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Twinning and Multiple Birth Rates According to Maternal Age in the City of São Paulo, Brazil: 2003–2014

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The present study investigates the twinning rates in the city of São Paulo, Brazil, during the years 2003–2014. The data were drawn from the Brazilian Health Department database of *Sistema de Informações de Nascidos Vivos de São Paulo—SINASC* (Live Births Information System of São Paulo). In general, more information is available on the incidence of twinning in developed countries than in developing ones. A total of 24,589 twin deliveries and 736 multiple deliveries were registered in 140 hospitals of São Paulo out of a total of 2,056,016 deliveries during the studied time period. The overall average rates of singleton, twin, and multiple births per 1,000 maternities (‰) were 987.43, 11.96 (dizygotic (DZ) rate was 7.15 and monozygotic (MZ) 4.42), and 0.36, respectively. We further regressed maternal age and historical time period on percentage of singleton, twin, and multiple birth rates. Our results indicated that maternal age strongly positively predicted twin and multiple birth rates, and negatively predicted singleton birth rates. The historical time period also positively, although weakly, predicted twin birth rates, and had no effect on singleton or multiple birth rates. Further, after applying Weinberg's differential method, we computed regressions separately for the estimated frequencies of DZ and MZ twin rates. DZ twinning was strongly positively predicted by maternal age and, to a smaller degree, by time period, while MZ twinning increased marginally only with higher maternal age. Factors such as increasing body mass index or air pollution can lead to the slight historical increase in DZ twinning rates. Importantly, consistent with previous cross-cultural and historical research, our results support the existence of an age-dependent physiological mechanism that leads to a strong increase in twinning and multiple births, but not singleton births, among mothers of higher age categories. From the ultimate perspective, twinning and multiple births in later age can lead to higher individual reproductive success near the end of the reproductive career of the mother.

■ **Keywords:** twinning rates, maternal age, reproductive fitness, zygosity, monozygotic, dizygotic

Twins and higher order multiple births are high-risk pregnancies for both mothers and infants (e.g., Guo & Grummer-Strawn, 1993), thus calling for increased societal and medical attention. Furthermore, twins are used as a specific investigative tool in biological, medical, and psychological research because they offer a unique opportunity to dissect the proportion of genetic and environmental influences on a diverse array of characteristics, including diseases and behavioral traits (Segal, 2000; 2012). However, the availability of basic data on twins, such as twinning rates, is relatively rare outside of North America and Western Europe (Polderman et al., 2015).

Worldwide changes in twinning rates have been observed during human history (e.g., Fellman & Eriksson, 2004; Madrigal, 2013; Razzaque et al., 1990) and especially over the last several decades (e.g., Pison & D'Addato, 2006). In developed countries (e.g., Denmark, Germany, Switzerland, Sweden, Holland, Italy, England, France, Wales, and United States), twin birth rates increased notably since the 1980s. This can be partly due to the prospective mother's delaying pregnancy and/or seeking assisted reproduction technologies (ART; e.g., Astolfi et al., 2003; Imaizumi, 1992;

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for a review, see Pison et al., 2015). Studies conducted in England and France have shown that the growth in twinning rates was impressive, especially among women older than 35 years; for women of lower maternal age (< 20 years), the increase was less pronounced.

This trend can be also observed cross-culturally, although some exceptions are known. For example, in Taiwan, a negative relationship between maternal age and twinning rates was reported by Chen et al. (1992). Furthermore, in Japan, after the introduction of ART, the dizygotic (DZ) twinning rate rose until 2005, but decreased after that. In contrast, the monozygotic (MZ) twinning rate remained stable before and after the introduction of ART (Imaizumi & Hayakawa, 2013). In the United States, the 2014 twinning rate was 33.9 per 1,000 births (‰),[1] which represented a new high. In contrast, the triplet and higher order multiple birth rate fell by 5% to 113.5 per 100,000 total births (Hamilton et al., 2015).

It is interesting to note that the rate of MZ twinning does not vary extensively around the world, whereas the DZ twinning rate shows considerable variation (e.g., Imaizumi, 1992). The mechanisms for MZ twinning are still being investigated, while more is known about the maternal factors that contribute to the higher frequency of DZ twins: for example, genetic history, advanced maternal age, increased parity, higher body stature, and higher body mass index (> 30; Hoekstra et al., 2008).

Available information on the incidence of twinning in developed countries with good birth registration is considerable. However, data remain scarce for developing countries. Twinning across the developing world was recently examined, based on demographic and health surveys conducted between 1987 and 2010 in 75 low- and middle-income countries (Smits & Monden, 2011), yielding an average of 13.1‰ or one twin birth in 76.3 births. Very low twinning rates are found in Asian and Latin American countries (6–9‰), in marked contrast with the very high twinning rates of African countries (above 18‰). The highest national twinning rate was reported in Benin, Africa (27.9‰), while the lowest DZ twinning rate was reported in India at approximately 7‰ (Satija et al., 2008). Similarly, African-Americans show the highest twinning rates, 13–15‰ (Eriksson et al., 1995; Pollard, 1995), whereas Asian-Americans show the lowest twinning rates, at 7‰, and White Americans show a twinning rate that is intermediate, 10‰ (Pollard, 1995). Previous studies have provided intermediate twinning rates (10–20‰) in Europe and also in the United States (Pison & D'Addato, 2006; Pison et al., 2004; Reynolds et al., 2003).

Only a few studies have investigated the twinning rates in Brazil, showing highly divergent results,[2] that is, between 4.7 and 29.0‰ (Colletto, 2003; Colletto & Rosario, 2004; Colletto et al., 2001; 2003; Geraldo et al., 2008, for details see, Table 1). These studies were conducted in only a few hospitals in the cities of São Paulo and Pelotas (located in southeast and south of Brazil, respectively). Consistent with studies conducted in other world regions, maternal age was one of the significant factors positively influencing twinning rates. Additionally, the investigations showed that twinning rates were positively correlated with the socioeconomic status of the mothers.

Considering that the previous Brazilian studies were limited to only a single or a few hospitals and areas of a city, it is of great importance to extend the number of hospitals in order to collect more representative and less biased data. São Paulo is the largest urban area in South America, and one of the biggest in the world, with living standards that range between moderate and high with large social inequality. Additionally, São Paulo is Brazil's melting pot, in that the population is highly ethnically diverse, with the majority of people being of mixed descent, mainly of European, African, native indigenous, Far and Middle Eastern origin. This population is, thus, relatively unique, and offers a rare opportunity to study a very large and heterogeneous sample of the population.

The main purpose of our study was to analyze the variation of singleton, twin, and higher order multiple birth rates and their relationship with maternal age in São Paulo, using the complete municipal database of individual live births from numerous hospitals. A second objective of the study was to analyze historical trends in birth rates during a time period of 12 years, between the years 2003 and 2014. Furthermore, we investigated the frequency of MZ and DZ twin births, and their association with maternal age and the specified time period. Based on the cited literature, we expected that twin births rates would increase with maternal age, and throughout the historical time interval. Furthermore, we hypothesized that the increase in twin birth rates would occur particularly among DZ twins, while MZ twin rates would show a more stable pattern across mothers' age categories and time period.

Materials and Methods

Data on the singleton, twin, and higher order multiple births (live-born individuals) from mothers residing in one of the 32 districts of São Paulo between years 2003 and 2014, from all 140 hospitals (56 public and 84 private), were extracted from an online database (*Sistema de Informações de Nascidos Vivos de São Paulo*—SINASC: Live Births Information System of São Paulo). SINASC is a Brazilian Health Department database that provides information about hospitals, other health establishments, and home deliveries. This database does not include stillbirths. Since multiple births are at higher risk and lead to higher mortality than non-twin births (e.g., Guo & Grummer-Strawn, 1993), our data might underestimate the rates of multiple births compared to other studies that included stillbirths (e.g., Colleto

[1] The data in this report were based on individual birth certificates.
[2] Despite the fact that all data were based on deliveries.

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TABLE 1

Description of Studies of Twin Delivery Rates Conducted in Brazil

Study	Year	City	Number of hospitals	Twinning rate ‰ (min–max)[a]	Total sample size
Colletto et al. (2001)	1995–1998	São Paulo	1 hospital	17.7–29.0	7,997
Colletto et al. (2003)	1990, 1996, 1999	São Paulo	4 hospitals	8.0–21.3	51,541
Colletto (2003)	1979–1998	São Paulo	1 hospital	6.7–15.4	89,491
Coletto and Rosario (2004)	1978–1999	São Paulo	1 hospital	4.7–14.1	50,122
Geraldo et al. (2008)	1993, 1997, 2003	Pelotas, RS	5 hospitals	7.3–11.1	16,924
Smits and Monden (2011)	1996	–	–	8.8[b]	11,099
The present study	2003–2014	São Paulo	148 hospitals	10.2–13.3	2,056,016

Note: [a]The twinning rate percentage refers to minimum and maximum rates within studied years.
[b]Smits and Monden (2011) report individual twin births, not pairs as the other studies mentioned.

et al., 2003). By this method, we were able to obtain a large number of cases: 2,081,282 individual births of singletons, twins, and higher order multiples. We have not included 264 births with unknown type of gestation into the analyses.

The majority of mothers (81.5%) had 8 or more years of education. The majority of mothers of singletons (55.4%) and twins (47.2%) indicated 8–11 years of education, versus 24.5% of mothers of multiples, who mostly (62.5%) reported 12 or more years of education.

The public database includes data on the number of individuals born, but does not offer information about the sex composition of siblings within twin and multiple births. Thus, the frequency of DZ and MZ pairs could not be estimated based on these data. We then contacted SINASC, and after receiving agreement from the Human Research Ethics Committee at the Institute of Psychology, University of São Paulo (Protocol Number 1,418,827), we received a more detailed database that conveyed not only the total numbers of individual births, but also name of the mother, date and hour of the delivery, place of the delivery, and register number. Based on these new data, we were able to collect information about every single delivery that allowed us to determine the sex composition of twin and multiple births. With this information, we could estimate the frequency of DZ and MZ twin pairs. However, the final twin rates vary slightly from those yielded from the first database.

Statistical Analysis

To compute maternity rates per 1,000 (‰), individual twin births were divided by two, and individual higher order multiple births were divided by three, assuming that the majority of multiple births were triplets. These rates of singleton, twin, and multiple maternities were then entered into the following formula: delivery rate = (1,000 × maternities)/total births. In Table 1, we present a description of single studies of twinning rates conducted in Brazil, including the current study.

The variability in rates across time was measured by nonparametric correlations involving year per 1,000 births separately for singleton, twin, and higher order multiple births. Furthermore, the variable of mothers' age was recoded into eight classes (< 15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44 and > 45 years). Rates on the different types of births were divided according to mothers' age, and correlational analyses were performed separately for each age category.

A stepwise multiple regression analysis model was used to investigate the independent influence of maternal age and time period on twin and multiple births. The rate of twin and multiple deliveries was entered as a dependent variable, and maternal age and year as independent variables.

To estimate the relative frequency of DZ and MZ twin pairs, we applied Weinberg's differential method (e.g., Fellman & Eriksson, 2006) that uses sex distribution among twins, and the probability of being male or female in each population. Following this method, the rate of DZ twinning is twice the rate of twin maternities in which the twins are of opposite sex, while the MZ twinning rate is computed as a difference between the total twinning rate and the DZ twinning rate. The rule has been shown to be a generally robust and reliable indicator of MZ and DZ twin rates, in particular when large birth registry data are considered (Fellman & Eriksson, 2006; Hardin et al., 2009).

Results

We found a positive correlation between the time period (2003–2014) and twinning rate percentage (Spearman's $\rho = 0.972$, $N = 12$, $p < .001$) and a negative correlation for both singleton rates (Spearman's $\rho = -0.944$, $N = 12$, $p < .001$) and higher order multiple rates (Spearman's $\rho = -0.699$, $N = 12$, $p = .011$). Figure 1 displays the temporal trends during the period of 2003–2014 for singleton and twin rates percentage, and higher order multiple deliveries per 10,000 births. The twin birth rate rose 30.8% from 2003 to 2014, increasing from 10.19‰ to 13.33‰ (see Table 2). The average rate of twin deliveries was 11.96‰, while the averages of singleton and multiple births were 987.42‰ and 0.36‰, respectively.

An upward trend over the years was noted in twinning rates according to mothers' age among women of age 25 years or more (see Table 3). An opposite pattern appeared to characterize the singleton mothers.

The final stepwise regression model for twinning rates was highly significant ($F = 80.36$, $df = 2.95$, $p < .001$),

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TABLE 2

Total Number Singleton, Twin, and Higher Order Multiple Births in São Paulo, 2003–2014

		Singletons		Twins		Multiples (triplets)	
Year	Total number of maternities	Total number	Maternities ‰	Total number	Maternities ‰	Total number	Maternities ‰
2003	172,789	170,880	988.95	1,761	10.19	77	0.44
2004	172,776	170,724	988.12	1,876	10.86	78	0.45
2005	169,484	167,433	987.90	1,962	11.58	60	0.35
2006	164,754	162,723	987.68	1,872	11.36	61	0.37
2007	169,493	167,374	987.50	1,971	11.63	56	0.33
2008	171,341	169,213	987.58	2,039	11.90	61	0.36
2009	171,535	169,290	986.91	2,153	12.55	84	0.49
2010	172,082	169,937	987.53	2,076	12.06	58	0.34
2011	174,184	171,956	987.21	2,159	12.39	54	0.31
2012	173,567	171,265	986.74	2,210	12.73	53	0.31
2013	170,669	168,417	986.80	2,202	12.90	36	0.21
2014	173,344	170,964	986.27	2,312	13.33	58	0.34
Total	2,056,016	2,030,176		24,593		736	
Average			987.43		11.96		0.36

Note: Maternities ‰ = rate per 1,000 maternities.

TABLE 3

Non-Parametric Spearman Correlations Between Time Period (Years 2003–2014) and Rate of Births Divided According to Mothers' Age Categories

Mothers' age	< 15	15–19	20–24	25–29	30–34	35–39	40–44	45+
Singletons	0.336	−0.273	0.245	−0.657*	−0.699*	−0.657*	−0.685*	–0.804**
Twins	−0.217	0.399	0.273	0.867**	0.881**	0.692*	0.825**	0.769**
Multiples	–	−0.289	−0.566	–0.790**	–0.748**	−0.364	−0.210	0.401

Note: The coefficients refer to non-parametric Spearman ρ.
*$p < .05$, **$p < .01$.

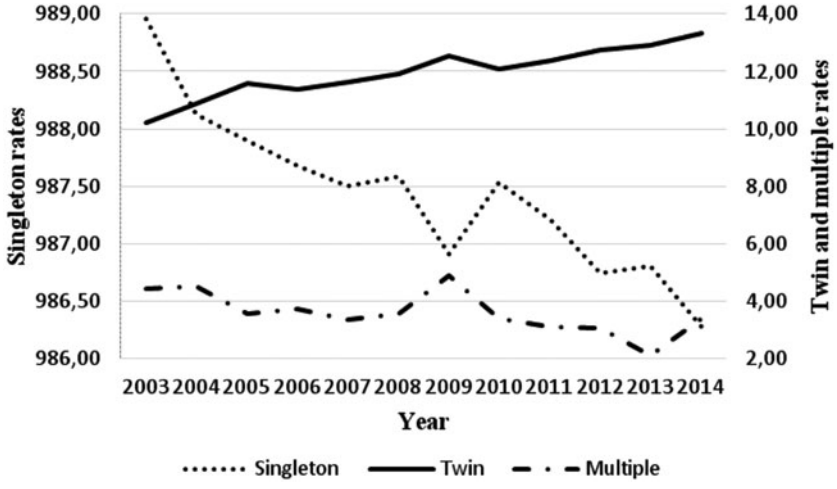

FIGURE 1

Temporal trends of singleton birth rates, twin birth rates, and multiple birth rates per 10,000 births in the city of São Paulo, Brazil.

explaining 63% of the total variation. Mothers' age appeared to be the strongest positive predictor ($B = 4.62$, $SE = 0.37$, $t = 12.49$, $p < .001$), with the time period also positively, albeit weakly, predicting the twinning rates ($B = 0.54$, $SE = 0.25$, $t = 2.19$, $p = .031$). The model for higher order multiple rates was also significant ($F = 31.63$, $df = 1.95$, $p < .001$), explaining 25% of the total variation. Mothers' age appeared to be the only positive predictor of the multiple birth rates ($B = 0.54$, $SE = 0.10$, $t = 5.62$, $p < .001$).

Further, using Weinberg's differential method, we estimated the average frequency of MZ and DZ twin rates to be 4.42‰ and 7.15‰, respectively. Spearman's correlations showed that the time period (2003–2014) positively and significantly correlated with both MZ (Spearman's $\rho = 0.720$, $n = 12$, $p = .008$) and DZ birth rates (Spearman's $\rho = 0.839$, $n = 12$, $p = .001$). Divided according to mothers' age, the mothers' age positively and significantly correlated with both MZ (Spearman's $\rho = 0.857$, $n = 8$, $p =$

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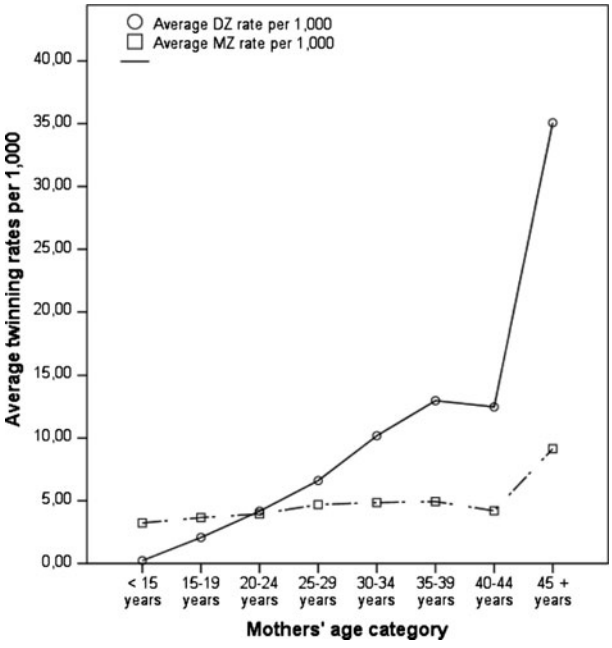

FIGURE 2

Dizygotic (DZ) and monozygotic (MZ) twin rates per 1,000 births as a function of mother's age.

.007) and DZ birth rates (Spearman's $\rho = 0.976$, $n = 8$, $p < .001$).

The stepwise regression model for DZ twinning rates was highly significant ($F = 73.55$, $df = 2.95$, $p < .001$), explaining 61.3% of the total variation. Mothers' age was the strongest positive predictor ($B = 3.88$, SE $= 0.33$, $t = 11.95$, $p < .001$), with the time period also positively, although weakly predicting the twinning rates ($B = 0.45$, $SE = 0.22$, $t = 2.07$, $p = .042$). The model for MZ twinning rates was also significant ($F = 19.40$, $df = 1.95$, $p < .001$), explaining 17.1% of the total variation. Mothers' age was the only positive predictor of the MZ twinning rates ($B = 0.56$, $SE = 0.13$, $t = 4.41$, $p < .001$; see Figure 2).

Discussion

The aim of this study was to investigate the effects of maternal age and time period (2003–2014) on singleton, twin, and higher order multiple birth rates on a complete live births registry from São Paulo, Brazil. The overall average rate of twin births per 1,000 maternities was 11.96 (the DZ rate was 7.15‰ and the MZ rate was 4.42‰), while the average rate of higher order multiple births was 0.36‰. As expected, twin and multiple birth rates increased with maternal age, and DZ twinning marginally increased throughout the historical time period. Singleton birth rates, however, decreased with maternal age, with no effect of the time period.

The overall average twin rate of 11.96‰ is higher than that found in an investigation by Smits and Monden (2011), focusing explicitly on developing countries, including Brazil. In that study, the 1996 twinning rate in Brazil was 8.8‰, a figure placing the country in the same category as Asian countries that are recognized as countries having the lowest twinning rates in the world. The discrepancy between Smits and Monden (2011) and our study may be partly attributed to different sample sizes (11,099 vs. 2,056,016 in the present study), different data collection methods (household interviews conducted as part of the USAID's Demographic and Health Survey vs. the Brazilian Health Department database of Live Births Information System of São Paulo SINASC in the present study), and the period of time sampled (1996 vs. 2003–2012). It should also be underlined that they report individual twin births, not pairs as we did.

Our data make evident that during the 12-year period from 2003 to 2014, the rate of twin births in the city of São Paulo rose by more than 30% from 10.19‰ to 13.33‰. This tendency may be partly linked to the general improvement in life quality and income of the citizens of São Paulo; for example, women with the financial means can seek assisted reproduction, if necessary. An investigation by Colletto et al. (2001) between 1995 and 1998, using only data from a private hospital in São Paulo (attended mostly by higher socio-economic status citizens), and providing very high twinning rates (18–29 twin births per thousand), generally confirms this view. In a recent review, Pison et al. (2015) model and discuss the role of ART in the increasing trend of twinning across a number of developed countries. The authors conclude that ART has had a strong impact on the increase in twinning during the last decades, although the relation is not straightforward and varies between countries. Of similar high importance would be studies that also consider underdeveloped countries or populations without access to ART, where other factors that influence twinning rates can be explored.

Further, results of our regression model clearly showed that the increase in twinning and multiple birth rates are substantially more influenced by mother's age than by the studied time period. In other words, the increased chance for twin maternity in older women is much stronger than, and independent of, the trend towards an increase in the twinning rate throughout the years. This can be due to age, per se, or any other factor correlated with age. For example, older women tend to seek ART more frequently than younger women, and, as a result, the higher twinning and higher order multiple births among older women can be attributed to this intervention (e.g., Reynolds et al., 2003; for a review, see Pison et al., 2015). However, in Brazil, assisted methods of fertilization are still restricted mostly to high social status women who represent a minority of the population (Corrêa & Loyola, 2015).

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Given the foregoing, factors other than assisted methods of fertilization are influencing the increased twinning rates among older mothers, for example, genetic history, increased parity, higher body stature, or higher body mass index (BMI; Hoekstra et al., 2008). Indeed, BMI in São Paulo has been increasing during the last 30 years (e.g., Cintra et al., 2007) and in women BMI is positively related to age and parity (Ferreira & Benicio, 2015). Thus, both BMI and parity can interact and contribute to age-related changes in twinning rates (see Sear et al., 2001). Another factor playing a role in twinning rates might be the air pollution of São Paulo which is known for its high levels of pollutants in the air due to various industries and traffic (Miranda et al., 2012). Lloyd et al. (1988) and Obi-Osius et al. (2004) have found increased twin rates in areas containing higher industrial and incinerator pollution in Scotland and Germany, respectively. Some of the pollutants' compounds have estrogenic properties; interestingly, there has been an increased number of twins among cattle living close to the polluted areas (Lloyd et al., 1988).

Moreover, the same trend in increasing twinning rates as a function of maternal age has been documented in many traditional societies with natural reproduction, such as in Gambia (Sear et al., 2001), agricultural areas of Costa Rica (Madrigal, 2013), 18th-century Sweden (Fellman & Eriksson, 2004), and in 17th- to 18th-century French Canadian immigrants (Nonaka et al., 1995). Therefore, our results, consistent with previous cross-cultural and historical research, support the existence of an age-dependent physiological mechanism that can lead to a strong increase in twinning and multiple births, but not singletons, among mothers of higher age categories. Thus, it seems that at least under some socio-ecological conditions, women near the end of their reproductive period tend to have more twins. Moreover, our data revealed that among older women there is an increased chance of both DZ and MZ twinning, although this effect is less pronounced for MZ twins. This might indicate that increasing multiple ovulations is not the only physiological mechanism by which older women could boost twinning maternities. Therefore, in a stable environment with basic nutrition, healthcare and relatively long life expectation, women near the end of the reproductive life might experience not only multiple ovulations, but also other physiological changes that tend to give rise to multiple gestations.

Thus, it has been suggested that the increase in DZ twinning may reflect more than just an age-related phenomenon. The association between advanced maternal age and increased twinning deserves additional attention from an evolutionary perspective. In diverse populations, such as in Europe, Africa, and America, it was found that despite its costs, twinning can increase mothers' fitness (e.g., Gabler & Voland, 1994; Helle et al., 2004; Robson & Smith, 2011; Sear et al., 2001). Forbes (1997) suggested that DZ twinning may represent an 'adaptive trade-off', such that successful pregnancies in older women may compensate for the increased risk of conceiving children with a genetic defect, such as trisomy 21. Furthermore, it was shown that mothers of twins have relatively higher phenotypic qualities, and can thus 'afford' a riskier but more advantageous reproductive strategy (e.g., Robson & Smith, 2011).

Some limitations of the present study should be noted. The classification of the ethnicity or socio-economic status of the twins was not recorded in the São Paulo database. While this information would have presented a more detailed view of recent twinning trends, the results are consistent with findings from developed countries where advanced maternal age has affected twinning rates. Furthermore, São Paulo differs from other Brazilian regions with its relatively high human development index (HDI), high number of inhabitants, high levels of education, and the largest economy as reflected by its gross domestic product (GDP) in Latin America and southern hemisphere. We, thus, cannot generalize the results of the current study to the entire Brazilian population.

To conclude, in agreement with studies from developed countries, the twinning rate in São Paulo, Brazil, has been increasing during the last few decades. We also showed that higher maternal age is an even stronger predictor of increased twinning than the historical period. We suggest that this age- and historical time-related increase in the twinning rate may be partly caused by proximate factors, such as improved socio-economic status, health and nutrition, increasing weight, parity, air pollution, and the use of assisted reproduction among older mothers. From the ultimate perspective, twinning can lead to higher individual reproductive success near the end of the reproductive period of the mother.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ethics

The study was approved by the Human Research Ethics Committee at the Institute of Psychology, University of São Paulo, Brazil (Protocol Number 1,418,827).

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