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Do the Causes of Infertility Play a Direct Role in the Aetiology of Preterm Birth?

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Abstract

Background: It is well established that singletons born of assisted reproductive technology are at higher risk of preterm birth and other adverse outcomes. What remains unclear is whether the increased risk is attributable to the effects of the treatment alone or whether the underlying causes of infertility also play a role. The aim of this study was to examine whether any of the six categories of causes of infertility were associated with a direct effect on preterm birth using causal mediation analysis.

Methods: We assembled a hospital-based cohort of births delivered at a large tertiary care hospital in Montreal, Canada between 2001 and 2007. Causes of infertility were ascertained through a clinical database and medical chart abstraction. We employed marginal structural models (MSM) to estimate the controlled direct effect of each cause of infertility on preterm birth compared with couples without the cause under examination.

Results: The final study cohort comprised 18 598 singleton and twin pregnancies, including 1689 in couples with ascertained infertility. MSM results suggested no significant direct effect for any of the six categories of causes. However, power was limited in smaller subgroup analyses, and a possible direct effect for uterine abnormalities (e.g. fibroids and malformations) could not be ruled out.

Conclusion: In this cohort, most of the increased risk of preterm birth appeared to be explained by maternal characteristics (such as age, body mass index, and education) and by assisted reproduction. If these findings are corroborated, physicians should consider these risks when counselling patients.

Keywords: *infertility, causes of infertility, preterm birth, marginal structural models, causal mediation analysis.*

It remains unclear to what extent the underlying conditions that cause infertility and assisted reproductive technology (ART) each contribute to the increased risk of complications observed in pregnancies conceived through infertility treatment. Beyond the increased risk due to multiple births, assisted reproduction has long been associated with preterm birth and other adverse outcomes among singletons.[1–7] To date, research has primarily focused on the potential side effects of the procedures involved in ART, with less attention devoted to assessing the role of the underlying conditions that lead to infertility.[1–6,8–13] As children conceived naturally by infertile couples also have an increased risk of several adverse outcomes,[1,12] it is plausible that part of the increased risk of treated pregnancies may be due to the causes of infertility.[1,10]

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We examined the risk of preterm birth as a function of six categories of infertility in a hospital-based cohort. We hypothesised that the causal pathway between infertility and preterm birth included both a direct and an indirect effect. We used marginal structural models (MSM) to control for complex confounding structures and estimated the controlled direct effect (CDE) of each cause of infertility that was not mediated through in-vitro fertilisation (IVF) and non-IVF-based assisted reproduction.[14,15]

Methods

Cohort formation

Births among women residing in Montreal, Canada, who delivered at a large tertiary care hospital between April 2001 and September 2007 were eligible. The McGill University Obstetrical and Neonatal Database (MOND) records maternal and neonatal events

for all live neonates and stillbirths >500 g. The database included 24 243 records during the study period. A priori, we applied the following exclusion criteria to mothers: residence outside Metropolitan Montreal, having been referred to the hospital because of high obstetric/neonatal risks, age ≤20 or ≥45 years, pre-existing medical conditions potentially associated with both infertility and preterm birth (human immunodeficiency virus, lupus, rheumatoid arthritis, multiple sclerosis, and breast cancer), and carrying triplets or higher-order multiples (see Supplementary Appendix Figure A1 and Appendix S1). This study was approved by the McGill University Health Centre Research Ethics Board.

Assessment of the causes of infertility (exposure)

Each pregnancy in MOND has a recorded dichotomous infertility variable based on the available history. The MOND medical archivist also records a specific diagnosis for the cause of infertility if the information is available. However, in approximately 30% of the pregnancies identified as infertile, an 'unspecified' cause was recorded because of insufficient information. We addressed this problem by requesting access to the medical charts from the hospital's infertility treatment centre (McGill Reproductive Centre) for women with an unspecified diagnosis. Furthermore, we wanted to assess the validity of relevant data in MOND, and therefore sampled additional charts from women who had at least three visits at the reproductive clinic (MRC) but who had not been identified as infertile within MOND and further sampled charts with causes other than 'unspecified'. In total, we abstracted data from 839/908 requested charts ($n = 1050$ births). For various administrative reasons, 69 charts were not found (see Appendix S2). We implemented a thorough chart abstraction protocol to obtain more detailed information (including ultrasound, biochemistry, hysterosalpingogram, and semen analysis) and identify a cause of infertility.

We used time to pregnancy (TTP) (calculated as number of months reported at initial visit plus number of months needed to conceive while at clinic) to verify the infertility status of each pregnancy. Couples with a total TTP ≥12 months were categorised as infertile. The 'fertile' category comprised: (1) 16 627 births with no indication of infertility in MOND or MRC, (2) 14 births with infertility indicated, but with a total TTP of less than 12 months and no recorded treatment in the MRC or MOND, and (3) 268 births to women who attended the MRC but had no indication of infertility in MOND and whose chart was not sampled.

We classified each of the births to infertile couples in one of the following categories: (1) ovulatory dysfunction: including diagnoses of polycystic ovarian syndrome, premature ovarian failure, anovulation, and other ovulation disorders; (2) endo-tubal: including endometriosis and tubal blockages (partial or complete, bi- or unilateral); (3) male factor: based on two or more consecutive abnormal semen analysis results; (4) uterine abnormalities: including diagnoses of fibroids, polyps, other acquired uterine conditions, or congenital uterine abnormalities (additional laparoscopic data was used for diagnosis); (5) unexplained: diagnosed as a failure to conceive after >12 months, despite regular ovulation, normal semen count, and bilateral patent tubes; and (6) unspecified (assigned in instances when a diagnosis was not identified, either due to insufficient information within a chart, or when the chart was not-requested/obtained). When more than one cause was identified, each was considered separately.

Assessment of preterm birth (outcome)

All births delivered between 20 and 37 weeks were considered preterm. Pregnancies ending before 140 days were considered miscarriages and excluded from the analysis (see Supplementary Appendix, Figure A1). We examined total preterm birth (<37 weeks), moderate (<35 weeks), and very preterm birth (<32 weeks). In all instances, the non-cases included only babies born at term (≥37 weeks of gestation).

Ascertainment of infertility treatment (mediator)

We defined infertility treatment as any type of assisted reproduction, including high-technology (IVF-based procedures), or low-technology (non-IVF-based, such as intra-uterine insemination and/or ovulation induction or stimulation) procedures. We considered both types of treatment a mediator on the pathway between the cause of infertility and preterm birth. Treatment status was determined by estimating the date of conception (calculated by subtracting gestational age from the infant's birth date). Based on this estimated date, pregnancies were considered as having been exposed to treatment if conception

occurred within 90 days of last reported treatment cycle (or last contact, if the couple left the clinic). Pregnancies were considered untreated if conceived within this period with no treatment indicated in the last recorded cycle in either MRC or MOND, or if conceived after this period with no treatment indicated in MOND.

Assessment of covariates

MOND included information on maternal age at birth, parity, education, smoking, and alcohol or substance use during pregnancy, and reported pre-pregnancy weight and height. Maternal education (<12 years; 12–16 years; ≥16 years) and parity (0, 1, 2, or more) were categorised as dummy variables. Body mass index (BMI) was calculated using pre-pregnancy weight and height and categorised (<20, ≥20 to <25, ≥25 to ≤30, and >30). Smoking and alcohol/substance use (yes/no) during pregnancy were self-reported, and we assumed that use also preceded pregnancy.

Data analysis

All statistical analyses were carried out using Stata 11.2 (StataCorp. 2013, College Station, Texas, USA). We estimated unadjusted and adjusted (total effects) risk ratios (RR) using generalised linear models for each definition of preterm birth by the six causes of infertility, compared with the reference category. The comparison group included pregnancies in the 'fertile' category, as well as those without the cause under examination. This was done because the positivity assumption requires that some instances with treatment be present in both the exposed and comparison groups.[14,16] Due to the high proportion (~50%) of missing weight and height combinations, and education (~10%), we used multiple imputation via chained equations procedures to impute missing values for continuous height or weight using linear regression, and generated 10 imputed data sets.[17] Total effects were estimated by adjusting for base covariates and imputed BMI and education using multiple-imputed modelling procedures. Covariates were selected a priori based on being risk factors for both infertility (or infertility treatment) and preterm birth.

Mediation analysis attempts to disentangle direct and indirect effects so as to produce estimates that can be interpreted causally.[18] Conventional regression to adjust for a mediator may produce biased estimates in the presence of interaction or mediator–outcome confounding.[19] Therefore, we applied MSM to control for potential mediator–outcome confounding and for the mediated effects of infertility treatment to estimate the CDE of each cause of infertility on the risk of preterm birth. The CDE is the effect that the cause of interest would have on preterm birth if we hypothetically set the mediator to no treatment.[14,20] We estimated inverse probability weights to control for identified exposure-outcome (causes→preterm birth) and mediator–outcome (treatment→preterm birth) confounders, including those that are affected by exposure, and fitted log-linear weighted MSMs (see Appendix S3 and Figure A2).

We carried out these analyses on all singleton births and then restricted to only first births. We then repeated the analyses, including twin pregnancies. Examining only first births allowed us to study the effect of primary infertility and additionally address the potential effect of clustering of pregnancies by mother.

Sensitivity analyses

We carried out additional pre-specified stratum-specific sensitivity analyses with different reference categories. We restricted to pregnancies conceived without treatment, and then to only those conceived with low-tech or high-tech treatment. These analyses triangulated those from the MSM by determining whether, under a specific treatment scenario, there was an additional risk associated with the cause of interest. We additionally performed the following comparisons restricted to infertile couples: (1) any female factor vs. male factor only, (2) more than one cause of infertility vs. a single cause, (3) TTP as an indicator of severity[21,22] [<12 months, 12–24 months, 24–36 months, >36 months] stratified by treatment (no, low-tech, and high-tech) and adjusted only for maternal age.

Results

After applying the exclusion criteria, the total study cohort comprised 18 598 births (reference, $n = 16\,909$; infertility-exposed, $n = 1689$). Characteristics of study participants and frequency of preterm birth by cause of infertility compared with the reference cohort among singletons are shown in Table 1 (see

Table 1. Maternal characteristics and study outcomes, according to reference and causes of infertility – all singleton pregnancies

		Infertile groups $n = 1435$					
Characteristics	Reference[a] $n = 16\,712$	Unexplained 348 (24.2)	Ovulatory 346 (24.1)	Endo-tubal 269 (18.7)	Male factor 299 (20.8)	Uterine 204 (14.2)	Unspecified 169 (11.8)
Age mean (SD)	31.58 (4.6)	35.02 (4.5)	32.60 (4.4)	35.06 (4.1)	34.94 (3.9)	35.09 (3.9)	34.1 (4.9)
Parity							
0	7754 (46.4)	205 (58.9)	214 (61.8)	158 (58.7)	191 (63.9)	126 (61.8)	121 (71.6)
1	6252 (37.4)	110 (31.6)	102 (29.5)	96 (35.7)	96 (32.1)	65 (31.8)	35 (20.7)
≥2	2706 (16.19)	33 (9.48)	30 (8.7)	15 (5.6)	12 (4.0)	13 (6.4)	13 (7.69)
Education (years)[b]							
<12	1794 (10.7)	23 (6.6)	52 (15.0)	27 (10.0)	28 (9.4)	17 (8.3)	14 (8.3)
12 to <16	5160 (30.9)	114 (32.8)	112 (32.4)	84 (31.2)	94 (31.4)	66 (32.3)	38 (22.5)
≥16	7949 (47.6)	174 (50.0)	147 (42.5)	140 (52.0)	149 (49.8)	102 (50.0)	97 (57.4)
BMI[c]							
<20	990 (5.9)	54 (15.5)	30 (8.7)	28 (10.4)	37 (12.4)	29 (14.2)	8 (4.7)
20 to <25	3130 (18.7)	169 (48.6)	112 (32.4)	125 (46.5)	111 (37.1)	84 (41.2)	42 (24.8)
25 to <30	1445 (8.6)	53 (15.2)	66 (19.1)	48 (17.8)	61 (20.4)	45 (22.1)	22 (13.0)
30+	793 (4.7)	22 (6.3)	70 (20.2)	18 (6.7)	47 (15.7)	31 (15.2)	15 (8.9)
Missing	10 354 (62.0)	50 (14.4)	68 (19.6)	50 (18.6)	43 (14.4)	15 (7.3)	82 (48.5)
Smoking[d]	956 (5.7)	42 (12.1)	30(8.7)	30 (11.1)	24 (8.0)	28 (13.7)	6 (3.5)
Substance use	384 (2.3)	5 (1.4)	4 (1.2)	6 (2.2)	4 (1.3)	1 (0.5)	3 (1.8)
TTP[e]		33 (23–47)	36 (25–58)	44 (31–66)	38 (25–58)	34 (25–58)	29 (21–33)
Treatment							
Untreated		175 (50.3)	135 (39.0)	139 (51.7)	114 (38.1)	128 (62.7)	35 (20.7)
Low tech		79 (22.7)	150 (43.3)	37 (13.7)	46 (15.4)	32 (15.7)	67 (39.6)
High tech		94 (27.0)	61 (17.6)	93 (34.6)	139 (46.5)	44 (21.6)	67 (39.6)
Preterm birth (weeks)							
<37	1202 (7.2)	30 (8.6)	27 (7.8)	28 (10.4)	36 (12.0)	32 (15.7)	18 (10.6)
<35	395 (2.5)	9 (2.8)	11 (3.3)	6 (2.4)	13 (4.7)	13 (7.0)	8 (5.0)
<32	161 (1.0)	6 (1.8)	8 (2.4)	4 (1.6)	7 (2.6)	5 (2.8)	4 (2.6)
Spontaneous	713 (59.3)	16 (53.3)	17 (63.0)	21 (75.0)	15 (41.7)	25 (78.1)	13 (72.2)

[a]Total singletons: $n = 18\,147$. Reference (includes only those in the 'fertile' group): $n = 16\,712$. Infertile groups: $n = 1435$. Unless otherwise stated, values are presented as *n* (%). Diagnosis categories are not mutually exclusive.
[b]Education: ≥16 years was the reference category.
[c]Body mass index (BMI): 20 to <25 was the reference category.
[d]Smoking and substance were self-reported.
[e]TTP = time to pregnancy (median 25–75%) was reported only for women seen at MRC. We present total TTP, which includes reported number of months trying to conceive before seeking treatment plus time until conception.
SD, standard deviation.

Tables A1–A4). Ovulatory dysfunction, unexplained infertility, and male factor were the most commonly diagnosed causes of infertility among couples with singleton births. The frequency of preterm birth (<37 weeks) was lower in the reference category than among the infertile group as a whole (7.2% vs. 10%, respectively). Differences in the frequency of preterm birth were observed by cause of infertility, with male factor, and uterine categories having the highest incidence (Table 1).

Results for the unadjusted, total, and CDE by cause of infertility on preterm birth among all singletons are reported in Table 2. Male factor infertility, uterine abnormalities, and unspecified categories were associated with an increased unadjusted risk of preterm birth at all three cut-points of gestational age (Model 1). Total effects (Model 2) were attenuated for all causes after adjustment for age, parity, smoking, alcohol/substance use, and BMI and education using multiple imputation. The point estimates remained elevated despite adjustment, particularly for male factor, uterine abnormalities, and unspecified causes (Table 2). Results from Model 3 for the CDE (the effect that the cause of interest would have on preterm birth

Table 2. Risk ratios for preterm birth by cause of infertility: unadjusted, total effect, and controlled direct effect – all singletons ($n = 18\,147$)

		All singleton births[a]		
Causes of infertility	*n*[e]	Model 1[b] Unadjusted effect RR [95% CI]	Model 2[c] Total effect RR [95% CI]	Model 3[d] Direct effects RR [95% CI]
Unexplained (weeks)	348			
<37	30	1.16 [0.82, 1.65]	1.07 [0.76, 1.52]	1.02 [0.44, 2.40]
<35	9	1.06 [0.55, 2.04]	0.95 [0.50, 1.82]	0.63 [0.24, 1.66]
<32	6	1.67 [0.74, 3.74]	1.47 [0.65, 3.33]	0.59 [0.14, 2.48]
Ovulatory dysfunction (weeks)	346			
<37	27	1.05 [0.73, 1.52]	0.97 [0.67, 1.40]	0.70 [0.40, 1.25]
<35	11	1.29 [0.72, 2.33]	1.09 [0.60, 1.97]	0.80 [0.36, 1.78]
<32	8	2.23 [1.11, 4.49]	1.79 [0.88, 3.64]	1.26 [0.48, 3.31]
Endo-tubal (weeks)	269			
<37	28	1.41 [1.00, 2.01]	1.31 [0.91, 1.87]	1.11 [0.51, 2.42]
<35	6	0.94 [0.42, 2.07]	0.83 [0.37, 1.84]	1.25 [0.26, 5.97]
<32	4	1.46 [0.55, 3.90]	1.26 [0.47, 3.40]	2.57 [0.42, 15.70]
Male factor (weeks)	299			
<37	36	1.64 [1.20, 2.23]	1.52 [1.11, 2.07]	0.87 [0.52, 1.47]
<35	13	1.84 [1.07, 3.16]	1.54 [0.98, 2.65]	1.15 [0.54, 2.46]
<32	7	2.36 [1.12, 4.96]	1.86 [0.87, 3.98]	1.09 [0.34, 3.46]
Uterine (weeks)	204			
<37	32	2.14 [1.55, 2.95]	1.94[1.40, 2.70]	1.44 [0.66, 3.15]
<35	13	2.76 [1.62, 4.70]	2.27 [1.32, 3.89]	2.43 [0.85, 6.93]
<32	5	2.55 [1.06, 6.13]	1.98 [0.82, 4.83]	1.10 [0.22, 5.62]
Unspecified (weeks)	169			
<37	18	1.44 [0.93, 2.23]	1.38 [0.90, 2.14]	1.22 [0.61, 2.44]
<35	8	1.95 [1.00, 3.87]	1.70 [0.87, 3.30]	1.80 [0.70, 4.62]
<32	4	2.32 [0.87, 6.18]	1.93 [0.72, 5.20]	0.86 [0.17, 4.38]

[a]All risk ratios (RR) estimate the effect of preterm birth for the specified cause of infertility as compared with births from the reference group ($n = 16\,712$) plus those among the infertile ($n = 1435$) without the specified cause of interest.
[b]Model 1: unadjusted model had no covariates.
[c]Model 2: total effect: multiple imputation model adjusted for maternal age, parity (except in male factor), smoking, substance use, and imputed BMI and education.
[d]Model 3: controlled direct effect: marginal structural model – stabilised inverse probability weights were used to adjust for exposure–outcome and mediator–outcome confounding.
[e]*n* represents total number of cohort participants per cause of infertility and number of cases of preterm birth at the three clinical cut-points within each cause of infertility.

when the mediator is hypothetically set to no treatment) showed potential associations for the unspecified category and uterine abnormalities at the higher gestational end points (uterine <35 weeks: 2.43 [95% CI 0.85, 6.93]) and, possibly, an increasing trend as the definition of preterm birth became more strict for endo-tubal factors. There was no clear trend for a direct effect among the other causes. Confidence intervals (CIs) were, however, wide.

Table 3 reports results restricted to singleton first births. Higher unadjusted RR (Model 1) of preterm birth were observed overall for male factor, ovulatory, uterine, unspecified, and unexplained categories, with uterine abnormalities showing significant associations at all three end points. Overall, total effects (Model 2) were reduced for all causes compared with the unadjusted results, but remained significant for uterine abnormalities at <37 weeks (1.83 [95% CI 1.20, 2.78) and <35 weeks (3.13 [95% CI 1.76, 5.58]), and for ovulatory dysfunction at <32 weeks (2.62 [95% CI 1.22, 5.67]). Estimates of CDE (Model 3) showed similar trends for uterine abnormalities and endo-tubal factors as those observed in Table 2 for all singletons, with less clear evidence of direct effects

Table 3. Risk ratios for preterm birth by cause of infertility: unadjusted, total effect, and controlled direct effect – first births ($n = 8651$)

		Singleton first births[a]		
Causes of infertility	***n***[e]	Model 1[b] Unadjusted effect RR [95% CI]	Model 2[c] Total effect RR [95% CI]	Model 3[d] Direct effect RR [95% CI]
Unexplained (weeks)	205			
<37	17	1.16 [0.73, 1.84]	1.00 [0.63, 1.60]	0.59 [0.26, 1.31]
<35	7	1.39 [0.66, 2.91]	1.25 [0.60, 2.64]	0.63 [0.19, 2.12]
<32	5	2.39 [0.98, 5.82]	2.10 [0.84, 5.23]	0.47 [0.06, 3.67]
Ovulatory dysfunction (weeks)	214			
<37	20	1.31 [0.86, 2.00]	1.20 [0.78, 1.84]	0.88 [0.48, 1.61]
<35	9	1.73 [0.91, 3.32]	1.46 [0.75, 2.82]	0.90 [0.37, 2.17]
<32	7	3.28 [1.54, 7.01]	2.62 [1.22, 5.67]	1.45 [0.51, 4.13]
Endo-tubal (weeks)	158			
<37	16	1.42 [0.89, 2.28]	1.20 [0.75, 1.94]	1.60 [0.63, 4.05]
<35	4	1.05 [0.40, 2.70]	0.92 [0.35, 2.46]	1.36 [0.25, 7.38]
<32	2	1.24 [0.31, 5.00]	1.07 [0.26, 4.35]	2.33 [0.35, 15.43]
Male factor (weeks)	191			
<37	21	1.55 [1.03, 2.34]	1.34 [0.88, 2.04]	0.75 [0.39, 1.44]
<35	9	1.97 [1.03, 3.77]	1.67 [0.86, 3.24]	0.76 [0.28, 2.06]
<32	4	2.10 [0.78, 5.66]	1.61 [0.58, 4.45]	0.63 [0.10, 3.97]
Uterine (weeks)	126			
<37	20	2.25 [1.49, 3.40]	1.83[1.20, 2.78]	1.58 [0.68, 3.67]
<35	12	4.07 [2.34, 7.08]	3.13 [1.76, 5.58]	2.66 [0.93, 7.60]
<32	4	3.35 [1.25, 8.96]	2.42 [0.88, 6.73]	1.05 [0.15, 7.56]
Unspecified (weeks)	121			
<37	11	1.27 [0.72, 2.24]	1.13 [0.64, 2.00]	0.95 [0.40, 2.29]
<35	6	2.01 [0.91, 4.43]	1.72 [0.77, 3.83]	1.43 [0.48, 4.28]
<32	4	3.22 [1.21, 8.65]	2.64 [0.97, 7.20]	0.80 [0.15, 4.35]

[a]All risk ratios (RR) estimate the effect of preterm birth for the specified cause of infertility as compared with births from the reference group ($n = 7754$) plus those among the infertile ($n = 897$) without the specified cause of interest.
[b]Model 1: unadjusted model had no covariates.
[c]Model 2: total effect: multiple imputation model (MI) adjusted for maternal age, parity (except in male factor), smoking, substance use, and imputed BMI and education.
[d]Model 3: controlled direct effect: marginal structural model – stabilised inverse probability weights were used to adjust for exposure–outcome and mediator–outcome confounding.
[e]*n* represents total number of cohort participants per cause of infertility and number of cases of preterm birth at the three clinical cut-points within each cause of infertility.

among the remaining causes restricting to singleton first births.

Results for unadjusted, total, and direct effects for the entire study cohort (singletons and twins) are reported in Table A2 (all births) and A3 (all first births) of the Supplementary Appendix. Relative to the unadjusted estimates, where all six causes showed association at all three preterm endpoints, total effects (Model 2) were substantially attenuated. However, associations persisted across most causes after multivariable adjustment for covariates. Most of these observed total effects are likely due to the presence of twins, as we observed reduced CDE within the total sample (Model 3). The direct effect associations remained among the unspecified category for both all births and all first births (Tables A2 and A3, Model 3).

Sensitivity analyses

When restricting the analysis to pregnancies conceived without treatment, only uterine abnormalities were associated with an increased risk of preterm birth. The remaining causes of infertility showed little association, with several point estimates below 1 (Table 4). When using births where male factor infertility was the only diagnosed cause as the reference

Table 4. Risk ratios of preterm birth according to cause of infertility among untreated infertile pregnancies only – total sample and singletons

	Total Sample		Singletons	
Causes of Infertility[a]	**All Births N**[b] **= 17,536** RR (95%CI)	**First Births N = 8,157** RR (95%CI)	**All Singletons N = 17,332** RR (95%CI)	**First Births N = 8,079** RR (95%CI)
Unexplained	n[c] = 177	n = 85	n = 175	n = 85
<37 weeks	0.63 (0.32–1.23)	0.44 (0.13–1.45)	0.66 (0.33–1.35)	0.46 (0.14–1.52)
<35 weeks	0.53 (0.11–2.47)	0.30 (0.04–2.36)	0.63 (0.13–3.06)	0.29 (0.36–2.36)
<32 weeks	0.46 (0.05–4.18)	n/a[d]	0.46 (0.05–4.18)	n/a
Ovulatory	n = 135	n = 69	n = 135	n = 69
<37 weeks	0.15 (0.04–0.63)	0.48 (0.11–2.05)	0.18 (0.04–0.74)	0.51 (0.12–2.17)
<35 weeks	0.39 (0.05–3.08)	0.72 (0.09–5.58)	0.42 (0.10–3.36)	0.72 (0.10–5.58)
<32 weeks	0.94 (0.11–8.07)	1.39 (0.16–12.14)	0.94 (0.11–8.00)	0.87 (0.09–8.01)
Endo-Tubal	n = 140	n = 67	n = 139	n = 67
<37 weeks	1.28 (0.70–2.32)	1.10 (0.45–2.68)	1.32 (0.71–2.48)	1.15 (0.46–2.84)
<35 weeks	0.26 (0.03–2.03)	0.38 (0.05–2.90)	0.26 (0.03–2.08)	0.38 (0.05–2.89)
<32 weeks	0.61 (0.07–5.14)	0.74 (0.09–6.4)	0.61 (0.10–5.14)	0.74 (0.08–6.41)
Male factor	n = 115	n = 64	n = 114	n = 64
<37 weeks	1.28 (0.68–2.42)	1.34 (0.53–3.42)	1.43 (0.75–2.75)	1.41 (0.55–3.62)
<35 weeks	1.61 (0.44–5.96)	3.11 (0.85–11.31)	2.05 (0.53–7.92)	3.11 (0.86–11.27)
<32 weeks	0.85 (0.10–7.5)	1.02 (0.10–10.6)	0.85 (0.10–7.51)	1.02 (0.09–10.62)
Uterine	n = 130	n = 68	n = 128	n = 68
<37 weeks	2.63 (1.55–4.49)	2.76 (1.26–6.04)	2.92 (1.67–5.11)	3.15 (1.41–7.04)
<35 weeks	4.84 (1.49–15.7)	7.10 (1.72–29.3)	6.05 (1.70–21.50)	7.10 (1.72–29.23)
<32 weeks	3.91 (0.79–19.4)	6.70 (1.06–42.4)	3.93 (0.80–19.50)	6.70 (1.06–42.40)
Unspecified	n = 37	n = 26	n = 35	n = 25
<37 weeks	3.11 (1.36–7.10)	0.91 (0.15–5.55)	2.04 (0.66–6.33)	n/a
<35 weeks	3.58 (0.65–19.8)	n/a	1.55 (0.15–16.61)	n/a
<32 weeks	n/a	n/a	n/a	n/a

[a]Risk ratios are for the comparisons among the untreated infertile pregnancies only, and between those with and without the cause of interest. Models used multiple imputed data and adjusted for age, education, BMI, smoking, substance use, and parity (except for in male factor).

[b]N represents total number of participants in sample; reference group = N-n.

[c]n represents total number of cohort participants by cause of infertility.

[d]n/a : no results generated due to small sample.

category, it did not appear that female causes as a whole were associated with an increased risk of preterm birth at any of the gestational age cut-points (Table 5). When comparing multiple female causes to a single cause, associations were observed among all singletons and in the total study cohort at <37 weeks (Table 5 and Table A4, respectively). Finally, when examining the association between TTP (as a measure of severity of infertility) and preterm birth, we did not observe any association with preterm birth within any strata of treatment (data not shown).

Comment

In this study, we saw limited evidence of a direct effect of the diagnosed cause of infertility on the risk of preterm birth, contrary to our expectation. Total effects were observed for uterine abnormalities, male factor, and ovulatory dysfunction among singletons. After accounting for the effect of infertility treatment and of mediator–outcome confounders (including those affected by the cause), we found some evidence of a CDE pathway for uterine abnormalities and, possibly

Table 5. Risk ratios of preterm birth: internal comparisons using infertile groups as the reference – singletons

	All singletons			First births		
	Model 1[b] Unadjusted effect		Model 2[c] Total effect	Model 1 Unadjusted effect		Model 2 Total effect
Comparisons[a]	***n***[d]	RR [95% CI]	RR [95% CI]	***n***	RR [95% CI]	RR [95% CI]
Any female vs. male factor (weeks)[e]						
<37	62	0.71 [0.46, 1.08]	0.65 [0.40, 1.07]	41	0.96 [0.54, 1.71]	1.06 [0.52, 2.15]
<35	22	0.73 [0.34, 1.56]	0.55 [0.23, 1.33]	18	1.17 [0.44, 3.08]	1.05 [0.30, 3.66]
<32	13	0.65 [0.25, 1.67]	0.53 [0.17, 1.65]	10	1.09 [0.30, 3.89]	1.48 [0.2, 11.11]
Multiple causes vs. one cause (weeks)[f]						
<37	16	2.03 [1.22, 3.39]	2.02 [1.20, 3.40]	8	1.55 [0.77, 3.12]	1.43 [0.70, 2.94]
<35	4	1.39 [0.49, 3.91]	1.26 [0.44, 3.63]	3	1.28 [0.40, 4.17]	1.03 [0.30, 3.51]
<32	3	2.07 [0.59, 7.26]	2.17 [0.60, 7.93]	2	1.79 [0.40, 8.06]	1.84 [0.40, 8.57]

[a]Risk ratios estimate the effect of preterm birth among subgroups of infertile only, using generalised linear models with a binomial link.
[b]Models 1: unadjusted crude model had no covariates.
[c]Models 2: total effects: multiple imputation model (MI) to adjust for maternal age, parity, smoking, substance use, and imputed BMI and education.
[d]*n* represents total number of cohort participants per cause of infertility (in bold) and number of cases of preterm birth at the three clinical cut-points within each cause of infertility.
[e]Any female factor included any of the following: uterine abnormalities, ovulatory dysfunction, or endo-tubal factors (all singletons $n = 619$; first births $n = 380$) compared with male factor only (reference category: all singletons, $n = 191$; first births, $n = 125$).
[f]Multiple causes included women with more than one of the female causes (uterine abnormalities, ovulatory dysfunction, or endo-tubal factors) (all singletons, $n = 91$; first births, $n = 51$) compared with women with only one cause (reference category: all singletons, $n = 636$; first births, $n = 395$).

endo-tubal factors. In the total study cohort, we observed elevated total effects for all six causes, largely due to the high proportion of twins in treated pregnancies. However, only the subgroup with unspecified causes showed evidence of a direct effect relative to those without the cause of interest, after using MSMs to account for the mediating effect of infertility treatment. These findings suggest that most of the observed risk in our cohort could be explained by maternal characteristics and by the effect of assisted reproduction, rather than by the underlying conditions causing infertility – at least as operationalised in this study.

The application of MSM allowed for an estimate of the CDE of the cause of infertility that is neither confounded by covariates nor mediated by assisted reproductive treatment (and thus by twinning in case of the total cohort). Although associations did not reach statistical significance, a direct effect for some of the causes could not be completely ruled out. In particular, there was evidence of a direct effect of uterine abnormalities in singleton first births and in the total cohort at <35 weeks (2.66 [95% CI 0.93, 7.60]) and (2.50 [95% CI 0.92, 6.80]), respectively. However, CIs were wide for most categories, thus preventing us from ruling out a possible effect.

When we examined the impact of causes only among pregnancies conceived without assisted reproduction, the results were consistent with those from the MSMs: only uterine abnormalities appeared to increase the risk of preterm birth in both the total study cohort and among singletons, across all three gestational cut-points (Table 4). Results from the sensitivity analysis also suggested no independent association of TTP on preterm birth, when stratified by treatment.

To the best of our knowledge, no prior study has examined the underlying causes of infertility and their independent association on the risk of preterm birth by using clinical data and applying causal inference methods to separate the effect of treatment from that of the diagnosed cause. The literature in this field, although extensive, has been mostly limited to examining the risk associated with a long TTP (or with infertility treatment). However, several studies still attempted to disentangle the impact of the infertility from that of ART.[3,8,23,24] Despite these attempts, many

of these studies have been criticised for not using methods that distinguish the effect of treatment from that of the underlying causes of infertility.[25,26]

Thomson *et al.* examined a hospital-based cohort and, although information on the cause of infertility was obtained, the authors concluded that there were no statistically significant differences in obstetric and neonatal outcomes by type of infertility. However, the data were not shown.[9] The study by Wang *et al*. reported an increased odds of preterm birth for any female factor infertility compared with male factor; however, such a finding was reported only among twins and did not take into account the use of ART.[27] More recently, Nelson and Lawlor used an IVF treatment registry to examine livebirth outcomes and examined, among other predictors of risk, the reasons for treatment. They observed an increased odds of preterm birth (<37 weeks) for cervical causes; however, this condition is not a well-defined cause of infertility, and the sample was restricted to IVF cycles.[28] A large registry-based study by Kawwass *et al*. examined pregnancies conceived through IVF in the United States between 2000 and 2010.[29] The authors reported a higher risk of preterm birth among pregnancies to women diagnosed with tubal factor infertility compared with pregnancies where the only identified cause was a male factor. Although several important confounders were adjusted for in the multivariable analysis, BMI, socio-economic status, and other variables were not included, and residual confounding may explain part of the observation. A study by Gibbons *et al*. examined different modes of IVF as well as causes of infertility to assess varying egg, sperm, and uterine conditions on pregnancy risk. They concluded that the uterine environment, including uterine causes, had a significant influence over obstetrical outcomes compared with egg or sperm quality.[30]

One of the strengths of our study was that a large proportion of the diagnosed causes of infertility were abstracted from medical charts (1050/1435 singleton pregnancies). We also included clinical diagnoses from women with infertility that conceived without the aid of treatment, and linked the clinical information from the charts to a large hospital-based database. Both sources provided rich data to not only adjust for basic factors such as age and parity, but also for other important covariates (BMI, education, smoking, alcohol/substance use). Such adjustment helped reduce residual confounding, thereby strengthening our inferences. We used causal mediation methods to assess the direct effect of each cause of infertility with the risk of preterm birth at different cut-points. We furthermore carried out several sensitivity analyses in order to control for underlying treatment conditions.

It was not feasible for us to examine every chart from the MRC, which likely resulted in some differences in accuracy between the diagnoses based on charts and those based only on MOND. Nevertheless, it is highly unlikely that misclassification of the cause was differential by outcome, as MOND is based on information recorded in the obstetric chart, and charts from the MRC were abstracted blindly to the outcome. There was a high degree of consistency (84–100%) between the MOND and study-assigned causes (see Supplementary Appendix, Table A5). We also verified the diagnosis of a sample of charts by a second rater and estimated an agreement of 89%. Thus, although our main exposure likely included a degree of error, such error would likely be non-differential. A degree of misclassification, however, may have been possible within our treatment groups, as we set an a priori period of 90 days from which treatment status would have been maintained. As such, rebound spontaneous pregnancies (conceived immediately following a failed treatment cycle) would have been classified as treated, with the majority (almost 95%) conceiving within that month. This approach had the advantage, however, of reducing the number of treated women being misclassified as untreated.

Furthermore, although our study question necessitated the use of causal mediation analysis, this method had a trade-off: in order to meet the positivity assumption, the comparison group had to include subjects that were exposed to treatment. As a result, the comparison group included infertile subjects without the cause being examined. Doing so resulted in a slightly higher baseline risk of preterm birth in the reference category, likely producing minor bias toward the null. When we ran the regression analyses without the other causes in the reference group, we indeed found some increases in the point estimates, but conclusions were materially unchanged.

Due to limited sample size, we could not fully examine spontaneous and induced preterm birth separately. However, induction is likely a consequence of infertility and infertility treatment. When we explored this aspect, however, only couples with

unexplained infertility or male factor had a higher proportion of induced preterm birth than the reference category (Table 1, Table A1), suggesting that our findings were likely not strongly affected by differences in induction between groups. Some pregnancies ending in a singleton birth may have started as multiples, which could have resulted in a higher risk among singletons than may be estimated in a setting where single embryo transfer is the rule. We performed several comparisons, thus potentially resulting in some estimates that would be significant by chance. Lastly, this study was carried out in a large tertiary care hospital, and there is the possibility that the baseline risk of preterm birth may have been higher than in the underlying population, which would again have resulted in a dilution of effect. However, the initial 10.3% preterm birth rate among all singletons was reduced to 7.2% after removing referred pregnancies and those from non-Montreal residents, an estimate close to that reported for Montreal in 2009.[31]

We used several approaches to determine whether underlying conditions that lead to infertility were driving part of the risk of preterm birth, as this notion has been proposed in the literature for some time.[1,8–10,12,13,28,32] Our study found that uterine abnormalities, which included fibroids, adhesions, and malformations, were the only causes potentially directly associated with a higher risk of preterm birth. This is somewhat consistent with findings from Gibbons *et al*.,[30] who showed that the uterine environment had an important effect on obstetric outcomes in ART births. The differences in risk observed between prior studies that looked at TTP and ours, which examined causes and found very little evidence of a direct effect, may be explained by two salient differences. Unlike previous work, our study was able to fully adjust for several important factors, such as BMI, smoking, alcohol, and substance use, which resulted in less residual confounding. We also believe that prior studies may have under-reported or misclassified non-IVF-based technologies, such as ovulation induction, whereas our study captured all forms of treatment. It is also possible that other mechanisms or causal entities such as underlying undiagnosed maternal disease (e.g. hypothyroidism or Crohn's disease) are responsible for the increased risk of adverse pregnancy outcome in couples taking a long time to conceive.[33,34] However, we were not able to capture such instances in our study.

The mechanisms underlying the increased risk associated with IVF and non-IVF-based assisted reproduction remain unclear. In a recent study, we found that both low-technology (non-IVF-based) and high-technology (IVF-based) treatments were associated with a similar increased risk of preterm birth at the three cut-points of gestational age.[35] Beyond the potential risk of laboratory procedures in ART (e.g. culture medium/time, fresh/frozen embryos),[36] there is evidence linking ovarian stimulation with oxidative stress and suboptimal endometrial development, conditions that may be implicated in poor implantation and placentation.[37–40] More research is needed to understand what specific aspects of various technologies are associated with risk, with human studies focusing on the consequences of treatment on the endocrine system and uterine environment. A larger cohort, with detailed information on both the underlying causes of infertility, as well as specific types of treatment, incorporating novel mediation methods, may result in a better understanding of infertility, its causes, and the impact of treatment.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure A1. Total study cohort – participant flow chart.
Figure A2. Hypothesized Directed Acyclic Graph (DAG).
Table A1. Maternal characteristics and study outcomes, according to reference and causes of infertility – all births.
Table A2. Risk ratios for preterm birth by cause of infertility: unadjusted, total effect, and controlled direct effect – all births.
Table A3. Risk ratios for preterm birth by cause of infertility: unadjusted, total effect, and controlled direct effect – all first births.
Table A4. Risk ratios of preterm birth: internal comparisons using the infertile groups as the reference.
Table A5. Consistency between MOND assigned causes and causes as determined through chart abstraction.
Appendix S1. Cohort formation and study participants.
Appendix S2. Selection of medical records for primary data abstraction.
Appendix S3. Marginal structural models.