



Article

Family History of Twinning and Fertility Traits in Nigerian Mothers of Dizygotic Twins

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Abstract

Familial twinning and fertility traits were investigated in Nigerian mothers of dizygotic (DZ) twins (MoDZT; $N = 972$) and controls ($N = 525$) who responded to our person-to-person interview, which included questions on pregnancy history and family history of DZ twinning. Controls were defined as women who are not twins themselves and do not have twins in their first-degree relatives. Over 95% of the participants were Yoruba. We found that Nigerian MoDZT had an average of 4.0 (± 2.6) pairs of twins among their relatives, and of these, the prevalence of DZ twins was significantly higher than that of monozygotic (MZ) twins (45.9% vs. 25.8%). Controls had an average of 0.5 (± 0.4) pairs, and over 95% of the controls had no twins in their relatives. These results suggest genetic influences on DZ twinning in Nigerians. MoDZT were significantly younger in their mean age at first child, and had higher parity than controls, suggesting increased fertility in MoDZT. As compared to mothers with a single set of twins, mothers ($N = 130$) with multiple sets had significantly more twins among their relatives (5.4 pairs vs. 3.7 pairs) and had their first twins at a younger age (28.4 vs. 30.7 years), indicating that mothers with multiple sets of twins might have higher genetic propensity for twinning associated with earlier age at twin pregnancy. Our findings argue for genomewide association studies for DZ twinning in Nigerians, and may help to develop intervention strategies to overcome infertility/subfertility problems.

Keywords: Dizygotic twinning; genetics; fertility; Nigerians; family history

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Spontaneous dizygotic (DZ) twins come from two separate ova that are released during the same menstrual cycle and fertilized by two sperms (Hall, 2003). The rate of DZ twinning varies considerably worldwide. For example, Africans, especially, Nigerians have rates of more than 20 pairs of spontaneous DZ twins per 1000 births. By contrast, East Asians show only about 2 to 3 pairs per 1000 births (Bulmer, 1970). Maternal age and parity have been shown to be major risk factors for DZ twinning (Bulmer, 1970; Hoekstra et al., 2008). In addition, family history is an important factor in conceiving DZ twins: the risk of conceiving twins for mothers, sisters, and daughters of a woman with DZ twins is about twice as high as the risk in the general population (Bulmer, 1970; Lewis et al., 1996).

While heritability studies of DZ twinning are relatively scarce, Duffy and Martin (2022) investigated seven large multigenerational pedigrees from Europeans and Africans and concluded that heritability of DZ twinning ranged from 8 to 20%. Interestingly, heritability estimates were similar between Europeans and Africans. However, as zygosity of same-sex twins was not available in the study, heritability was likely underestimated as long as the sample included monozygotic (MZ) twins, which is known to be a

largely random event. In a genomewide association study (GWAS) based on mothers of DZ twins in Australia, the Netherlands and United States, Mbarek et al. (2016) identified associations of DZ twinning with genetic variants close to Follicle-Stimulating Hormone Beta Subunit (*FSHB*) on chromosome 11 and SMAD Family Member 3 (*SMAD3*) on chromosome 15, both expressed in the human ovary. These risk alleles increased the frequency of twin births in the Icelandic population by 18% and 9% respectively (Mbarek et al., 2016). A larger GWAS meta-analysis further revealed that gonadotropin-releasing hormone 1 (*GNRH1*) and the follicle stimulating hormone receptor (*FSHR*) genes were associated with DZ twinning (Gordon et al. 2023; Mbarek et al., 2023; van Dongen et al., 2023).

As compared to other women, mothers of DZ twins tend to be taller, have earlier ages at menarche, first child and menopause, and more children (Hoekstra et al., 2008; Tong et al., 1997). As these characteristics are associated with increased fertility, DZ twinning has been suggested to be a marker of high fertility (Tong et al., 1997). Indeed, Mbarek et al. (2016) found that r11031006-G allele near *FSHB* implicated in DZ twinning was also associated with earlier ages at breast development, menarche, menopause, earlier first child, and higher lifetime number of children in a GWAS of European mothers of DZ twins, indicating genetic relationships between DZ twinning and increased fertility. However, one of the important limitations of these studies is that the samples were mostly based on Europeans, and whether the

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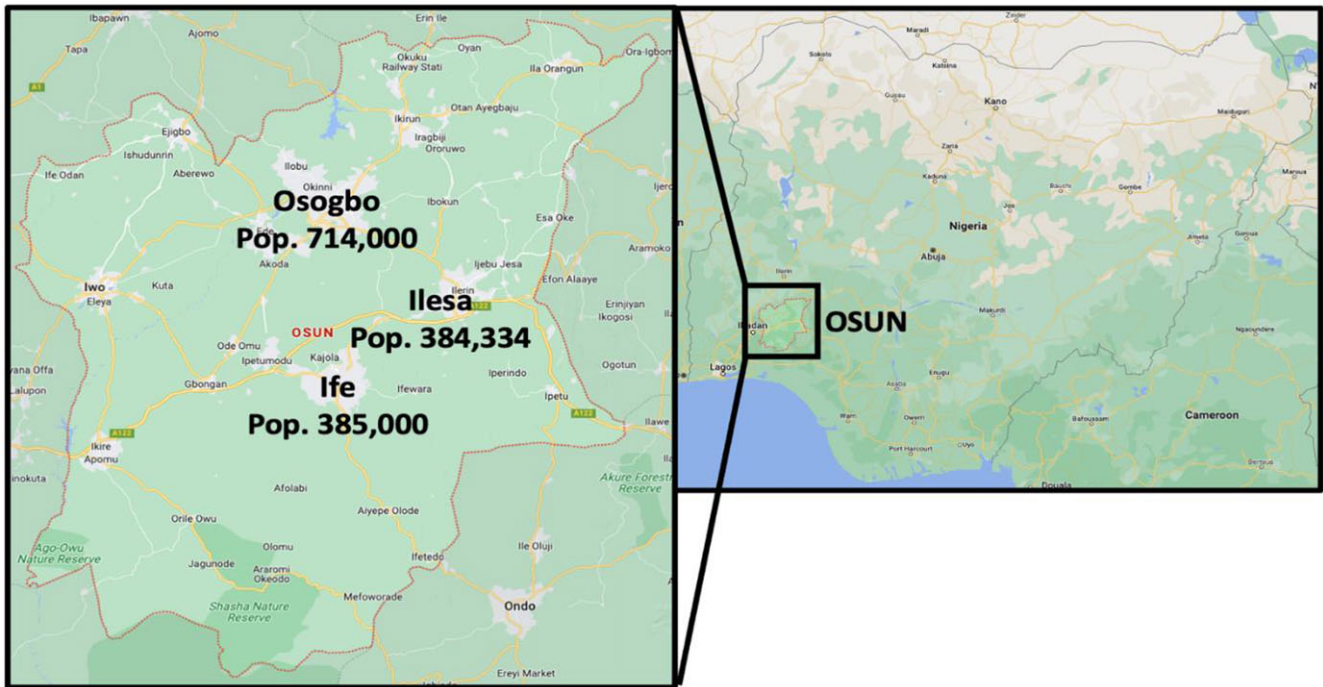


Figure 1. Osun State, Nigeria.

findings can be generalized to other populations needs to be investigated.

The aims of the present study were to examine the family history of DZ twinning and to compare fertility traits between Nigerian mothers of DZ twins and control women who are not twins themselves and do not have twins in their first-degree relatives. Although Nigeria is known to have one of the highest twin birth rates in the world (MacGillivray, 1988), familial DZ twinning and characteristics of mothers of DZ twins in Nigeria remain largely unknown because few studies have been carried out in Nigerian mothers of twins. Nylander (1979) suggested that a high twinning incidence in Nigeria might be attributable to the presence of estrogen-like substance in the food (e.g., yam) that Nigerians eat on a daily basis. Such substance could increase FSH levels in Nigerian women, resulting in increased DZ twinning rates. Nigerian mothers of twins are also unique in that there are many mothers with multiple sets of twins. For this reason, Nigerian mothers of DZ twins provide a valuable opportunity to examine the characteristics of mothers with multiple sets of twins.

Materials and Methods

Procedures

Recruitment procedures. We obtained ethics approvals from the Obafemi Awolowo University Teaching Hospital (OAUTH), and approvals from the Ministry of Education and Universal Basic Education Commission offices to conduct research in schools in Osun state, Nigeria. We set up three major data collection sites: two offices at the OAUTH in Ife and Ilesa, and the assembly hall at the Osogbo Grammar School in Osogbo (see Figure 1). We called and visited schools (mainly at Ile-Ife, Ilesa, and Osogbo) and asked the principals to send mothers of DZ twins attending their schools to come to our data collection sites. We also identified mothers who gave birth to opposite-sex dizygotic (OSDZ) twins from the

maternity section of the OAUTH and invited them to our offices by telephone calls. We also distributed flyers to people in busy markets and invited mothers of OSDZ twins to our offices. Also, mothers with twins who participated in our study told other mothers in their neighborhood about our study and they participated in our study.

To recruit controls, we explained our research to the staff at the OAUTH and schoolteachers, and invited women who met the selection criteria to our data collection sites. The selection criteria were those women who were not twins themselves and had no twins among their first-degree relatives. The control sample mainly consisted of hospital staff, schoolteachers and their acquaintances. Controls were not collected through markets, out of concern for possible cheating.

Questionnaire

Demographic questions. Mothers were asked to report their father's, mother's, and own ethnic background (tribe), birth dates, and the highest level of education they had completed. As the question on the level of education was added later in the study, only 76% of the participants ($n = 1138$) responded to the question.

Pregnancy history and fertility traits. Mothers were asked to report age at menarche, number of children, age at each pregnancy, outcome of each pregnancy, and whether they had ever used fertility treatment. The response categories of the pregnancy outcome were 'male singleton', 'female singleton', 'male same-sex twins', 'female same-sex twins', 'opposite sex twins', and 'triplet or higher'. Mothers were also asked to indicate stillbirth, miscarriage, or abortion, if applicable.

Family history of twinning. Mothers were asked to indicate whether they were twins themselves, had twins in their first-degree (sibling, parent, children), second-degree (grandparent, grandchild, aunt, uncle, nephew, niece), and third-degree (cousin) relatives, and

Table 1. Demographic characteristics of the sample

	Mothers of OSDZ	Mothers of SSDZ	<i>p</i>	Total MoDZT	Controls	<i>p</i>
<i>N</i>	732	240		972	525	
Mean \pm SD age in years	41.9 \pm 10.5	41.2 \pm 9.2	.32	41.8 \pm 10.2	40.3 \pm 10.4	.01
(%) Yoruba	95.9	92.9	.08	95.2	96.4%	.36
(%) Postsecondary education [†]	25.9	24.8	.75	25.5	54.0	<.01
Mothers themselves twins (%)	8.3 (MZ=4.3, DZ=4.1)	7.1 (MZ=2.1, DZ=5.0)	.53	8.0 (MZ=3.7, DZ=4.3)	0.0	<.01

Note: OSDZ, opposite-sex dizygotic; SSDZ, same-sex dizygotic; MoDZT, mothers of dizygotic twins; MZ, monozygotic twins; DZ, dizygotic twins.
[†]Only 76% (*n* = 1138) of the sample answered the question because the question was implemented later in the study.

all other relatives. We asked about relatives on both mother's and father's sides. Mothers were also asked to indicate how many twins they had in each relative category and whether the twins were 'identical', 'non-identical', 'triplet or higher', or 'unknown'.

Data collection procedures. Three steps were taken to collect the questionnaire data and saliva samples from the volunteer women. First, we did a screening interview with mothers with DZ twins and controls. Controls were defined as women who are not twins themselves or do not have twins among their first-degree relatives. In the screening interview, we checked birth certificates, photos of twin children, teacher's letter to certify twin status or twin children that mothers brought to verify their status as mothers of DZ twins. If the twins were opposite sex, they were certainly DZ twins. If they were of same sex, at least two assistants were asked to agree that the twins were sufficiently different in appearance to be DZ (either by photo or in person). For controls, we included questions about family history of twinning and whether they were twins themselves. Because we were not able to verify the eligibility of the control sample, we had to rely on their self-reports. Second, we explained the purpose and the procedures of the study again, obtained informed consent from each subject, and then collected saliva samples from all volunteers. Finally, trained interviewers who can speak both Yoruba and English fluently interviewed mothers with DZ twins and controls about demographic information, pregnancy history, family history of twinning, and reproductive traits. During the interview, interviewers typed responses from the subjects directly into excel spreadsheets in the laptop computers. At the end of the interview, 3000 Naira (about USD6) was given to each subject to compensate for participation.

Results

Sample

In total, 1518 women participated in the present study. Of these, 1497 women consisting of 732 mothers with OSDZ, 240 mothers with same-sex dizygotic (SSDZ) twins, and 525 controls completed the questionnaire interview and provided their saliva samples (Table 1). The remaining 21 women consisting of 15 mothers with OSDZ, 3 mothers with SSDZ twins, and 3 controls only offered saliva samples because they left early without waiting for their turns for interview.

As shown in Table 1, mean (SD) ages of mothers with OSDZ and SSDZ were 41.9 (\pm 10.5) years and 41.2 (\pm 9.2) years, respectively, which were not significantly different. However, the mean age of mothers of all DZ twins was significantly higher than that of controls (41.8 vs. 40.3 years). Over 95% of mothers of all DZ twins and controls were Yoruba. Mothers with OSDZ did not differ

from mothers with SSDZ in percentages of completion of post-secondary education, whereas controls were significantly more educated than mothers of all DZ twins (54.0% vs. 25.5%). This difference was consistent with the finding that Nigerian women in the lowest social class had higher twinning rates compared with those in the highest social class (Nylander, 1979). However, this difference may be due largely to the fact that we recruited controls primarily from hospital staff and schoolteachers, as noted above. Eight percent of mothers of all DZ twins reported that they were twins themselves. While none of SSDZ mothers had triplet or higher order children, 20 OSDZ mothers reported that they had triplet or higher order children. Due to the small number of mothers of triplets or higher order multiples, we only focused on twins in our data analysis.

Frequencies of Twin Pairs Among Children of Mothers with DZ Twins

Among twin mothers, 90.8% of SSDZ mothers and 85.2% of OSDZ mothers had one pair of twin children; 7.9% of SSDZ and 12.6% of OSDZ mothers had two pairs; and the remainder had three or four pairs of twin children. Frequencies of twin pairs between mothers with SSDZ and OSDZ were not significantly different ($\chi^2_{(3)} = 5.06$, $p = .17$).

Frequencies of Twin Pairs in All Relatives

Over 96% of the control group had no twins among their relatives; only 3.1% had one to four pairs of twins in their second- or third-degree relatives. Regardless of zygosity, SSDZ mothers had a mean of 4.0 (\pm 2.8; range = 1 to 15) pairs of twins in all of their relatives, and OSDZ mothers, a mean of 3.9 (\pm 2.6; range = 1 to 18) pairs of twins in all of their relatives. As these two groups were not significantly different from each other, their data were combined, which yielded a mean of 4.0 (\pm 2.6) pairs of twins in all relatives.

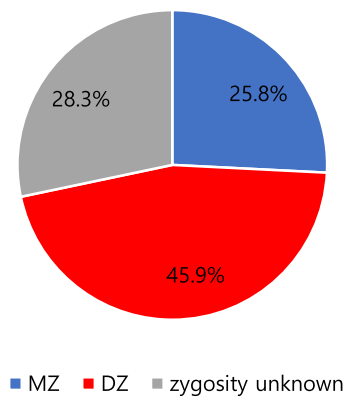
Prevalence of MZ and DZ Twins in All Relatives of Mothers With DZ twins

The prevalence of MZ and DZ twins in all relatives was not significantly different between mothers with SSDZ and OSDZ twins (MZ = 23.7%, DZ = 45.9% in SSDZ; MZ = 22.1%, DZ = 42.9% in OSDZ) and so data from the two groups were combined. Among all relatives of all mothers of DZ twins, the prevalence of MZ and DZ twins was 25.8% and 45.9%, respectively (Figure 2). The remainder of 28.3% was categorized as the 'zygosity unknown' group. There were only 27 sets of triplets reported in all relatives of twins (< 3%). Because the zygosity of these sets was unknown, they were categorized as the 'zygosity unknown' group.

Table 2. Means (SD) for fertility traits between mothers with DZ twins and controls

Fertility traits	Mothers of OSDZ	Mothers of SSDZ	<i>p</i>	Total MoDZT	Controls	<i>p</i>
Age at first pregnancy of twins	30.2 (±6.1)	31.0 (±6.5)	.07	30.4 (±6.2)	—	
Age at first pregnancy	24.3 (±4.6)	24.3 (±4.5)	.96	24.3 (±4.6)	26.0 (±4.1)	<.00
Parity	3.1 (±1.4)	3.3 (±1.3)	.15	3.2 (±1.4)	2.5 (±1.3)	<.00
Age at menarche	15.9 (±2.0)	16.1 (±2.0)	.31	15.9 (±2.0)	15.6 (±2.1)	<.01

Note: OSDZ, opposite-sex dizygotic; SSDZ, same-sex dizygotic; MoDZT, mothers of dizygotic twins.

**Figure 2.** Prevalence (%) of monozygotic (MZ) and dizygotic (DZ) twins in all relatives of mothers with DZ twins.

Comparisons of Fertility Traits Between Mothers With DZ Twins and Controls

Table 2 shows descriptive statistics for age at menarche, age at first pregnancy with twins, age at first pregnancy, and parity among mothers with SSDZ and OSDZ twins and controls. Mothers of OSDZ and SSDZ twins were not significantly different in any of these traits. The mean age at first pregnancy was significantly lower in mothers of all DZ twins than in controls, that is, mothers with DZ twins had their first child at an earlier age than the controls in this study. Mothers with all DZ twins had significantly higher parity than controls. Given that mothers with DZ twins were significantly older and more educated, parity was corrected for age of mothers and educational attainment ($r = .45$ between age of mothers and parity; $r = -.27$ between education and parity). The difference in parity between mothers with all DZ twins and controls was still significant ($p < .001$) even when parity was corrected for age and education. Unexpectedly, the mean age at menarche was slightly but significantly higher in mothers with DZ twins than in controls. Of note is that because there were some unrealistically late menarcheal ages in the responses, we excluded responses with greater than 20 years ($n = 18$). Due to cohort effects on menarcheal age and the large age range of the sample (17 to 85 years), the menarcheal age was significantly positively associated with age ($r = .18$, $p < .01$) in the present sample. Because mothers with DZ twins were significantly older than controls, we adjusted menarcheal age for current age. However, the results were the same. Except for age at menarche, Table 2 suggests

that DZ twinning in Nigerian mothers was highly associated with female fertility traits.

Comparisons of Mothers With Multiple Sets and a Single Set of Twins

Of the total mothers with DZ twins, 13.4% were mothers with more than one set of twin children, irrespective of zygosity. When we compared the mean number of twin pairs in all relatives between mothers with multiple sets and a single set of twins, the mean was significantly higher in the former than in the latter group (5.4 pairs vs. 3.7 pairs, $p < .01$) (Figure 3). However, the mean numbers of MZ or DZ pairs were not significantly different between mothers with multiple sets and a single set. These results suggest that mothers with multiple sets of twin children may have stronger genetic predisposition to twinning, regardless of zygosity, as compared to mothers with a single set of twins.

The mean age at the first pregnancy of twins, the mean age at the first pregnancy, and age at menarche were also compared between mothers with multiple sets and a single set of twins. Mean age at the first pregnancy of twins was significantly lower in mothers with multiple sets than mothers with a single set (28.4 years vs. 30.7 years). The mean age of the first pregnancy was not significantly different between the two groups (23.9 years vs. 24.4 years; Figure 4a, 4b), indicating that mothers with multiple sets of twins start their pregnancy of twins at younger age, as compared with mothers with a single set of twins. The two groups were not significantly different in age at menarche.

Discussion

We compared family history of spontaneous DZ twinning and fertility traits between Nigerian mothers with spontaneous DZ twins and control women who are not twins themselves and do not have twins among their first-degree relatives. Mothers with SSDZ and OSDZ twins in our sample were not significantly different in demographic characteristics, family history of twinning and fertility traits, suggesting that SSDZ twins were correctly assigned as DZ twins. Nigerian mothers with DZ twins had an average of 4.0 (±2.6) pairs of twins among their relatives, regardless of zygosity. Among the twins, the prevalence of DZ twins was significantly higher than that of MZ twins (45.9% vs. 25.8%), suggesting genetic influences on DZ twinning, consistent with those found in Western samples. Genetic influence on MZ twinning is still controversial: Some researchers argue that MZ twinning has a very different etiology than DZ twinning (e.g., Hall, 2003), whereas others found a modest genetic influence on MZ twinning in a European sample (e.g., Parisi et al., 1983). Our Nigerian sample showed substantial numbers of MZ twins in relatives of mothers with DZ twins (25.8%), indicating possible genetic and environmental overlaps between MZ and DZ twinning. Note, however, that our sample included many 'zygosity unknown' twins (28.3%), which limited drawing a strong conclusion.

Mothers of DZ twins had significantly lower mean age at first child, and had higher parity than controls, indicating that mothers of DZ twins may have higher level of fertility than controls. These findings were consistent with the relationship between DZ twinning and female fertility found in Western samples (e.g., Hoekstra et al., 2008; Mbarek et al., 2016). As compared to mothers with a single set of twins, mothers with multiple sets had significantly more twins among their relatives and had their first twins at a younger age, indicating that mothers with multiple sets of twins may have higher genetic loadings for twinning and that greater

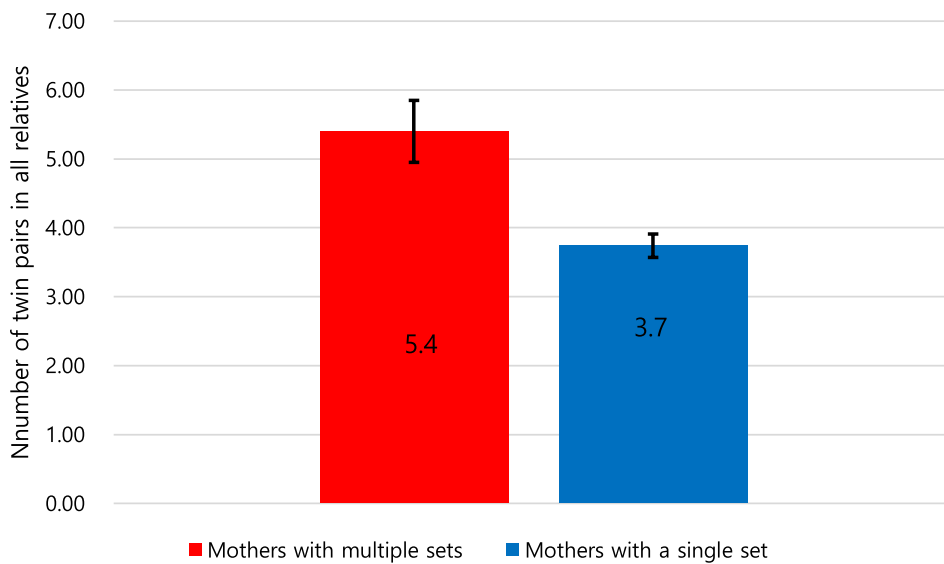


Figure 3. Mean number of twin pairs and their 95% CI in all relatives of mothers with multiple sets and a single set of twins.

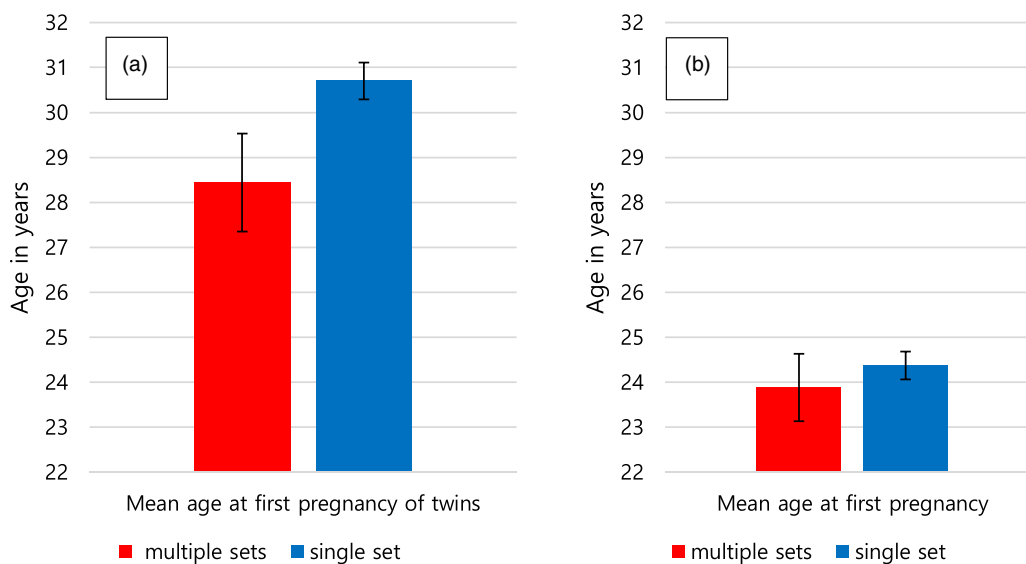


Figure 4. 4a. Mean ages at first twin pregnancy and their 95% CI in mothers with multiple sets and a single set of twins; 4b. Mean ages at first pregnancy and their 95% CI in mothers with multiple sets and a single set of twins.

genetic propensity for twinning may be associated with earlier age at twin pregnancy. These findings were consistent with Nylander's report (1979) that the mean FSH levels were much higher in women with two sets of twins than women with one set of twins in the Western Nigerian population. Interestingly, however, the age of the first pregnancy and the age at menarche were not significantly different between the two groups, indicating that fertility may not be significantly different between the two groups. Taken together, these results suggest that there are genes unique to twinning, although genes for twinning are overlapping with those for female fertility.

There are several limitations in the present study. First, this study used self-report to measure familial twinning in controls as well as in mothers with DZ twins. It is difficult to verify validity of responses in our sample. However, differences in fertility traits between controls and mothers with DZ twins were generally in line with our expectation. It is possible that mothers with DZ twins more likely judged the twins of her relatives as DZ, resulting in greater prevalence of DZ twins in their relatives. To examine this bias, we compared frequencies of DZ and MZ twins in the relatives

between mothers who were DZ twins and those who were MZ twins. Although the small sample size precluded a definitive conclusion ($N = 78$), there were no significant differences in the prevalence of MZ and DZ twins between the two groups. Age at menarche was also self-reported in the present study. Given the age of the sample (range = 17 to 84 years), error of recall may have influenced the results. To examine this bias, we only included mothers under the age of 35 in data analysis. However, the effect size of the difference in menarcheal age between mothers with DZ twins and controls was still the same ($d < 0.20$). To investigate whether our finding is unique to Nigerian women or sampling error, we plan to increase our sample in the future and analyze the data again. We are also collecting the same data from Korean women with twins to increase generalizability of our findings. Second, our controls were not a random sample because they were drawn mainly from hospital staff and schoolteachers and selected to have no twins in their families or first-degree relatives. Mothers with DZ twins were ascertained from school children, markets, hospital staff and maternity records. Although both samples were

based on the population in the Osun State, the differences we found in fertility traits and familial DZ twinning may be partly due to different ascertainment schemes we used. Finally, for fertility traits, we only examined age at first child, age at menarche, and parity. Although these variables are known to be good indices of fertility, future research should investigate other fertility variables such as age at first sexual intercourse, age at menopause, and polycystic ovarian syndrome in mothers of DZ twins, to better understand the relationship between DZ twinning and fertility.

In conclusion, we found that DZ twinning is familial in Nigerians, and that fertility is generally higher in Nigerian mothers with DZ twins compared to controls. These findings were consistent with those found in mothers with DZ twins in European countries. We also showed that mothers with multiple sets of twins had higher genetic propensity for twinning and this propensity was associated with earlier age at pregnancy of twins. This finding awaits replications in mothers with multiple sets of twins in other populations. Our results of familial DZ twinning and increased fertility in Nigerian mothers of DZ twins justify genomewide association studies for DZ twinning in Nigerians, and have implications in improving family planning and reproductive health in Nigerians and developing intervention strategies to overcome infertility/subfertility problems in females.

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