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Trends in Dizygotic and Monozygotic Spontaneous Twin Births During the Period 2007–2017 in Lombardy, Northern Italy: A Population-Based Study

Giovanna Esposito¹, Michela Dalmartello¹, Matteo Franchi^{2,3}, Paola Agnese Mauri^{1,4}, Sonia Cipriani⁴, Giovanni Corrao^{2,3} and Fabio Parazzini¹

¹Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy, ²Unit of Biostatistics, Epidemiology and Public Health, Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan, Italy, ³National Centre for Healthcare Research and Pharmacoepidemiology, Milan, Italy and ⁴Department of Obstetrics, Gynecology and Neonatology, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Abstract

In this study, we analyzed the estimated frequency of monozygotic (MZ) and dizygotic (DZ) spontaneous twins in Lombardy during the period 2007–2017. This is a population-based study using the regional healthcare utilization databases of the Lombardy Region. The total number of spontaneous twin deliveries, in separate strata of like and unlike sex, was obtained. Moreover, estimates of DZ and MZ twin births were calculated using Weinberg's method. The standardized rates (SRs), adjusted for maternal age, of DZ and MZ twin births were computed according to calendar period. The twinning rates were calculated among strata of parity and maternal age. Finally, DZ:MZ ratio was calculated. Among the 734,278 spontaneous deliveries, 9176 (12.5 out of 1000 births) couples of twins were identified. In the three periods considered (i.e. 2007–2010, 2011–2014 and 2015–2017), no trend in the SRs of MZ twins was observed, respectively 0.41 (95% CI [0.40, 0.43]), 0.43 (95% CI [0.42, 0.45]) and 0.43 (95% CI [0.42, 0.45]). Differently, a slightly decreasing trend was observed in DZ twins SRs, respectively 0.87 (95% CI [0.84, 0.89]), 0.81 (95% CI [0.79, 0.83]), and 0.78 (95% CI [0.76, 0.80]). As concerns parity and maternal age, the rate of DZ twin births was consistently higher in nulliparae women aged 35 years or more. In our cohort, despite the increase of maternal age, a decline of spontaneous twin births emerged, especially due to the downward trend of DZ twins.

Keywords: spontaneous twins; twin births; monozygotic; dizygotic; twinning rate

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The natural twinning rate among young mothers and the ratio of dizygotic (DZ) to monozygotic (MZ) twins have been suggested to be reliable monitors of a population's fertility and its reproductive health (James, 1998; Tong, 2000; Tong et al., 1997; Tong & Short, 1998). Mothers of DZ twins have been alleged to be more fertile in comparison to women who achieve singleton pregnancies, in particular, due to the established association between a long waiting time to pregnancy and a reduced probability of DZ twins (Basso et al., 2004; Ferrari et al., 2007; Hoekstra, Willemsen et al., 2008; Zhu et al., 2007). Regarding male fertility, there is evidence that a better semen quality is related to a higher spontaneous DZ twinning rate (Asklund et al., 2007). Thus, the analysis of trends of twin pregnancies acquires a relevant interest in order to investigate population fertility. In addition, multiple gestations represent a health issue due to their increased risk of preterm birth, restricted

intrauterine growth, birth defects, cerebral palsy, and perinatal mortality and morbidity in comparison to the singleton ones.

A good assessment of the trends in multiple births has been proposed up to the years 1990–2000 for selected countries, although no updated evidence is available for the last years. In high-income countries, the frequency of twin pregnancies began to decline at the beginning of the 1900s and continued until the late 1970s. In Italy, as well as in most Western countries, the cultural conditions and the new role of women in the society modified reproductive strategies (Astolfi et al., 2003). During the last 40 years, the twinning rate has displayed a reversal of tendency (Dawson et al., 2015; Eriksson & Fellman, 2007). This upward trend was mainly due to the spreading use of assisted reproductive techniques (ART) (Dawson et al., 2015; Fuster et al., 2008). In Italy during the last century, despite the overall increasing trend, a constant decline of spontaneous twins, mainly of DZ ones, was observed until the late 1980s (Parazzini et al., 1989; Parazzini et al., 1994; Parazzini et al., 1991). Similar trends for spontaneous twin births were documented from other countries (James, 1986). The rate of spontaneous twinning became stable during the last decades of the 20th century (Murphy et al., 2000). For example, an analysis on twinning rates in Denmark

Author for correspondence: Giovanna Esposito, Email: giovanna.esposito@unimi.it

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showed that the decline in natural twinning has ended and suggested an increase in natural twinning rates in the early years of this century (Eriksson & Fellman, 2007). Moreover, a Belgian study (Derom *et al.*, 2011), conducted during the period 1969–2009, after allowing for ART and age, observed a stable natural DZ twinning rate.

Spontaneous MZ twin rates were generally unchanged over time (Parazzini *et al.*, 1991), but few data are available from different countries and, in particular, with reference to the last decades (Eriksson & Fellman, 2007; Otta *et al.*, 2016).

In this article, we analyzed the estimated frequency of spontaneously conceived MZ and DZ twins in the Lombardy Region, Northern Italy, during the period 2007–2017.

Materials and Methods

Cohort Selection

We retrieved data from the regional healthcare utilization databases of the Lombardy Region, including an archive of residents who received National Health Service (NHS) assistance, which reported demographic and administrative data, the standard form used to register all discharges from public or private hospitals (scheda di dimissione ospedaliera [SDO]), and the specific form filled out by midwives at delivery (Certificato di assistenza al parto [CedAP]) reporting information about maternal characteristics, pregnancy and delivery. A deterministic record linkage between databases through a unique identification code included in all databases allowed the identification of large and unselected birth cohorts. A more exhaustive explanation of methodology and databases is reported elsewhere (Parazzini *et al.*, 2015).

We included all the deliveries that occurred in Lombardy between January 1, 2007, and December 31, 2017. Out of these, we excluded (1) deliveries that did not match to a SDO related to childbirth, (2) deliveries of women not beneficiaries of NHS and not resident in Lombardy, (3) deliveries of women aged less than 13 or more than 55, (4) deliveries that did not reach 22 to 42 weeks of gestation, (5) deliveries in which the infant could not be linked to the mother because of a missing identification code, (6) deliveries with missing values on at least one of this information: mode of conception (i.e. spontaneous and assisted) or vitality status, (7) deliveries after ART, (8) triplets or quadruplets, and (9) deliveries with missing values on the information concerning the sex of at least one newborn.

Statistical Analysis

The total number of spontaneous twin deliveries, in separate strata of like and unlike sex, was obtained. In addition, the estimated number of DZ and MZ twin births was calculated using Weinberg's method (Weinberg, 1901). According to this rule, the total number of DZ twin pregnancies is twice the number of twin pregnancies with unlike-sex twin sets. The number of MZ twin sets is the difference between the number of like and unlike-sex twin sets. The method is based on the following assumptions: (1) the sex ratio in DZ twins is 0.5 and (2) the sexes of DZ twins are determined independently and with the same probability in all parents; thus, there should, on average, be equal numbers of like-sexed and unlike-sexed DZ twins.

At all stages of the analysis, the unit of the study was the delivery.

The total twin delivery rates, separately for MZ and DZ, were calculated by dividing the estimated number of twins by the total

number of deliveries according to maternal age and calendar period. Four maternal age groups were considered (i.e. <30, 30–34, 35–39, ≥40), and the study period was divided into three intervals (i.e. 2007–2010, 2011–2014 and 2015–2017). In order to take into account the potential confounding effect of age of mothers, standardized rates (SRs) were calculated through a direct method of standardization. Corresponding 95% confidence intervals (CIs) for each rate were calculated.

MZ and DZ rates were estimated also according to parity (two categories: nulliparae and multiparae) and maternal age (two categories: <35 and ≥35). Nulliparity was defined as having no previous delivery. Finally, the DZ:MZ ratio was calculated.

Ethical Approval

Analysis of an administrative, anonymous database does not require ethical approval in Italy. All data retrieved from the mentioned databases were anonymous.

Results

A total of 913,726 deliveries that occurred in Lombardy from January 1, 2007, to December 31, 2017, were identified from the CedAP database. We subsequently excluded (1) 7685 records because they did not match a SDO related to childbirth, (2) 143,788 records of women not resident in Lombardy, (3) 839 records because the mother was younger than 13 years or older than 55 years, (4) 2891 records because the gestational age was too short (<22 weeks) or too long (>42 weeks), (5) 458 records because the infant could not be linked to the mother because of a missing identification code, (6) 3353 records because of missing values on mode of conception (i.e. spontaneous and assisted) or vitality status, (7) 20,208 records referred to deliveries after ART (8) 124 triplets or quadruplets, and (9) 102 records because of a lack of information concerning the sex of at least one newborn. Thus, we obtained a final study cohort including 734,278 deliveries.

Overall, during the study period, 9176 (12.5 out of 1000 births) spontaneous twin deliveries were recorded.

The distribution of maternal characteristics of singleton and twin births is provided in Supplementary material (Table S1). Twin deliveries were more frequent among nulliparae ($p < .0001$) and women aged more than 35 years ($p < .0001$). Mean age at delivery was respectively 33.2 ± 4.9 among women who conceived twins and 32.6 ± 5.1 among those who had singleton pregnancies (p value $< .0001$), and the proportion of women aged more than 35 years increased from 33.8% in 2007 to 37.3% in 2017 ($p < .0001$).

Figure 1 shows the trend of twinning rates according to calendar year. A fluctuant trend with a tendency of decreasing of a borderline statistical significance emerged (p value for trend = 0.06).

In the three periods considered (i.e. 2007–2010, 2011–2014, 2015–2017), the SRs adjusted for age of MZ twins were respectively 0.41 (95% CI [0.40, 0.43]), 0.43 (95% CI [0.42, 0.45]) and 0.43 (95% CI [0.42, 0.45]). No significant differences were observed. Otherwise, a slightly decreasing trend was observed in DZ twins' SRs, being in the three periods respectively 0.87 (95% CI [0.84, 0.89]), 0.81 (95% CI [0.79, 0.83]) and 0.78 (95% CI [0.76, 0.80]); see Table 1.

The trends were similar when we calculated the rates according to maternal age and parity (Figure 2). Table 2 provides twinning rates according to zygosity, parity, and maternal age. While the

Table 1. Twinning rates according to zygosity, maternal age and calendar period

| | Dizygotic twins | | | Monozygotic twins | | |
|-----------|------------------|------------------------------|--------------------------|-------------------|------------------------------|--------------------------|
| | No. ^a | Rate per 100 births (CI 95%) | SR ^b (CI 95%) | No. ^a | Rate per 100 births (CI 95%) | SR ^b (CI 95%) |
| 2007–2010 | | | | | | |
| <30 | 388 | 0.57 (0.52, 0.63) | 0.87 (0.84, 0.89) | 287 | 0.42 (0.38, 0.48) | 0.41 (0.40, 0.43) |
| 30–34 | 916 | 0.87 (0.82, 0.93) | | 466 | 0.44 (0.40, 0.49) | |
| 35–39 | 876 | 1.02 (0.96, 1.09) | | 334 | 0.39 (0.35, 0.43) | |
| ≥40 | 158 | 1.49 (1.26, 1.73) | | 28 | 0.26 (0.18, 0.38) | |
| 2011–2014 | | | | | | |
| <30 | 430 | 0.59 (0.53, 0.64) | 0.81 (0.79, 0.83) | 361 | 0.49 (0.44, 0.55) | 0.43 (0.42, 0.45) |
| 30–34 | 796 | 0.80 (0.75, 0.86) | | 454 | 0.46 (0.42, 0.50) | |
| 35–39 | 844 | 0.94 (0.87, 1.00) | | 348 | 0.39 (0.35, 0.43) | |
| ≥40 | 172 | 1.30 (1.11, 1.51) | | 32 | 0.24 (0.17, 0.34) | |
| 2015–2017 | | | | | | |
| <30 | 256 | 0.50 (0.44, 0.57) | 0.78 (0.76, 0.80) | 238 | 0.47 (0.41, 0.53) | 0.43 (0.42, 0.45) |
| 30–34 | 544 | 0.80 (0.73, 0.87) | | 313 | 0.46 (0.41, 0.51) | |
| 35–39 | 568 | 0.94 (0.87, 1.03) | | 238 | 0.40 (0.35, 0.45) | |
| ≥40 | 104 | 1.00 (0.82, 1.21) | | 25 | 0.24 (0.17, 0.34) | |

Note:

^aEstimated number according to Weinberg's method.

^bSR, standardized rate.

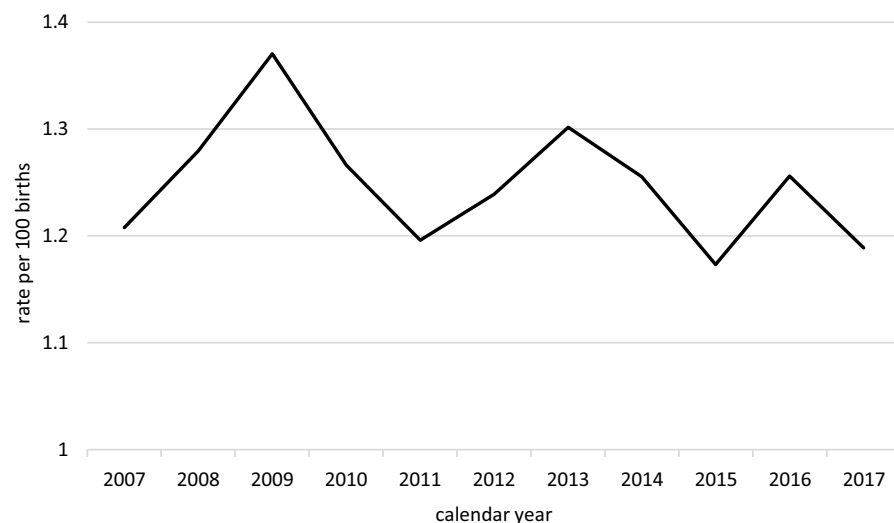


Fig. 1. Trend of twinning rates according to calendar year.

DZ twinning rate was higher among nulliparae women aged 35 years or more (1.28 per 100 births, 95% CI [1.21, 1.35]), MZ twinning rate was higher among younger nulliparae women (0.51 per 100 births, 95% CI [0.48, 0.54]). When we took into account only parity regardless of maternal age, no material differences were found in both DZ and MZ twinning rates.

The DZ:MZ ratio according to calendar year (represented in Figure 3) rose from 1.7 in 2007 to 2.4 in 2008 and then described a fluctuant trend, declining to 1.5 in 2017.

Discussion

This population-based analysis of temporal trends of twin deliveries among natural conceptions suggested that in the

Lombardy Region of Northern Italy, the frequency of spontaneous twins was not steady over the period 2007–2017, describing a fluctuant decline. As the MZ twinning rate was stable in our cohort, this trend was substantially due to a decreasing trend of DZ twins.

Available data from different countries on the trends in natural twinning rates are scanty and partially inconsistent, especially with reference to the last decades.

As MZ twinning generally occurred at a constant rate of 4 per 1000 maternities around the world (Tong et al., 1997), the fluctuation in twinning rate has been generally considered the result of the variation in DZ rates.

Twinning rates vary considerably across time and place. To our knowledge, the longest available historical descriptions regarding the changes in the twinning rates are from the Nordic countries

Table 2. Twinning rates according to zygosity, parity and maternal age

| | Dizygotic twins | | Monozygotic twins | |
|-------------------|------------------|------------------------------|-------------------|------------------------------|
| | No. ^a | Rate per 100 births (CI 95%) | No. ^a | Rate per 100 births (CI 95%) |
| Multiparae | | | | |
| Overall | 2996 | 0.80 (0.78, 0.83) | 1370 | 0.37 (0.35, 0.39) |
| <35 | 1532 | 0.76 (0.73, 0.80) | 784 | 0.40 (0.36, 0.42) |
| ≥35 | 1464 | 0.85 (0.81, 0.90) | 586 | 0.34 (0.32, 0.37) |
| Nulliparae | | | | |
| Overall | 3046 | 0.84 (0.81, 0.87) | 1764 | 0.49 (0.47, 0.51) |
| <35 | 1788 | 0.68 (0.65, 0.71) | 1345 | 0.51 (0.48, 0.54) |
| ≥35 | 1258 | 1.28 (1.21, 1.35) | 419 | 0.43 (0.39, 0.47) |

Note:
^aEstimated number according to Weinberg’s method.

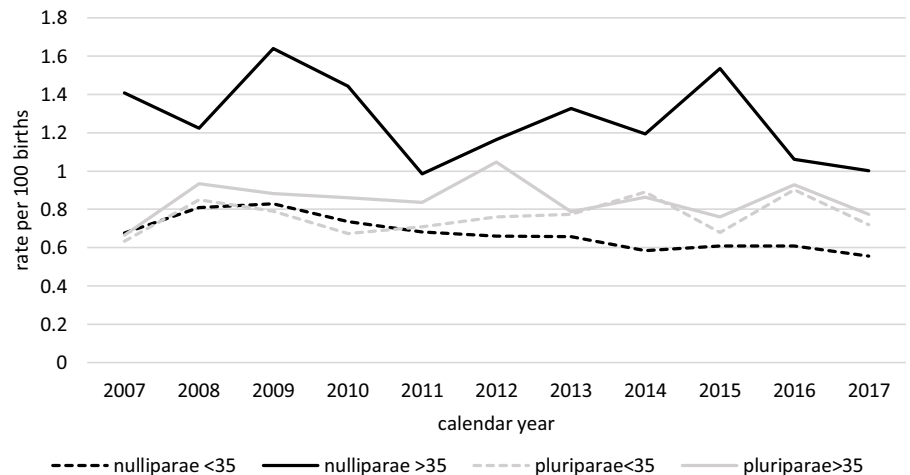


Fig. 2. Trend of dizygotic twinning rates according to maternal age and parity.

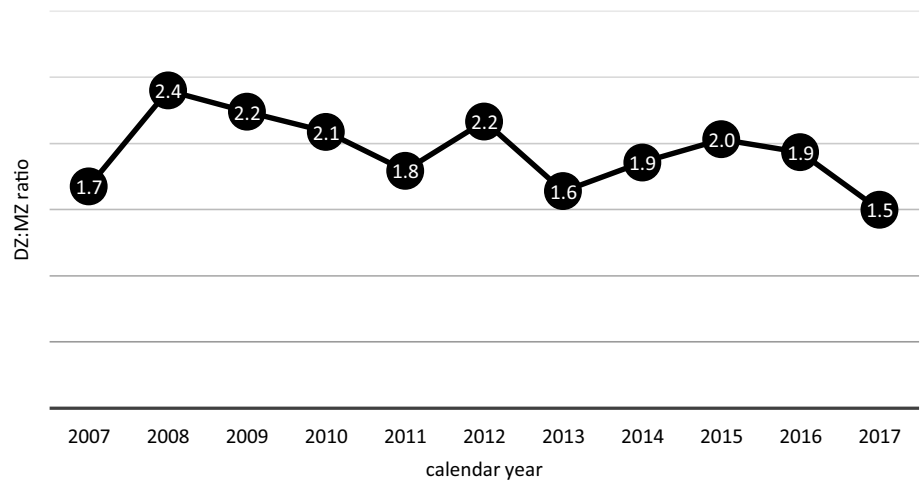


Fig. 3. Dizygotic (DZ):monozygotic (MZ) ratio according to calendar year.

(Eriksson, 1962; Eriksson *et al.*, 1976; Eriksson & Fellman, 1973), starting from the middle of the 18th century in Sweden and from the middle of the 19th century in Finland and Denmark. The Danish twinning rate increased strongly at the end of the 19th century and the beginning of the 20th century, peaking around the 1920s at a rate of 16.6 per 1000 births; then, from the 1930s,

a continuously falling trend was observed until the second half of the 1970s. Similar downward and upward trends were observed in most of the high-income countries, even if twinning rates varied across different areas; for example in the USA, the decline started much earlier, around 1900 (Jeanneret & Macmahon, 1962). In Italy, an extensive analysis (Parisi & Caperna, 1981, 1982) of vital

statistics regarding the period 1868–1979 confirmed that a decline in twinning rate began from the 1920s, and it was reported that in the most industrialized regions, such as Lombardy and Piemonte, the decline started early and had been continuous since 1870. Among high-income countries, the proportion of twin births declined to a minimum in 1970s and 1980s and began to increase again by the second half of 1980s until the first years of the new millennium (data retrieved by published figure) (Hoekstra, Zhao et al., 2008). In Western European countries, twinning rates rose from 9–11 per 1000 births in the 1970s to 15–18 per 1000 births in 2001 (MacFarlane & Blondel, 2005). In the USA, the number of twin births has increased from 18.9 per 1000 births in 1980 to 33.2 per 1000 births in 2009 (Kogan et al., 2000; Martin et al., 2012; Martin et al., 2011). Similar temporal increases in the rate of twinning have been documented in other industrialized countries, such as Australia, Finland, Norway, Sweden, Singapore, and Japan (Imaizumi, 1997).

The international variation in twinning rate was previously synthesized into three groups: low (twinning rate of 2–7 per 1000 births), intermediate (9–20 per 1000 births), and high prevalence (over 20 per 1000 births). The low prevalence group included East Asia, the intermediate prevalence group included the rest of Asia, North Africa, America, Oceania, and Europe, and the high prevalence group included countries from Africa (Little, 1988). Also within the same continent, twinning rates have been reported to be considerably different. In Europe at the beginning of century, the incidence of twinning rates decreased from north to south, being lower in Luxembourg and Portugal and higher in Nordic countries (Astolfi et al., 2003; Hall, 2003).

A wide range of aspects is involved in the variability of twinning rates over the time period. At first, the study of changes in twin maternities should be contextualized in their historical context. Genetic and environmental factors, including urbanization and industrialization, play a role of paramount importance in the fluctuation of twinning rate across countries, and several biological factors (e.g. gonadotrophin levels and sperm count) are related to intra-population variability. In the literature, no exhaustive interpretations of the role of all components and their interactions are available, especially regarding the more recent findings.

Researchers have explained the incremental rate of twinning in the 1980s occurred with the large diffusion of fertility treatments in these years (Fauser et al., 2005; Hoekstra, Zhao et al., 2008; Martin et al., 2005). A study conducted in Italy in 1950–1996 found that the twinning rate in 1996 did not reach the rate of the 1950s before the decline, supporting that the spontaneous twinning had not yet recovered from the dip of the late 1970s and the rise in twin births was due to the spread of ART, at least for older mothers (Astolfi et al., 2003). However, Lambalk et al. (2004) showed that the increase in opposite-sex twins in the period 1995–2002 was mainly caused by natural conception and not by ART. Along this line, Faisel et al. (2008) analyzed the twinning rates in Cardiff and Vale of the Glamorgan area of South Wales during the period 1965 to 2004 and reported a continuous and gradual increase of twinning rate from 10.0 per 1000 spontaneous pregnancies in 1980–1984 to 13.3 per 1000 in 2000–2004 (p value $<.0001$). Another study, conducted in Norway in the same period, reported a constant increasing frequency of twins among natural conception, the rate being around 10 per 1000 births in 1967 and 15 per 1000 in 2001 (Tandberg et al., 2007). No evidence regarding the natural twinning rate was found in later years as more recent studies have focused on the role of ART. One study conducted in North Europe (i.e. Norway,

Denmark and Sweden) observed a decline in the twinning rate from the early 2000s (data estimated by published figures) and suggested that the improved treatment fertility techniques may avoid multiple pregnancies (Fellman, 2016). Instead, in South America from 2000 to 2014, the twinning rate increased from about 8.5 per 1000 pregnancies to 10.2 per 1000 in Brazil (Otta et al., 2016) and 12.9 in Uruguay (Gomez et al., 2019), where probably the access to the fertility procedures had widened in recent years.

Nowadays, the greater urbanization and cultural changes in society may have had an impact on the decline of the twinning rate in the last years. In an Italian study conducted in the 1980s, it was suggested that an urban, sedentary lifestyle could increase the risk of spontaneous abortions among twin pregnancies (Parisi & Caperna, 1981). Along this line, women living in rural areas seemed to have a stronger genetic disposition for twinning and better physical conditions to carry twin pregnancies in comparison to women living in urbanized areas (Eriksson et al., 1995). In addition, the attitude of Italian women, and in general of women of high-income countries, toward family and work has undergone profound changes, such as the achievement of higher qualifications and the desire for a satisfactory job position, leading to new reproductive strategies, including a delayed first childbirth and the reduction of family size.

When searching for possible etiological factors leading to twin births, the association between maternal age and parity has been known for a long time (Bonnelykke, 1990).

In the literature, a positive association between natural DZ twinning and advancing maternal age was found (Abel & Kruger, 2012; Beemsterboer et al., 2006; Bonnelykke, 1990; Bortolus et al., 1999; Derom et al., 2011; Hoekstra, Zhao et al., 2008; Tandberg et al., 2007). A hormonal reason underlying this relation was established: under equal ovarian feedback conditions, older mothers experience hyperstimulation by endogenous follicle-stimulating hormones due to neuroendocrine, hypothalamic or pituitary mechanisms (Lambalk et al., 1998). According to a study that aimed to quantify the contribution of advancing maternal age to the increase in multiple pregnancies, in England and Wales, France and the USA, one fourth to one-third of the increase in twin or triplet pregnancies was attributable to the advanced maternal age (Blondel & Kaminski, 2002). If monitoring the natural DZ twinning rate is a good marker of fecundity, a paradox emerges: the advancing maternal age results in both decreased fecundity and increased DZ twin pregnancies. However, Derom et al. (2011) observed that when adjusted for the nonspontaneous conceiving and the maternal age at birth, the observed increase in the natural DZ twinning rate disappeared and a constant time trend remained, indicating a stable population fecundity regardless of the advancing maternal age. Moreover, according to Beemsterboer et al. (2006), there is a greater probability of poliovalulation in fertile women of advanced age. Our results were consistent with previous evidence: the higher DZ twinning rates were observed among older mothers (aged more than 35 years), and the MZ twinning rate was almost stable across classes of maternal age and dramatically declined in women aged 40 years or more. In addition, despite the proportion of women aged over 35 years that increased from 2007 to 2017, a decreasing trend in DZ twins emerged when the rate was standardized.

According to parity, evidence is dated and controversial (Bonnelykke, 1990). We did not observe significant differences in the distribution of twin pregnancies according to parity. This may suggest that parity is not a direct determinant of twins. However, when we related parity to maternal age, we registered a consistently higher DZ twinning rate among nulliparae women

aged 35 years or more and a higher MZ twinning rate among younger nulliparae women.

Several other factors were associated with the risk of natural (mainly DZ) twin pregnancies, such as pollution (Obi-Osius *et al.*, 2004), smoking (Hoekstra *et al.*, 2010; Parazzini *et al.*, 1996; Smits & Monden, 2011), folic acid supplementation (Kallen, 2004; Muggli & Halliday, 2007), or use of oral contraceptives (Hoekstra, Zhao *et al.*, 2008). The temporal trends in these factors may change over the years and influence the twinning rates.

Also, biological factors should be considered. Spontaneous DZ twinning reflects the presence of the following conditions: a double ovulation and a successful fertilization of two ova (Lazar *et al.*, 1978). A positive association between DZ twinning and shorter waiting time to pregnancy, as a marker of couple fecundity, was found (Basso *et al.*, 2004; Ferrari *et al.*, 2007; Hoekstra, Willemsen *et al.*, 2008; Zhu *et al.*, 2007). Regarding male fecundity, there is evidence that a better semen quality is related to higher spontaneous DZ twinning rate (Asklund *et al.*, 2007). Other authors (Richiardi *et al.*, 2004) reported fewer twins among men who subsequently developed testicular cancer, which is associated with subfertility.

Thus, as the DZ twinning rates among young women have been suggested to be associated with the fertility of a population (James, 1998; Tong, 2000; Tong *et al.*, 1997; Tong & Short, 1998), the decreasing tendency in the number of twins and in the DZ:MZ ratio may reflect the fact that Italy is characterized by very low fertility rates compared to the rest of Europe and the USA. For example, the average number of children of an Italian woman is lower than that of a Swedish, American or French one; in 2017, the respective total fertility rates were 1.32, 1.78, 1.80 and 1.90 (<https://ec.europa.eu/eurostat>; <https://www.istat.it/>; <https://www.statista.com/>).

Some weaknesses of our study warrant consideration. As the information about zygosity was not available, we estimated the number of DZ or MZ twin births using Weinberg's method. This may cause a substantial amount of misclassification of like-sexed DZ twins into the group of MZ twins. Anyway, the validity of the method has been critically scrutinized and has been considered robust and reliable in its estimation of MZ twins (Fellman & Eriksson, 2006).

Among the strengths of this analysis, we have to consider the large sample size and the population-based design. In this perspective, we have included only deliveries of women residing in Lombardy in order to consider a well-defined population.

In conclusion, despite the increasing mean maternal age, a downward trend of twinning among natural conception emerged in Italy during the decade 2007–2017. Monitoring the trends in naturally conceived twin births may be useful to identify trends in fertility across different countries.

Supplementary Material. To view supplementary material for this article, please visit <https://doi.org/10.1017/thg.2022.19>

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Roche, AMGEN and BMS). He also received honoraria as a member of the advisory board to Roche. The other authors declare no potential conflict of interests.

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