Our finding that the association was attributable to DZ twinning is consistent with the general belief of spontaneous DZ twinning as a marker of high fecundity. DZ twins involve multiple ovulations, successful fertilization of at least two ova, multiple implantations and maintenance of a multiple pregnancy up to the time of delivery. The decline of DZ twinning with TTP could be due to a decline in the rate of multiple ovulations, fertilization or implantation, or to an increased rate of fetal loss or vanishing twin phenomenon. Since no decline was seen in MZ twin deliveries with TTP, and since infertility treatment increased the frequency of DZ twin deliveries by ovulation stimulation or transferring more than one embryo, it is possible that the decline in DZ twin deliveries is due to a decline in multiple ovulations. The

Table III. ORs with 95% CIs of DZ and MZ twinning in untreated couples, according to TTP (reference group: TTP of 0–2 months)

| TTP (months) | No. of singletons | DZ twins | | MZ twins | |
|---|---|---|---|---|---|
| | | No. of (%)[a] pairs | Adjusted OR (95% CI) | No. of (%)[a] pairs | Adjusted OR (95% CI) |
| 0–2 | 16 794 | 166 (1.0) | 1.0 | 44 (0.3) | 1.0 |
| 3–5 | 7 724 | 69 (0.9) | 0.9 (0.7–1.2) | 23 (0.3) | 1.1 (0.7–1.9) |
| 6–12 | 5 553 | 30 (0.5) | 0.5 (0.4–0.8) | 11 (0.2) | 0.8 (0.4–1.5) |
| >12 | 3 320 | 12 (0.4) | 0.3 (0.2–0.6) | 11 (0.3) | 1.3 (0.7–2.6) |
| Test for trend | | | $P < 0.0001$ | | $P = 0.83$ |

[a]Percentages were calculated on the basis of all deliveries, including singletons and twins (DZ, MZ and unknown).

**Table IV.** ORs with 95% CIs of DZ twinning for different types of treatment, based on zygotic information from the Danish Twin Register, 1997–2000 (reference group: fertile couples)

| Types of treatment | No. of singletons | No. of (%)[a] pairs of DZ twins | Crude OR | Adjusted OR (95% CI) |
|---|---|---|---|---|
| ICSI | 194 | 56 (21.5) | 32.8 | 33.4 (23.7–46.9) |
| IVF | 774 | 199 (19.7) | 29.2 | 29.5 (23.5–37.0) |
| IUI | 761 | 80 (9.4) | 11.9 | 12.0 (9.1–15.7) |
| HT | 611 | 48 (7.1) | 8.9 | 9.0 (6.5–12.4) |

ICSI, Intracytoplasmic sperm injection; IVF, *in vitro* fertilization; IUI, intrauterine insemination; HI, hormonal treatment.
[a]Percentages were calculated on the basis of all deliveries, including singletons and twins (DZ, MZ, and unknown).

'insurance ova' hypothesis (Andersen, 1990) views multiple ovulations as a mechanism that compensates fertilization failure or fetal loss. According to this hypothesis, DZ twins would be a by-product of the selective pressure favouring multiple ovulations. The true incidence of multiple ovulations may be much higher than that seen at birth, since loss of one twin has been observed more commonly than was originally thought (Landy and Keith, 1998). Other mechanisms may, however, be responsible for the decline in twinning among infertile women: the rate of multiple fertilizations or implantations may decrease or, if they remain constant, it is possible that the vanishing twin phenomenon becomes more frequent among women with infertility and multiple implantations, since the quality of the ova may be poorer and embryo quality appears to be an important factor in the maintenance of a twin pregnancy during the first few weeks (Lambers *et al.*, 2006).

Since in this study we were only able to examine twins who survived to birth, however, we can only speculate about the actual mechanisms behind this association.

It is well known that assisted reproductive technology increases the twinning prevalence, mainly the DZ twinning prevalence (Lambert, 2002; Pinborg, 2005; Wright *et al.*, 2005). In the case of IVF and ICSI, a Danish law from October 1997 restricts the number of embryos transferred to two, with three in some special cases (Westergaard *et al.*, 2000). In Denmark, IUI using husband semen often involves the use of clomiphene citrate and/or FSH analogs. We found that couples undergoing IVF or ICSI had three times the prevalence of DZ twinning than couples using IUI and HT. We did not find a statistically significant increase in MZ twin deliveries for infertility treatment as a whole, and the sample size was too small for any treatment-specific analysis.

Eligible women were invited by their general practitioner to participate in the DNBC. About half of all general practitioners in the country took part in the recruitment and ∼60% of the invited women accepted the invitation. As a result, 30–35% of all eligible pregnant women took part in the study. Unless participation in the cohort depended on being pregnant with twins and on infertility status, it is unlikely that this introduced bias in our estimates. The overall frequency of twin deliveries in the DNBC was 2.2%, in agreement with the Danish twinning prevalence (1.9–2.2%) between 1997 and 2001 (the homepage of the Danish Society of Obstetrics and Gynaecology, http://www.dsog.dk).

We used a twin-similarity questionnaire to classify zygosity in this young cohort of twins. It has been shown that zygosity can be estimated by a simple questionnaire with a risk of misclassification below 5% (Christiansen *et al.*, 2003), even in very young twins (6 months to 6.5 years) (Bonnelykke *et al.*, 1989). The zygosity classification for same-sex twins was incomplete (only available for 74%) in our study. However, it is unlikely that this has biased our results either, since we found no significant difference in the determination rate among the study groups and we obtained similar estimates by using only opposite-sex twins as the outcome (data not shown).

The prevalence of DZ twinning decreased with TTP and substantially increased with infertility treatment, whereas the prevalence of MZ twinning remained constant. This, together with the association of maternal age and DZ twinning (but not MZ twinning), suggests that MZ twinning may be a stochastic event, whereas environmental and genetic factors play an important role in DZ twinning.

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