Original Research

Risk of Adverse Pregnancy Outcome After Paternal Exposure to Methotrexate Within 90 Days Before Pregnancy

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OBJECTIVE: To study the association between paternal exposure to methotrexate within the 90-day period before pregnancy and congenital malformations and stillbirth in the offspring.

METHODS: We conducted a nationwide register study. Our cohort consisted of all live births in Denmark between 1997 and 2011 identified from the Medical Birth Registry. Methotrexate-exposed fathers were identified from the National Prescription Registry. From the national Hospital Registry we identified paternity, live births, and stillbirths as well as discharge diagnoses on congenital malformations.

RESULTS: We identified 849,676 live births with known paternity. There were 127 live births of methotrexate-exposed fathers. Of these, four (3.2%) had major malformations compared with 28,814 (3.4%) of the unexposed. The odds ratio (OR) for major congenital malformation among exposed fathers compared with unexposed was 0.93 (95% confidence interval [CI] 0.34–2.51) and when adjusted for year of birth, maternal age, educational length, household income, and parity, the adjusted OR was 1.01 (95% CI 0.37–2.74). There were no stillbirths in the methotrexate-exposed group compared with 2,541 (0.3%) in the unexposed group and no increased risk of preterm birth (adjusted OR 1.31, 95% CI 0.66–2.59) among the children from exposed fathers.

CONCLUSION: We found no association between paternal exposure to methotrexate within 90 days before pregnancy and congenital malformations, stillbirths, or preterm birth. Available data suggest that prepregnancy paternal methotrexate exposure should not be of major concern. Multinational recommendations should be changed accordingly.

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Methotrexate is used as first-choice treatment for rheumatoid arthritis.[1] Methotrexate is furthermore used to treat several malignant diseases, inflammatory bowel disease, psoriasis as well as to terminate extrauterine pregnancies.[2–6] Multinational recommendation is to discontinue methotrexate at least 3 months before planned pregnancy for both men and women. Women should additionally not use methotrexate during pregnancy and breastfeeding.[1]

Methotrexate is a folic acid antagonist that works by inhibiting the dihydrofolate dehydrogenase and thus blocking the reduction of dihydrofolate to tetrahydrofolate. By blocking this enzyme, methotrexate leads to the depletion of cofactors required for synthesis of DNA and RNA and consequently an inhibitory effect on protein synthesis and cell proliferation.[6] Methotrexate-induced cell damage during spermatogenesis has raised concerns about the effect on pregnancy outcome such as congenital malformations despite the lack of evidence.[7]

Several studies have found a decrease in the number of functional sperm cells[8] after paternal

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methotrexate exposure. Only a few observational studies have discussed the issue of a possible teratogenic effect of men using low-dose methotrexate, usually 7.5–25 mg weekly, in the 90 days before pregnancy.[4,5,9–12] The multinational recommendations emphasize the lack of evidence concerning consequences of paternal peripregnancy methotrexate use. Larger studies are thus needed to ascertain a possible association between paternal prepregnancy exposure to methotrexate and congenital malformations.[1,12] The primary objective of this epidemiologic observational study was to study the association between paternal exposure to methotrexate in the 90 days before pregnancy and congenital malformations in the offspring.

MATERIALS AND METHODS

We conducted a nationwide register study. To investigate the risk of major congenital malformations, we constructed a cohort, including all live births from 1997 to 2011 allowing 1 year of follow-up to identify diagnosis of malformations within the first year of life. To investigate the risk of stillbirths, we constructed another cohort, including both live and stillbirths from 1997 to 2011. All records of live births and stillbirths were identified from the Medical Birth Registry.[13] Only those birth records with information on the identity of the father were included.

Information on paternal methotrexate exposure was obtained from the National Prescription Registry.[14] The National Prescription Registry contains data on all prescription drugs dispensed in all pharmacies in Denmark since 1995. Pharmacies are required by law to register all redeemed prescriptions to receive reimbursement from the Danish government. This ensures highly accurate prescription data and completeness has previously been estimated to be 97.5%.[15] The registry has no information on use of over-the-counter drugs on an individual level or drugs dispensed in hospitals. In the study period, 72% of the methotrexate use was dispensed in pharmacies and the users thereby identifiable.[16] If hospital use could bias the analysis, it would have only minor influence on the result.

The National Hospital Registry contains information on all hospitalizations in Denmark, including admittance data and discharge diagnoses.[17] It holds more than 99% of all discharge records from Danish hospitals since 1977.[18] From this register we identified all registered diagnoses on congenital malformations according to the European Surveillance of Congenital Malformations.[19]

The Medical Birth Registry holds individual-level data on all births in Denmark, including information on the mother, offspring, and assumed father. From this register we identified a unique identification number on the mother, father, and offspring; the maternal age; previous births; and gestational age of the offspring at birth. Time of conception is based on ultrasonic estimates or information of the last menstruation.[13] Since 1978 births in Denmark are registered in the Medical Birth Registry with a completeness of more than 99.5%.[20] Information on education was from the Populations Education Register.[21] A standardized educational length in months on the highest completed education was used to classify educational level. Information on income was from the Income Statistics Registry, which holds information on different income groupings, taxes, and public transfer payments.[22]

All records are registered with a unique and personal identification number given to all citizens at birth or migration. This allowed us to link records from different registries on an individual level.[23]

Exposure was defined as paternal dispensing of a prescription of methotrexate for systemic use (Anatomical Therapeutic Chemical Classification L01BA01 or L04AX03) within 90 days before pregnancy.

The primary outcome was any major congenital malformations diagnosed within the first year after birth according to the European Surveillance of Congenital Malformations classification system.[19] The secondary outcomes were subgroupings of major congenital malformations, minor malformations as well as risk of stillbirth after paternal methotrexate exposure. To avoid compromising the anonymity of the participants, the Danish registries restrict presenting subgroupings to those with three or more cases. To investigate whether prepregnancy paternal methotrexate use was associated with minor congenital malformations, we analyzed the odds of the combined endpoint of both major and minor malformations. All primary and secondary diagnoses of congenital malformations were included. The diagnoses of congenital malformations have previously been validated and completeness has been found to have a predictive value of 88% with a completeness of 90%. If there are any misclassifications, they are most probably random.[24] The other secondary outcome, stillbirth, was defined as a birth with no signs of life. In Denmark, the definition of stillbirth changed April 1, 2004. Fetal deaths occurring before April 2004 were considered stillbirths if death occurred after 28 completed weeks

of gestation. From April 2004, all fetal deaths were recorded as stillbirths after 22 completed weeks of gestation.[25] Preterm birth was defined as birth before 37 weeks of gestation and was performed as a post hoc analysis.

The population included all live births from 1997 to 2011, allowing 1-year follow-up for diagnosis of neonatal malformations. All children were included even if they did not survive to year one. We excluded births with coding errors and without information on the father. Pregnancies in which it was noted in the registries that the father had prior sperm donation or the mother had undergone in vitro fertilization or insemination in the month before pregnancy were included in the unexposed group, even if the father had filled a prescription for methotrexate. We excluded 11,334 births as a result of coding errors and 116,696 births resulting from missing information on the father. In the secondary analysis of stillbirth and preterm birth, the same cohort was used but with the addition of pregnancies complicated by stillbirth.

We used two logistic regression models. The first model was unadjusted and the second was adjusted for maternal parity (four categories, zero, one, two, three or greater), maternal age (five categories given, younger than 20, 20–24, 25–29, 30–34, and 35 years or older), smoking during pregnancy (yes or no), household income (categorized as quartiles), educational length (four categories, 12 or less, 12–13, 13–15, 15 years or greater), and year of birth (three categories, 1997–2001, 2002–2006, and 2007–2011). All variable had less than 0.5% missing values except for maternal education (2.7% missing values) and maternal smoking during pregnancy (3.2% missing values). To address missing data in the multivariable analyses, we performed the regression analyses as complete case and as sensitivity analyses with the missing observations as an individual category. The results of the sensitivity analyses addressing correlation between variables and missing data were all inconsequential for the results interpretation.

All analyses and data management were performed using SAS 9.4. For all analyses, a two-sided value of $P<.05$ was considered statistically significant, and all odds ratios (ORs) are presented with 95% confidence intervals.

The study was approved by the Danish Data Protection Agency (2015-41-4309). In Denmark, the Act on Processing of Personal Data does not require obtained consent or ethical approval in anonymized retrospective registry studies. We report our findings according to Strengthening The Reporting of OBservational studies in Epidemiology.[26]

RESULTS

There were 977,706 live births in Denmark from 1997 to 2011. We excluded 11,334 as a result of coding errors and 116,996 resulting from missing paternity information, leaving 849,676 live births. Of these, 139 children had fathers exposed to methotrexate within the 90 days before pregnancy. Twelve of these were excluded as a result of paternal sperm donation or maternal fertility treatment, leaving 127 children in the study group.

Exposed fathers were more likely to be older compared with unexposed fathers (mean 35.6 compared with 32.8 years; $P<.001$; Table 1). Women having a child with a man exposed to methotrexate within the 90 days before pregnancy were more likely to be older (mean 31.7 compared with 30.1 years; $P=.002$) and were more likely to have given birth previously (1.1 compared with 0.9 years; $P=.001$). There were no differences in maternal educational length (mean 12.8 compared with 13 years; $P=.59$), household income (median $107,814 compared with $108,487; $P=.72$), or maternal smoking (22.6% compared with 18.2%; $P=.20$). Fathers exposed to methotrexate were more likely to be exposed to other drugs such as antirheumatic drugs ($P<.001$), antipsoriatic drugs ($P<.001$), corticosteroids ($P<.001$), folic acid ($P<.001$), and intestinal anti-inflammatory agents ($P<.001$) (Table 2). The days in which fathers redeemed prescriptions for methotrexate were distributed throughout the 90-day period before pregnancy (Fig. 1).

Among the 127 children with fathers exposed to methotrexate before pregnancy, only four (3.2%) were diagnosed with a major congenital malformation compared with 28,814 (3.4%) children with fathers not exposed to methotrexate before pregnancy. The unadjusted OR was 0.93 (95% confidence interval [CI] 0.34–2.51) of having a diagnosis of major malformation among children to exposed fathers compared with unexposed. When adjusting for year of birth, maternal age, maternal educational length, household income, and maternal parity, the OR was 1.01 (95% CI 0.37–2.74). To analyze the association with minor congenital malformations, we included in a combined model both minor and major malformations. Five children (3.9%) with fathers exposed to methotrexate were born with either a major or a minor congenital malformation compared with 42,245 (5.0%) among children to unexposed fathers, resulting in an adjusted OR of 0.86 (0.35–2.10).

Table 1. Basic Characteristics of the Exposed and Unexposed Groups

Characteristic	Paternal Use of Methotrexate Within the 90 d Before Pregnancy	No Paternal Use of Methotrexate Within the 90 d Before Pregnancy	*P*
Paternal age (y)			<.001
Younger than 20	0 (0.0)	2,690 (0.3)	
20–24	4 (3.2)	42,530 (5.0)	
25–29	18 (14.2)	198,039 (23.3)	
30–34	38 (29.9)	315,682 (37.2)	
35 or older	67 (52.8)	290,608 (34.2)	
Maternal age (y)			.002
Younger than 20	0 (0.0)	10,145 (1.2)	
20–24	11 (7.9)	90,843 (10.7)	
25–29	25 (19.7)	283,421 (33.4)	
30–34	57 (44.9)	312,809 (36.8)	
35 or older	34 (26.8)	152,331 (17.9)	
Maternal parity			.001
1	29 (22.8)	337,663 (39.9)	
2	62 (48.8)	332,782 (39.4)	
3	25 (19.7)	128,257 (15.2)	
4 or greater	11 (8.7)	46,900 (5.6)	
Maternal education (y)			.59
Less than 12	32 (26.9)	193,942 (23.5)	
12–13	14 (11.8)	131,640 (15.9)	
13–15	36 (30.3)	248,915 (30.1)	
Greater than 15	37 (31.1)	252,463 (30.5)	
Household income (quartile)			.72
Lowest	37 (29.1)	212,506 (25.0)	
Low	28 (22.1)	212,350 (25.0)	
Medium	31 (24.4)	212,346 (25.0)	
High	31 (24.4)	212,347 (25.0)	
Maternal smoking during pregnancy	28 (22.6)	149,415 (18.2)	.20

Data are n (%) unless otherwise specified.

As a result of the small number of congenital malformations in the exposed group, we were able to analyze only the risk of congenital malformations of the heart (Table 3). There was no significant difference in the number of major malformations of the heart when comparing the unexposed and exposed groups (Table 3). We found an unadjusted OR of 2.66 (0.85–8.30) and an adjusted OR of 2.79 (0.89–8.78).

Among pregnancies of unexposed fathers, 2,541 (0.3%) births resulted in stillbirths (Table 4). Among the pregnancies of methotrexate-exposed fathers, there were no stillbirths (0.0%). We would have expected between 0 and 1 stillbirth in the exposed group if the risk of stillbirth was the same as in the unexposed group.

Post hoc we analyzed the risk of preterm birth in children to fathers exposed to methotrexate before

Table 2. Other Paternal Medication in the 12-Week Period Before Pregnancy

Medication	Fathers Exposed to Methotrexate	Fathers Not Exposed to Methotrexate	*P*
Antibacterials for systemic use	14 (11.0)	60,317 (7.1)	.09
Anti-inflammatory and antirheumatic drugs, non-steroid	34 (26.8)	34,307 (4.0)	<.001
Antipsoriatic drugs	8 (6.3)	1,287 (0.2)	<.001
Dermatologic corticosteroids	19 (15.0)	20,329 (2.4)	<.001
Corticosteroids for systemic use	24 (18.9)	6,539 (0.8)	<.001
Folic acid	34 (26.8)	373 (0.0)	<.001
Drugs for acid-related disorders	8 (6.3)	12,474 (1.5)	<.001
Intestinal anti-inflammatory agents	9 (7.1)	1,861 (0.2)	<.001

Data are n (%) unless otherwise specified.

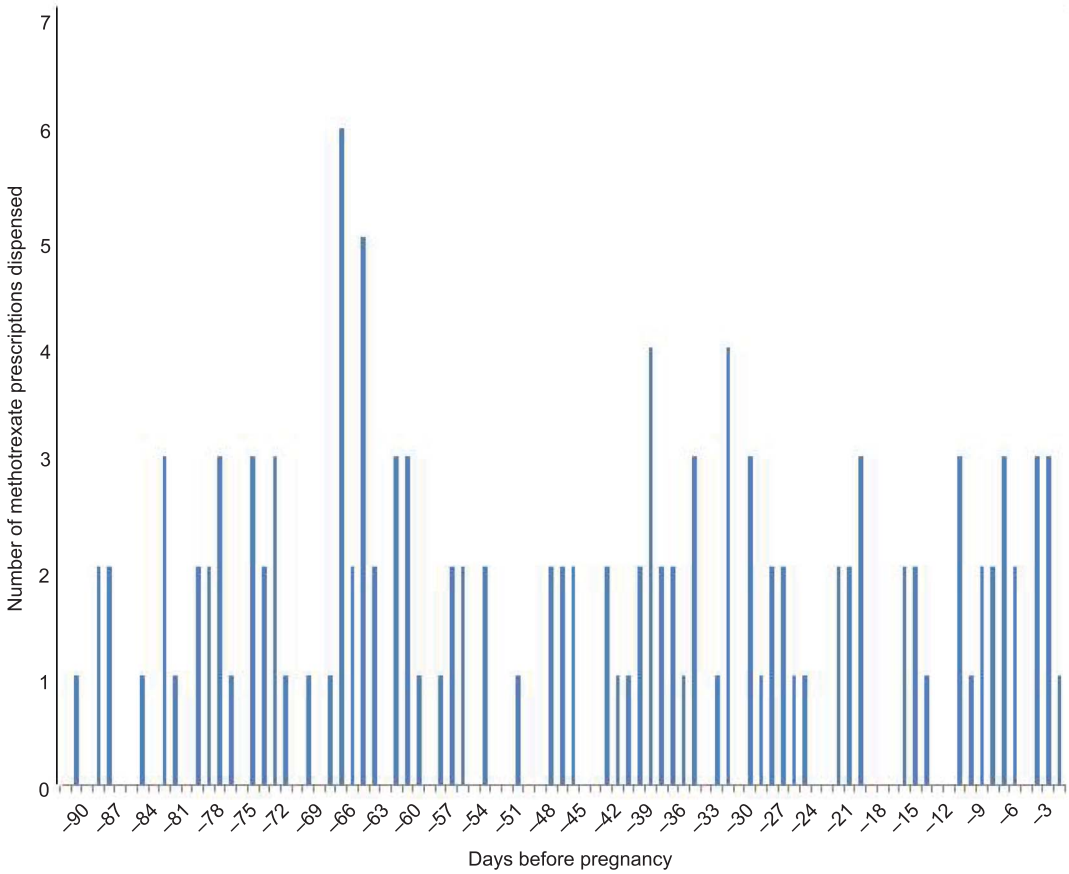

Fig. 1. Day of dispensation of methotrexate before pregnancy (–90 to 0 days). N=127 pregnancies.
Eck. Pregnancy Outcome After Paternal Exposure to Methotrexate. Obstet Gynecol 2017.

pregnancy. A total of 6.5% (55,171) were born before 37 weeks of gestation among the unexposed compared with 7.1% (9) among methotrexate-exposed fathers (unadjusted OR 1.11, 95% CI 0.56–2.18) and an adjusted OR of 1.31 (0.66–2.59) (Table 4).

DISCUSSION

In the present study we found no increased risk of major or minor congenital malformation nor any increased risk of stillbirth or prematurity when fathers were exposed to methotrexate in the 90

Table 3. Paternal Exposure to Methotrexate Within 90 Days Before Pregnancy and the Risk of Congenital Malformations

	No. of Offspring Diagnosed With a Major Malformation		OR (95% CI)	
Type of Major Malformation	Paternal Use of Methotrexate 90 d Before Pregnancy (n=127)	No Paternal Use of Methotrexate 90 d Before Pregnancy (n=849,549)	Unadjusted	Adjusted*
All major congenital malformations	4 (3.2)	28,814 (3.4)	0.93 (0.34–2.51)	1.01 (0.73–2.74)
Major and minor congenital malformations	5 (3.9)	42,245 (5.0)	0.73 (0.32–1.92)	0.86 (0.35–2.10)
Major congenital malformations of the heart	3 (2.4)	7,751 (0.9)	2.66 (0.85–8.30)	2.79 (0.89–8.78)

OR, odds ratio; CI, confidence interval.
Data are n (%) unless otherwise specified.
* Adjusted for year of birth, maternal age, maternal educational length, household income, and maternal parity.

Table 4. Paternal Exposure to Methotrexate Within 90 Days Before Pregnancy and the Risk of Stillbirth and Preterm Birth

Adverse Outcome	No. of Offspring With an Adverse Outcome		OR (95% CI)	
	Paternal Use of Methotrexate 90 d Before Pregnancy (n=127)	No Paternal Use of Methotrexate 90 d Before Pregnancy (n=852,090)	Unadjusted	Adjusted*
Stillbirth	0 (0.0)	2,541 (0.3)	—	—
Preterm birth	9 (7.1)	55,171 (6.5)	1.11 (0.56–2.18)	1.31 (0.66–2.59)

OR, odds ratio; CI, confidence interval.
Data are n (%) unless otherwise specified.
* Adjusted for year of birth, maternal age, maternal educational length, household income, and maternal parity.

days before pregnancy. As a result of the small number of congenital malformations, the only subgrouping we were able to evaluate was congenital malformations of the heart. We found a nonsignificant OR for having major malformations of the heart.

Methotrexate-exposed fathers were more likely to be exposed to other medicine than the nonexposed group. They had a higher intake of antirheumatic drugs, antipsoriatic drugs, intestinal anti-inflammatory agents, corticosteroids, and folic acid. These fathers have more chronic illnesses, which may potentially be a risk factor for poor pregnancy outcomes; even so, the exposed group did not have a higher risk of the study outcomes.

The results are in accordance with other publications concerning paternal exposure to methotrexate before pregnancy. In 2011 a French study of 40 fathers exposed to methotrexate before pregnancy (42 pregnancies with 36 live births) observed no congenital malformations.[5] A Norwegian study from 2012 with a cohort of 50 fathers who were treated with methotrexate 3 months before pregnancy found two orofacial malformations.[12] In the present study we did not find any children of fathers exposed to methotrexate diagnosed with an orofacial malformation. Another Norwegian study with five methotrexate-exposed fathers did not show any difference in birth outcomes when compared with the birth outcomes of unexposed fathers.[27] A more recent case–control study from 2013, including 87 liveborn children with methotrexate-exposed fathers, also concluded that there was no increased teratogenic risk.[4] The study also analyzed the potential association between methotrexate and miscarriage and found no association between paternal methotrexate and miscarriage.[4] In the present study we did not have the opportunity to repeat these analyses although information on miscarriage is available in Danish registers.[28–30] Information on fatherhood in Denmark is stored only if a child is born, and it is not possible to link miscarriage to the father. As such, we cannot rule out whether paternal methotrexate is associated with miscarriage.

In the present study there are several limitations. Purchase of the drug was used as a proxy for use of the medication. If the father filled the prescription but did not use it, the exposure status would be misclassified. In Denmark, registration of paternity is required only at birth; records of miscarriage or induced abortion do not include the identity of the father. If women terminated pregnancies complicated by fetal malformation, the rate of malformations would be underestimated in both the exposed and unexposed populations.

There has been a change of registration of stillbirths in the study period. From 2004 and onward, the stillbirth definition was changed from birth after 28 weeks of gestation to births after 22 weeks of gestation.[25] This might have underestimated the outcome because births of neonates without signs of life between 22 weeks of gestation and 28 weeks of gestation in the years from 1997 to 2004 were registered as miscarriages and therefore have no registered paternity.

According to Danish legislation, fathers are registered on the birth certificate as the assumed father as long as there is no dispute of paternity. Births with artificial insemination by donor are not identified in the birth registry with the donor as the father. Therefore, we classified all pregnancies with mothers given a diagnosis of fertility procedures such as in vitro fertilization and intracytoplasmic sperm injection in the month before the current pregnancy as the nonexposed. The same applied for fathers given a diagnosis with previous sperm donation. This may lead to misclassification of

exposure, but only 12 pregnancies were affected. Furthermore, misclassification of exposed could occur if the registered father was not the genetic father. No studies have investigated the nonpaternity rate in Denmark, but previous studies have estimated the rate of nonpaternity in the Western population to be less than 1%.[31] Another limitation to this study is lack of dosing information of methotrexate precluding an analysis of a potential dose–response effect.

The main strength of the present study is the use of the unique Danish nationwide registries. As described in the Methods sections, their quality is extremely high and the validation upholds the accuracy of the given rates and thereby strengthens our results.

In this study we found no association between paternal redemption of a prescription of methotrexate within 90 days before pregnancy and major congenital malformations in the offspring. The result is supported by all other published retrospective studies addressing this issue. This study and available data suggest that prepregnancy paternal methotrexate exposure should not be of major concern. Multinational recommendations for paternal use of methotrexate before pregnancy should be changed accordingly.

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