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Article

Maternal Characteristics in Natural and Medically Assisted Reproduction Dizygotic Twin Pregnancies

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Abstract

Previous studies have shown that mothers of naturally conceived dizygotic (DZ) twins tend to be taller, older, and smoke more than mothers of naturally conceived monozygotic (MZ) twin and mothers of singletons. Here, we investigate whether mothers of naturally conceived DZ twins differ from mothers who conceived their DZ twins after medically assisted reproduction (MAR) in eight maternal traits related to fertility based on observational survey data. We include data from 33,648 mothers from the Netherlands Twin Register (NTR) and 1660 mothers of twins from the Norwegian Mother, Father and Child Cohort Study (MoBA). We contrast mothers of naturally conceived DZ twins with mothers of MAR DZ twins. Next, we further segment the MAR group into mothers who underwent hormonal induction of ovulation but not in vitro fertilization (IVF) and those who IVF twins, comparing them both to each other and against the mothers of naturally conceived DZ twins. Mothers of naturally conceived DZ twins smoke more often, differ in body composition, have a higher maternal age and have more offspring before the twins than mothers of MZ twins. Compared to MAR DZ twin mothers, mothers of naturally conceived DZ twins have fewer miscarriages, lower maternal age and increased height, more offspring and are more often smokers. BMI before the twin pregnancy is similar in both natural and MAR DZ twin mothers. Mothers who received hormonal induction of ovulation (OI) have a lower maternal age, fewer miscarriages, and a higher number of offspring before their twin pregnancy than twin mothers who received IVF and/or intracytoplasmic sperm injection (ICSI) treatments. Our study shows that twin mothers are a heterogenous group and the differences between twin mothers should be taken into account in epidemiological and genetic research that includes twins.

Keywords: Twins; Medically assisted reproduction; IVF; ICSI; Maternal characteristics; Twin pregnancy

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Medically assisted reproduction (MAR) techniques have been employed since the 1970s to treat infertility (Fauser et al., 2005; Hall, 2003; van Zonneveld et al., 1999) and include a range of treatments, such as intra-uterine insemination (IUI), hormonal induction of ovulation (OI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). Globally, MAR has become common and has contributed to an increase in the prevalence of twin pregnancies primarily caused by the transfer of multiple embryos during IVF to enhance the likelihood of a successful pregnancy resulting in dizygotic (DZ) twins (Kulkarni et al., 2013; Monden et al., 2021). In the Netherlands, up to 22% of twin pregnancies occurred after MAR in the early 2000s, but in recent years, this percentage has fallen due to changes in MAR protocols, leading to more single embryo transfers (ESHRE et al., 2023).

Non-MAR, or naturally occurring twin pregnancies, have intriguing relations with (in)fertility-related traits (Rickard et al., 2022; Tong et al., 1997; van Dongen et al., 2023). A number of maternal traits and characteristics that are associated with DZ twinning are also linked to infertility, such as increased maternal age, increased BMI and smoking (Beemsterboer et al., 2006; Kinney et al., 2007; Rockhill et al., 2019). However, having DZ twins can also be seen as an index of super fertility as mothers of DZ twins experience spontaneous multiple ovulation and have an increased number of children born before the twin pregnancy (Hall, 2003; Martin et al., 1984). Understanding the similarities and differences between twin mothers who received MAR (from now on referred to as mothers of MAR twins) and mothers of naturally conceived twins may aid in understanding fertility and infertility problems and have implications for improving fertility treatments (Mbarek et al., 2016).

A previous study in the Netherlands by Hoekstra et al. (2010) showed that mothers of naturally conceived DZ twins were taller,

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had a higher BMI, and smoked more often before the twin pregnancy than mothers of naturally conceived MZ twins, consistent with observations in other studies (Campbell et al., 1974; Hoekstra et al., 2010). As far as we are aware no studies compared these maternal characteristics between mothers of naturally conceived DZ twins and MAR DZ twins. A study by Mbarek et al. (2016), reporting on a gene finding study for DZ twinning, found a large effect of including a small number of mothers who conceived after MAR. Genetic associations for being a mother of a DZ twin disappeared when MAR DZ mothers were included in the case sample (Mbarek et al., 2016). This finding points to potentially large differences between mothers who naturally conceive DZ twins and MAR DZ twin mothers, which could be related to the spontaneous double ovulation that sets the mothers of naturally conceived DZ twins apart from the other twin mothers.

Here, we compare mothers of naturally conceived DZ twins with mothers of naturally conceived MZ twins and MAR DZ twins registered with the Netherlands Twin Register (NTR), on eight maternal characteristics. We extend our previous analyses in mothers of naturally conceived DZ and MZ twins (Hoekstra et al., 2010), and for the first time compare these traits in mothers of naturally and MAR conceived DZ twins. We repeated these analyses in a sample of twin mothers who participated in the Norwegian Mother, Father and Child (MoBa) Cohort Study. Whereas the NTR is a volunteer register with registration based on being a multiple or being related to a multiple, MoBa is a population-based register including 41% of all children born in Norway between 1999 and 2009 (Magnus et al., 2016).

At the NTR, we stratified the MAR group into OI and IVF/ICSI conceived pregnancies. Infertile couples are a heterogenous group, with pregnancy challenges resulting from female infertility, male infertility, or a combination of both. MAR mothers who exclusively received OI to induce ovulation are likely to represent a group with female-related infertility, whereas IVF, ICSI and IUI more likely indicate a combination of female and male or exclusively male fertility problems. OI is a hormonal approach that mimics the mechanism of natural DZ twinning as multiple oocytes mature in one menstrual cycle (Boomsma, 2020; Fauser et al., 2005). We thus predict that mothers who underwent OI treatments will exhibit the most noticeable contrast with mothers of naturally conceived DZ twins. To test this hypothesis, we identified the DZ twin mothers who exclusively received OI treatment and compared them to mothers of naturally conceived DZ twins, and to DZ twin mothers who received IVF/ICSI treatments.

Methods

Netherlands Twin Register (NTR)

Participants. Data were obtained from mothers of twins registered with the NTR, which is a nationwide twin-family register established in the late 1980s (Boomsma et al., 1992; Ligthart et al., 2019). We analysed information from multiple NTR surveys including a 2005 survey aimed at all mothers of young and adult twins (Hoekstra et al., 2008); the first survey that mothers of twins receive when joining the NTR (van Beijsterveldt et al., 2013); and the first survey adult twins receive after registration (Willemsen et al., 2013). There were 57,996 mothers, 33,648 of whom could be included in this study. Major reasons for exclusion were missing information on the mode of conception (22,981), unknown zygosity of the twin offspring (378), not being the biological mother (87), being a mother of both MZ and DZ twins (64) or being a mother of MAR MZ twins (838). The research protocols of the study have been reviewed and accepted by the medical ethics review committee of the University Medical Centre of Amsterdam (Numbers 2003/161 and 548 6565).

Mode of conception. MAR was defined as the use of IVF, ICSI, OI and Intrauterine insemination (IUI). The mode of conception of the first twin pregnancy (natural/MAR) was based on the questions: 'How did you get pregnant with your first multiples?', and 'Was the pregnancy conceived spontaneously?'. Multiple replies were possible. A natural pregnancy was determined by the answer 'Spontaneous/Naturally' or 'Yes' and a MAR pregnancy was determined by the answers 'IVF', 'ICSI', 'IUI', 'OI with tablets/hormone injections' or 'No'. MAR was introduced in the Netherlands in the early 1970s, with the introduction of Clomid in the late 1960s (van Zonneveld et al., 1999), thus mothers were classified as non-MAR when the twins were born before 1970. We created three groups: mothers of naturally ($N = 14,684$) and MAR ($N = 8,255$) conceived DZ twins and mothers of naturally conceived MZ twins ($N = 10,699$). We further classified the mothers of MAR DZ twins into two non-overlapping groups: OI ($N = 3326$) based on the answers 'Hormonal induction with tablets' and 'OI with daily hormone injections' and IVF/ICSI ($N = 2808$), based on the answer 'IVF' and/or 'ICSI' in one of the surveys. We shall henceforth refer to the IVF/ICSI group as simply IVF. Mothers were excluded from the secondary analyses if they answered 'IUI' or if the MAR method was unclear or they indicated more than one MAR method.

Traits. The survey data included information on eight traits: maternal age, age at first birth, height, BMI before the twin pregnancy, number of older siblings, primigravida (first pregnancy yes/no), number of miscarriages before the twin pregnancy and smoking before the twin pregnancy (Supplementary Data 1).

MoBa

Participants. MoBa is a Norwegian pregnancy cohort with a trio-design, and includes data from more than 114,000 children, 95,000 mothers and 75,000 fathers (Magnus et al., 2016). The mothers were recruited nationwide between 1999 and 2009 at their birth clinic. Maternal characteristics were obtained from questionnaires or from the Medical Birth Registry of Norway. Zygosity was determined through either genotyping (MZ or DZ twins) or by sex discordancy (DZ twins) when genotyping was not available for both twins. Data were available for 1964 twin pregnancies, and zygosity was available for 1681 pairs. We excluded mothers of MAR MZ twin pregnancies, leading to a total sample of 1660 participants with complete information on six maternal phenotypes: maternal age, height, BMI before pregnancy, number of older siblings, primiparous status (first child yes/no) and smoking before pregnancy (Supplementary Data 2).

Mode of conception. Mode of conception was reported by record linkage to the Medical Birth Registry of Norway, which contains information on IVF/ICSI/OI use, as reported by the gynecologist.

Statistical Methods

We conducted logistic regression analyses with the NTR data, corrected for family clustering with the R4.3.2 geepack package (function geeglm; Supplementary Table 1). We performed separate analyses for each of the eight predictors, as the pattern of

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Table 1. Overview of the five analyses performed in the Netherlands Twin Register data

Analysis	Group 1	Group 2
1	Mothers of naturally conceived DZ twins	Mothers of naturally conceived MZ twins
2	Mothers of naturally conceived DZ twins	Mothers of MAR DZ twins
3	Mothers of naturally conceived DZ twins	Mothers of OI DZ twins
4	Mothers of naturally conceived DZ twins	Mothers of IVF DZ twins
5	Mothers of OI DZ twins	Mothers of IVF DZ twins

Note: DZ, dizygotic; MAR, medically assisted reproduction; IO, hormonal induction of ovulation; IVF, in-vitro fertilization

missingness differed substantially among the eight traits. The analyses were repeated with the MoBa data for six predictors. The data from the two cohorts were analysed separately.

In the NTR, a total of 40 statistical tests (8 traits x 5 analyses) were performed (Table 1), leading to a Bonferroni corrected significance threshold for these analyses of $1.25 \times 10e^{-3}$. For MoBa, a total of 18 (6 traits x 3 analyses) tests were performed, leading to a Bonferroni corrected significance threshold for these analyses of $2.78x10e^{-3}$.

Results

Mothers of Naturally Conceived DZ Versus Naturally Conceived MZ Twins

Within the NTR, mothers of naturally conceived DZ twins are older when conceiving their first child and at their first twin pregnancy compared to mothers of naturally conceived MZ twins (Table 2). No difference was found in the number of miscarriages. Mothers of DZ twins have more children before their twin pregnancies. Mothers of DZ twins are almost 1 cm taller (170.55 vs. 169.63 cm) and have a higher BMI (23.79 vs 23.32). We also observed that mothers of naturally conceived DZ twins smoke more before the twin pregnancy compared to mothers of naturally conceived MZ twins (43.32% vs. 40.43%).

MoBa data replicated the findings of a higher maternal age in mothers of DZ twins (see Table 3, columns 1 and 2). For the other traits, the direction of the effects were consistent with findings from the NTR, but were not significantly different between the two groups, consistent with a much smaller sample size.

Mothers of Naturally Conceived DZ Versus MAR DZ Twins

In the NTR (Table 4, columns 1 and 2), mothers of naturally conceived DZ twins are on average younger when conceiving their first child (28.86 vs. 31.35) and at the conception of their first twin pregnancy (30.5 vs. 32) compared to MAR DZ mothers. Miscarriages are less often reported by the mothers of naturally conceived DZ twins (0.34 events) compared to the mothers of MAR DZ twins (0.45 events). Having more children before the twin pregnancy and smoking before the pregnancy are also associated with being a mother of naturally conceived DZ twins. The mothers of naturally conceived DZ twins are also taller, but do not have a higher BMI.

In MoBa we replicated the finding that mothers of naturally conceived DZ twins are younger (30 vs. 32) at their twin pregnancy (Table 3, columns 1 and 3) and had more children before their twin pregnancy than mothers of DZ MAR twins. We did not observe a significant association with height, BMI or smoking, though the findings point in the same direction as in the NTR.

Mothers of Naturally Conceived DZ Twins Versus Mothers of OI and of IVF DZ Twins

Table 4 summarizes the tests comparing three groups in the NTR: mothers of naturally conceived DZ twins and mothers who received OI and IVF treatments. Compared to mothers of naturally conceived twins, mothers of OI twins are on average older at their first pregnancy, but not at their twin pregnancy (Table 4, columns 1 and 3). They have fewer children before the twin pregnancies and report more miscarriages. The mothers of OI twins are on average shorter (169.88 cm vs. 170.75 cm), but do not differ in BMI. In addition, the mothers of OI twins smoke less often before the twin pregnancy than the mothers of naturally conceived DZ twins. Compared to mothers of naturally conceived DZ twins, mothers of DZ IVF twins are on average older at their first and their twin pregnancy. The DZ IVF mothers have fewer children before the twin pregnancy and more miscarriages (Table 4, columns 1 and 4). Also, the mothers of IVF DZ twins are shorter, have a smaller BMI before the twin pregnancy and smoke less often before the twin pregnancy.

Mothers Who Received OI Versus IVF Treatments

When comparing DZ mothers who received OI and IVF treatments to each other (see Table 5), results showed that OI mothers are on average younger when conceiving their first child and their twin pregnancy compared to the IVF mothers. They are shorter (169.89 vs. 169.98 cm) and have a higher BMI before the twin pregnancy compared to the IVF mothers. Mothers who received OI treatments have more (0.37) children before their twin pregnancy compared to IVF DZ twin mothers (0.22) and smoke less often before the twin pregnancy. The two groups do not differ in the number of miscarriages they reported.

Discussion

We confirmed differences in maternal profiles between mothers of naturally conceived DZ and MZ twins for body size, smoking behavior and total number of children before the twin pregnancy. The results support our hypothesis that the differences in reproductive history and body composition are also present between mothers of naturally conceived DZ twins and mothers who received MAR treatments. MAR is an umbrella term that can represent a wide array of underlying fertility problems. We find that mothers who received OI treatments, representing a group with maternal fertility problems, differ in characteristics such as body composition, smoking, maternal age and reproductive history from other MAR twin mothers who received IVF. Our main analyses compared mothers of naturally conceived DZ twins to mothers of MAR DZ twins. We observed an increased BMI of the mothers of naturally conceived DZ twins when compared to mothers of IVF DZ twins and naturally conceived MZ twins. The body composition features are often attributed to a greater capacity to carry a twin pregnancy to term, but this cannot be the sole explanation as mothers of MZ are also capable to carry a demanding pregnancy to term. The larger body size of the mothers of naturally conceived DZ twins might be associated with different

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Table 2. Results of logistic regression comparing mothers of naturally conceived dizygotic (DZ) and naturally conceived monozygotic (MZ) twins

NTR	DZ Natural		MZ Natural				Total
Trait	***N***	Mean	***N***	Mean	Beta (95% CI)	***p* value**	***N***
Maternal age (years)	14,682	30.58 (4.05)	10,692	29.96 (4.17)	**−0.037 (−0.043, −0.031)**	***p* < 1e-5**	25,374
Age first birth (years)	11,996	28.86 (4.09)	9187	28.40 (4.03)	**−0.028 (−0.035, −0.021)**	***p* < 1e-5**	21,183
Height (cm)	14,071	170.55 (6.49)	10,305	169.63 (6.47)	**−0.022 (−0.026, −0.018)**	***p* < 1e-5**	24,376
BMI	12,122	23.79 (4.14)	8953	23.32 (3.77)	**−0.030 (−0.037, −0.023)**	***p* < 1e-5**	21,075
Miscarriages	9418	0.34 (0.69)	7.775	0.32 (0.66)	−0.048 (−0.094, −0.003)	.033	17,193
Older sibs	9120	0.79 (0.89)	7583	0.67 (0.85)	**−0.157 (−0.194, −0.120)**	***p* < 1e-5**	16,703
Primigravida (% Yes)	11,931	43.32%	9149	40.43%	−0.078 (−0.135, −0.022)	.006	21,080
Smoking (% Yes)*	13,470	24.91%	9756	17.19%	**−0.469 (−0.536, −0.402)**	***p* < 1e-5**	23,496

Note: Results that are significant based on the alpha level of 1.25e-3 are presented in bold. *Smoking before or during the pregnancy.

Table 3. Logistic regression of mothers of naturally conceived dizygotic (DZ) twins compared to mothers of naturally conceived monozygotic (MZ) twins and to mothers that conceived DZ twins though medically assisted reproduction (MAR) within MoBa.

MoBa	DZ natural		MZ natural				DZ MAR				Total
Trait	***N***	Mean	***N***	Mean	Beta (95% CI)	***p* value**	***N***	Mean	Beta (95% CI)	P value	N
Maternal age (years)	881	31.34	383	29.83	**−0.082 (−0.112, −0.052)**	***p* < 1e-5**	396	32.42	**0.067 (0.039 – 0.098)**	**p < 1e-5**	1,660
Height (cm)	881	168.9	383	167.6	**−0.037 (−0.058, −0.016)**	**3.70^{e}−4**	396	168.8	−0.009 (−0.030 – 0.012)	0.380	1,660
BMI	881	24.66	383	23.95	−0.037 (−0.069, −0.005)	.021	396	24.10	−0.030 (−0.060 – 0.001)	0.046	1,660
Older sibs	881	1.90	383	1.73	−0.213 (−0.361, −0.065)	.004	396	1.43	**−0.779 (−0.970 - −0.587)**	**p < 1e-5**	1,660
Primiparous (% Yes)	881	60.27%	383	50.65%	**−0.391 (−0.638, −0.144)**	**1.55^{e}−3**	396	36.11%	**−0.987 (−1.240 - −0.737)**	**p < 1e-5**	1,660
Smoking (% Yes)*	834	77.46%	370	80.27%	0.169 (−0.141, 0.478)	.275	376	76.06%	−0.078 (−0.372 – 0.215)	0.284	1,580

Note: Results that are significant based on the alpha level of $2.78e^{-3}$ are presented in bold. *Smoking ever.

hormone levels. Earlier studies indicate that mothers of naturally conceived DZ twins have higher follicle-stimulating hormone (FSH) levels than controls (Lambalk et al., 1998) and genetic association studies identify genetic variants located near the *FSHB* gene to be associated with natural DZ twinning (Beck et al., 2021; Mbarek et al., 2016).

Smoking cessation and current smoking are associated with dysregulation of the hypothalamic-pituitary-ovarian axis, increased levels of FSH, and decreased fertility (Kinney et al., 2007; Varvarigou et al., 2009). An association of DZ twinning and smoking behavior has been reported before in epidemiological studies (Hoekstra et al., 2010). Based on results of genetic association studies, Mbarek et al. (2024) also reported a positive genetic correlation between spontaneous DZ twinning and smoking. One proposed explanation for this positive correlation is that mothers of DZ twins possess such high fertility that even if they smoke, they still conceive easily, suggesting a survivorship bias in the relationship between smoking and DZ twinning. We indeed observed that mothers conceiving DZ twins naturally smoke more compared to other mothers of twins, while those undergoing ovulation induction (OI) treatments smoke the least among all twin mothers. OI treatments emulate the hormonal effects observed in natural DZ twinning by substituting FSH with artificial alternatives. A potential next step in unravelling the connection between smoking and DZ twinning could involve Mendelian randomization (MR) studies to investigate whether the association between smoking and DZ twinning is causal (Lawlor et al., 2008). A MR study would not, however, directly indicate increased FSH levels, but would give a direction to the relation between smoking and DZ twinning.

Our results further indicate that DZ MAR mothers have a higher maternal age and age at first birth compared to the mothers of naturally conceived DZ twins. The increased maternal age of the MAR mothers could be related to the time it takes to start MAR treatments and the low success rates of infertility treatments, especially IVF and ICSI, leading to multiple attempts before successful implantation (Doody, 2021). Another explanation may be that mothers of MAR DZ twins start trying to have children at an older age, as increased age is associated with lower fertility (Beemsterboer et al., 2006). A final factor potentially contributing to the increased maternal age and age at first birth is the increased number of miscarriages of the MAR mothers compared to the mothers of naturally conceived DZ twins.

The data of the NTR do not include information on the reasons why MAR techniques were necessary, that is, whether maternal, paternal or a combination of the two led to fertility problems. Because the MAR group is likely heterogeneous, we separated the MAR treatments in the NTR sample into OI and IVF (World Health Organization [WHO], 2023). Mothers who receive OI and still fail to get pregnant usually continue their treatments with IVF or ICSI (Fauser et al., 2005). Therefore, we hypothesized that the couples who successfully got pregnant after OI more likely experienced female fertility problems as otherwise further treatments would have been necessary. Interestingly, our results show that the DZ mothers who received OI treatments do not show the same maternal characteristics as mothers of naturally conceived

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Table 4. Logistic regression in the Netherlands Twin Register (NTR): Predicting being the mother of DZ naturally conceived twins, of DZ twins born after hormonal induction of ovulation (IO) or in-vitro fertilization (IVF) within the NTR

NTR	DZ natural		DZ MAR				DZ OI				DZ IVF			
Trait	*N*	Mean	*N*	Mean	Beta (95% CI)	*P* value	*N*	Mean (*SD*)	Beta (95% CI)	*P* value	*N*	Mean (SD)	Beta (95% CI)	*P* value
Maternal age (years)	14,682	30.58 (4.05)	8251	32.08 (3.90)	**0.094 (0.087, 0.101)**	**$p < 1e\text{-}5$**	3325	30.81 (3.79)	0.014 (0.005, 0.023)	.002	2806	32.77 (3.51)	**0.141 (0.131, 0.152)**	**$p < 1e\text{-}5$**
Age first birth (years)	11,996	28.86 (4.09)	7097	31.35 (4.00)	**0.151 (0.143, 0.159)**	**$p < 1e\text{-}5$**	2839	29.97 (3.82)	**0.068 (0.058, 0.078)**	**$p < 1e\text{-}5$**	2328	32.28 (3.62)	**0.215 (0.202, 0.227)**	**$p < 1e\text{-}5$**
Height (cm)	14,071	170.55 (6.49)	7965	170.23 (6.44)	**−0.008 (−0.012, −0.003)**	**3.03e-4**	3216	169.88 (6.28)	**−0.016 (−0.022, −0.010)**	**$p < 1e\text{-}5$**	2711	169.98 (6.54)	**−0.014 (−0.020, −0.007)**	**2.67e-5**
BMI	12,122	23.79 (4.14)	7054	23.90 (4.33)	0.006 (−0.001, 0.013)	.092	2848	24.07 (4.75)	0.015 (0.005, 0.025)	0.002	2327	23.39 (3.86)	**−0.025 (−0.037, −0.013)**	**2.30e-5**
Miscarriages	9418	0.34 (0.69)	5485	0.45 (0.84)	**0.185 (0.141, 0.230)**	**$p < 1e\text{-}5$**	2286	0.42 (0.82)	**0.145 (0.085, 0.205)**	**$p < 1e\text{-}5$**	1361	0.51 (0.91)	**0.263 (0.195, 0.330)**	**$p < 1e\text{-}5$**
Older sibs	9120	0.79 (0.89)	5307	0.32 (0.57)	**−0.910 (−0.968, −0.852)**	**$p < 1e\text{-}5$**	2223	0.37 (0.58)	**−0.779 (−0.853, −0.706)**	**$p < 1e\text{-}5$**	1241	0.22 (0.48)	**−1.269 (−1.340, −1.141)**	**$p < 1e\text{-}5$**
Primigravida (% Yes)	11,931	43.32%	7056	21.43%	**0.990 (0.921, 1.059)**	**$p < 1e\text{-}5$**	2824	25.11%	**−0.784 (−0.878, −0.689)**	**$p < 1e\text{-}5$**	2310	13.64%	**−1.536 (−1.663, −1.410)**	**$p < 1e\text{-}5$**
Smoking (% Yes)*	13,470	24.91%	7610	14.28%	**−0.688 (−0.765, −0.612)**	**$p < 1e\text{-}5$**	3099	13.46%	**−0.758 (−0.870, −0.645)**	**$p < 1e\text{-}5$**	2526	20.55%	**−0.249 (−0.355, −0.143)**	**$p < 1e\text{-}5$**

Note: MAR, medically assisted reproduction. Results that are significant based on the alpha level of $1.25e^{-3}$ are presented in bold. *Smoking before or during the pregnancy.

and IVF DZ twins. For example, the OI mothers appear shorter and smoke less before the twin pregnancy compared to both DZ mothers who naturally conceived and who conceived after IVF. Compared to the mothers who naturally conceived MZ twins, the mothers of naturally conceived DZ twins were older, taller, had a higher BMI, smoked more before the pregnancy and had more children before their twin pregnancy. These results are in line with the earlier study of Hoekstra et al. (2010), which included a subsample of the NTR data. As the NTR is a volunteer twin-family cohort, we repeated our analyses in the population cohort MoBa, in which twins were not targeted but included as part of the general population. We replicated findings concerning maternal age. For the remaining characteristics we found the same directions of effects; for example, DZ mothers being taller and heavier, having more offspring before their twin pregnancy and being more likely to smoke, thereby further confirming the classical risk factors associated with DZ twinning. The fact that statistical tests were not significant may be due to small effects that require large study samples (NTR vs. MoBA >33,000 mothers vs. 1660).

In interpreting the results, we should consider that we mainly rely on information gathered through surveys. The phenotypic information in the NTR was checked for consistency throughout several surveys and by multiple reporters. Previous studies showed that parents are willing and able to provide correct answers concerning the mode of conception of the twin pregnancies and that the NTR participants reported their height and weight correctly (Estourgie-van Burk et al., 2006; van Beijsterveldt et al., 2008). Unfortunately, within the NTR we had to exclude over one third (22,981 of 57,996) of twin mothers, because they did not receive questions about the mode of conception. The survey data also did not indicate the reasons behind the use of MAR. We find evidence of several differences between groups of twin mothers based on mode of conception, even within the MAR subgroup and recommend inclusion of questions about the mode of conception in future twin studies.

Criteria of the MAR and natural pregnancy status were stricter in the NTR then in MoBa. In the NTR, mothers were only included when information was present about the first twin pregnancy and mothers were excluded from the naturally conceived twin groups if an earlier pregnancy was established after the use of MAR techniques. In MoBa this information was not always present, so no selection could be made based on these criteria. Still, the results on MoBa further strengthen our hypotheses as we observe the same directions of the effects.

Conclusion

This study shows that mothers of naturally conceived DZ twins differ from mothers of naturally conceived MZ and MAR DZ mothers in both a Dutch and Norwegian sample. Our results confirm natural DZ and MZ mothers differ in maternal characteristics such as increased maternal age, height, BMI, and smoking behaviors. Compared to MAR DZ twin mothers, our study also finds that mothers of naturally conceived DZ twins have a lower maternal age and fewer miscarriages, but increased height, number of previously born children, and smoking prevalence. The mothers of DZ twins who receive OI treatments do not possess the same 'classical DZ twinning' characteristics. The results may indicate that characteristics associated with being a mother of naturally conceived DZ twin are linked to differences in hormone levels and spontaneous double ovulation. The findings indicate that twin mothers form a heterozygous group and should be

Table 5. Logistic regression of twin mothers for dizygotic (DZ) twins within the Netherlands Twin Register (NTR). The mothers used either hormonal induction of ovulation (OI) or other in-vitro fertilization (IVF) with their DZ twin pregnancy

NTR	DZ OI		DZ IVF				Total
Trait	***N***	Mean	***N***	Mean	Beta (95% CI)	***p* value**	***N***
Maternal age (years)	3325	30.81 (3.79)	2806	**32.77 (3.51)**	**0.145 (0.132, 0.160)**	***p* < 1e-5**	6094
Age first birth (years)	2839	29.97 (3.82)	2328	**32.28 (3.62)**	**0.166 (0.149, 0.182)**	***p* < 1e-5**	5167
Height (cm)	3216	169.88 (6.28)	2711	**169.98 (6.54)**	0.003 (−0.006, 0.011)	.536	5927
BMI	2848	24.07 (4.75)	2327	**23.39 (3.86)**	**−0.036 (−0.049, −0.023)**	***p* < 1e-5**	5175
Miscarriages	2286	0.42 (0.82)	1361	0.51 (0.91)	0.115 (0.036, 0.194)	.004	3647
Older sibs	2223	0.37 (0.58)	1241	**0.22 (0.48)**	**−0.526 (−0.680, −0.374)**	***p* < 1e-5**	3464
Primigravida (% Yes)	2824	25.11%	2310	**13.64%**	**−0.753 (−0.902, −0.604)**	***p* < 1e-5**	5134
Smoking (% Yes)*	3099	13.46%	2526	**20.55%**	**0.509 (0.365, 0.653)**	***p* < 1e-5**	5625

Note: Results that are significant based on the alpha level of $1.25e^{-3}$ are presented in bold. * Smoking before or during the pregnancy.

considered as such in future epidemiological and genetic research involving twin mothers.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/thg.2024.26.

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Data sharing statement. The data of the Netherlands Twin Register (NTR) may be requested through the NTR data access committee (https://tweelingenregister.vu.nl/information_for_researchers/working-with ntr-data). Procedures for access to data from the Norwegian Mother, Father and Child Cohort study can be found at https://www.fhi.no/en/ch/studies/moba/for-forskere-artikler/research-and-data-access/.

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