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Pregnancy Outcome After Methotrexate Treatment for Rheumatic Disease Prior to or During Early Pregnancy

A Prospective Multicenter Cohort Study

Corinna Weber-Schoendorfer,[1] Christina Chambers,[2] Evelin Wacker,[1] Delphine Beghin,[3] Nathalie Bernard[4] on behalf of the Network of French Pharmacovigilance Centers, Svetlana Shechtman,[5] Diana Johnson,[2] Benedikte Cuppers-Maarschalkerweerd,[6] Alessandra Pistelli,[7] Maurizio Clementi,[8] Ursula Winterfeld,[9] Georgios Eleftheriou,[10] Anna Pupco,[11] Kelly Kao,[2] Heli Malm,[12] Elisabeth Elefant,[3] Gideon Koren,[11] Thierry Vial,[4] Asher Ornoy,[5] Reinhard Meister,[13] and Christof Schaefer[1]

Objective. **High-dose methotrexate (MTX) exposure during pregnancy is associated with embryopathy. The teratogenic potential of MTX at dosages typically used in the treatment of rheumatic diseases remains uncertain. The aim of this study was to evaluate the risk of spontaneous abortion, major birth defects, elective termination of pregnancy, shortened gestational age at delivery, and reduced birth weight in women exposed to MTX.**

Methods. **Pregnancy outcome in women taking MTX (≤30 mg/week) either after conception or within the 12 weeks before conception was evaluated in a prospective observational multicenter cohort study. Pregnancy outcomes in the MTX group were compared to outcomes in a group of disease-matched women and a group of women without autoimmune diseases (neither group was exposed to MTX).**

Results. **The study sample included 324 MTX-exposed pregnancies (188 exposed post-conception, 136 exposed pre-conception), 459 disease-matched comparison women, and 1,107 comparison women without autoimmune diseases. In the post-conception cohort, the cumulative incidence of spontaneous abortion was 42.5% (95% confidence interval [95% CI] 29.2–58.7), which was significantly higher than the incidence of spontaneous abortion in either comparison group. The**

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[1]Corinna Weber-Schoendorfer, MD, Evelin Wacker, PhD, Christof Schaefer, MD, PhD: Charité-Universitätsmedizin Berlin and Pharmakovigilanz und Beratungszentrum Embryonaltoxikologie, Berlin, Germany; [2]Christina Chambers, PhD, MPH, Diana Johnson, MS, Kelly Kao, BA: University of California at San Diego, La Jolla, California; [3]Delphine Beghin, PhD, PharmD, Elisabeth Elefant, MD: Centre de Référence sur les Agents Tératogènes, Hôpital Universitaires Paris Est, AP-HP, Paris, France; [4]Nathalie Bernard, PharmD, Thierry Vial, MD: Hospices Civils de Lyon and Centre Régional de Pharmacovigilance, Lyon, France; [5]Svetlana Shechtman, PhD, Asher Ornoy, MD: Israel Ministry of Health and Israeli Teratology Information Service, Jerusalem, Israel; [6]Benedikte Cuppers-Maarschalkerweerd, PharmD: Netherlands Pharmacovigilance Centre Lareb, MH 's-Hertogenbosch, The Netherlands; [7]Alessandra Pistelli, MD, PhD: Azienda Ospedaliero Universitaria Careggi and Centro di Riferimento Regionale di Tossicologia Perinatale, Florence, Italy; [8]Maurizio Clementi, MD: University of Padua and Servizio di Informazione Teratologica, Padua, Italy; [9]Ursula Winterfeld, PhD: University Hospital, Lausanne and Swiss Teratogen Information Service, Lausanne, Switzerland; [10]Georgios Eleftheriou, MD, MSc, PhD: Ospedali Riuniti and Poison Control Center and Teratology Information Service, Bergamo, Italy; [11]Anna Pupco, MD, Gideon Koren, MD: Hospital for Sick Children, Toronto, Ontario, Canada; [12]Heli Malm, MD, PhD: HUSLAB and Helsinki University Central Hospital, Teratology Information, Helsinki, Finland; [13]Reinhard Meister, PhD: Beuth Hochschule für Technik Berlin, Berlin, Germany.

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Address correspondence to Corinna Weber-Schoendorfer, MD, Charité-Universitätsmedizin Berlin, Pharmakovigilanz und Beratungszentrum Embryonaltoxikologie, Spandauer Damm 130, Haus 10, 14050 Berlin, Germany. E-mail: corinna.weber-schoendorfer@charite.de.

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risk of major birth defects (7 of 106 [6.6%]) was elevated compared to both the cohort of women without autoimmune diseases (29 of 1,001 [2.9%]) (adjusted odds ratio [OR] 3.1 [95% CI 1.03–9.5]) and the disease-matched cohort (14 of 393 [3.6%]) (adjusted OR 1.8 [95% CI 0.6–5.7]). None of the malformations were clearly consistent with MTX embryopathy. Neither the cumulative incidence of spontaneous abortion (14.4% [95% CI 8.0–25.3]) nor the risk of major birth defects (4 of 114 [3.5%]) was increased in the pre-conception cohort. Elective termination rates were increased in both of the MTX-exposed cohorts. There were no other significant differences among groups in other study end points.

Conclusion. **Post-conception administration of MTX at dosages typically used in the treatment of rheumatic diseases was associated with an increased risk of major birth defects and spontaneous abortion. Such evidence was not found among women in our pre-conception cohort.**

The folic acid antagonist methotrexate (MTX) is used to treat a variety of health conditions, including malignancies and rheumatic or inflammatory autoimmune disorders; it is also used for the nonsurgical treatment of ectopic pregnancy and elective termination of pregnancy. Depending on the treatment indication, dosages range from 2.5 mg/week to 30 mg/week (for the treatment of rheumatoid arthritis [RA]) to 1,000 mg/m^2 of body surface area (for the treatment of certain types of cancer).

High doses of MTX have been shown to be teratogenic in humans (1–4). The MTX embryopathy shows a phenotype similar to that observed in subjects taking the MTX precursor aminopterin. The pattern of anomalies consists of skull, limb, and other skeletal defects, some minor craniofacial abnormalities, and growth restriction. However, limited data are available on the fetal risks of low-dose MTX exposure. This is an important question given that the dosage of most human teratogens is a factor that can influence the risk of birth defects (5).

In a small French study, it was found that 28 low-dose MTX–exposed pregnancies resulted in 19 live births with no major birth defects (6). In 4 small case series (totaling 23 MTX-exposed pregnancies), there were 5 elective terminations of pregnancy (1 of which involved a fetus with MTX embryopathy) (7), 4 spontaneous abortions, and 14 deliveries (13 healthy infants and 1 infant with a tentative diagnosis of cystic fibrosis) (8–10). Among case reports involving 37 MTX-exposed fetuses/infants with birth defects (1–4,7,11–32) only 1 fetus/infant was described with a phenotype typical of MTX embryopathy after low-dose therapy (12) (data available upon request from the corresponding author). We are unaware of any studies that have examined pre-conception exposure to low-dose MTX. The current recommendation is to avoid pregnancy exposure to MTX at any dose, and to discontinue the medication at least 3 months before attempting to conceive (33).

To assess the risk associated with MTX at weekly dosages typically used in the treatment of rheumatic diseases, taken either during pregnancy or shortly before conception, we designed a prospective multicenter cohort study. We evaluated the risk of spontaneous abortion, major birth defects, elective termination, shortened gestational age at delivery, and reduced birth weight.

PATIENTS AND METHODS

Data on MTX-exposed and comparison women were obtained from pregnancies identified by members of the European Network of Teratology Information Services (ENTIS) (Finland [n = 49], France [n = 596], Germany [n = 555], Israel [n = 237], Italy [n = 120], The Netherlands [n = 71], and Switzerland [n = 35]), as well as from the Organization of Teratology Information Specialists (OTIS) (US and Canada [n = 227]). Teratology information service centers offer risk assessment to health care providers and pregnant women who spontaneously seek consultation regarding a pregnancy. The mission and structure of teratology information service centers have been described elsewhere (34).

This prospective observational cohort study involved 2 MTX-exposed cohorts of subjects treated for rheumatic/inflammatory diseases and 2 comparison cohorts. The first group of subjects was composed of pregnant women who were exposed to MTX after conception (i.e., ≥1 dose 2 weeks after the first day of the last menstrual period). The second group of exposed subjects was composed of pregnant women who were exposed to MTX only before conception (i.e., between 10 weeks prior to the last menstrual period and no more than 1 week plus 6 days after the last menstrual period). According to multinational evidence-based recommendations for the use of MTX in RA and other rheumatic diseases, a maximum dosage of 30 mg/week was chosen as the upper limit for inclusion in the study (33).

The first comparison group was composed of pregnant women with rheumatic/inflammatory diseases who did not take MTX during pregnancy or during the 12 weeks before conception. These women had either been treated with other immunomodulatory drugs or the physician had decided not to prescribe any of these drugs after performing an individual risk–benefit consultation. The second comparison group was composed of pregnant women identified through teratology information service centers during spontaneous consultations for other conditions or exposures, such as use of hair dye, nonteratogenic infections, back pain, vaginosis, and asthma. Subjects in the second comparison group were not affected by

rheumatic/inflammatory diseases and were not taking any immunomodulatory drugs during pregnancy, with the rare exception of glucocorticoids for other indications. For all groups, exclusion criteria included exposure to other known major teratogens or fetotoxicants, as well as malignancies. Although classified as a category X drug by the US Food and Drug Administration, leflunomide exposure did not meet exclusion criterion because leflunomide has not been confirmed to be teratogenic in humans (35,36).

Data on pregnant women enrolled at teratology information service centers between 1994 and 2011 were used for this study. Each teratology information service center identified pregnancies that met the MTX-exposure criteria. From the eligible pool within each center, pregnancies in the disease-matched cohort were randomly matched with pregnancies in the MTX group for disease and year of enrollment at a ratio of ~1:1. Similarly, pregnancies in the cohort of women without autoimmune diseases were randomly matched within the centers with the MTX-exposed cohorts for year of ascertainment at a ratio of ~3:1. The data were then combined across centers, with the target sample size of at least 250 exposed women, 300 disease-matched women, and 900 comparison women without autoimmune diseases. All pregnancies were prospectively ascertained, indicating that neither the outcome of the pregnancy nor the results of prenatal diagnosis were known at the time of enrollment.

Data on exposure and outcome were obtained via structured telephone interviews and/or mailed questionnaires completed by the mother and/or her physicians (oral informed consent was obtained). Data were collected on demographics, maternal age, pregnancy history (including previous number of children with birth defects), pre-pregnancy body mass index, prescription and over-the-counter medications (including specific dosages and dates of exposure), smoking history and alcohol consumption, and details regarding the course of pregnancy. Major birth defects were defined as structural abnormalities of medical, surgical, or cosmetic relevance with special attention to anomalies consistent with MTX embryopathy. Birth defects were classified as major or minor by 2 of the authors (CW-S and CS) according to 2 standard classification systems (37,38). The classifications were performed independently and exposure data were evaluated under blinded conditions. In the case of disagreement between the 2 authors (2 of 106 cases), consensus was achieved through discussion.

Duration of gestation was calculated (in weeks) using ultrasound performed during the first trimester or, if not available, based on the date of the last menstrual period. Spontaneous abortion was defined as spontaneous loss of a fetus weighing <500 gm (or <23 weeks of completed gestation after the last menstrual period). Gestational age at delivery and birth weight were measured as continuous variables.

The study protocol was approved by the joint scientific committees of ENTIS and OTIS, by the ethics committee at the Charité-Universitätsmedizin Berlin, and by ethics committees of other teratology information service centers, as required. The study was registered with the German Clinical Trial Register and the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (online at www.encepp.eu).

Logistic regression was used to evaluate the risk of major birth defects. Crude birth defect rates were calculated by dividing the number of infants and fetuses with birth defects by the number of all live births plus the number of stillbirths/aborted fetuses with birth defects. Birth defects known to be of genetic etiology, such as Down syndrome, were considered separately. The final analysis involved propensity score adjustment for bias reduction, classifying pregnant women into 5 strata defined by the quintiles of the propensity score (39). Propensity score estimation used boosted regression trees (40), including maternal age, alcohol consumption, smoking status, and parity as covariates, and was repeated for each analysis. For analyses involving the disease-matched cohort, taking disease-modifying drugs or systemic glucocorticoids was additionally included in the propensity score estimation. For analyses involving comparison of the MTX cohorts to the cohort of women without autoimmune diseases, taking disease-modifying antirheumatic drugs (DMARDs) or systemic glucocorticoids was used as a covariate directly in the analysis, in addition to propensity score stratification, to account for effects of these concomitant medications. Propensity score stratification was used as described above in all multivariate analyses.

Table 1. Drug treatment indications in the MTX-exposed cohort and the disease-matched cohort*

Drug treatment indication	MTX-exposed cohort (n = 324)	Disease-matched cohort (n = 459)
Rheumatoid arthritis	202 (62.3)	265 (57.7)
Psoriasis/psoriatic arthritis	35 (10.8)	42 (9.2)
Systemic lupus erythematosus	19 (5.9)	45 (9.8)
Inflammatory bowel disease	15 (4.6)	36 (7.8)
Ankylosing spondylitis/Bechterew's disease	13 (4.0)	33 (7.2)
Other	40 (12.4)	38 (8.3)

* Values are the number (%). MTX = methotrexate.

The cumulative incidences of spontaneous abortion and elective termination were assessed using survival analysis (while accounting for left truncation due to varying time of gestation at enrollment) (41). Hazard ratios (HRs) were then estimated using Cox proportional hazards models. Similarly, Cox proportional hazards models were used to examine gestational age at delivery, taking into account delayed entry into the study. For the comparison of birth weights between groups, live births from all centers were classified according to newborn birth weight percentile categories (42). A score was determined through standardization and included in a linear regression model as the dependent variable.

For all models that included covariates, missing values were addressed through multiple imputation using chained equations, assuming that the data were missing at random (43). Fifty imputed data sets were generated per outcome. The models of multiple imputation were based on the respective outcomes and the covariates used to estimate the propensity score. For each imputed data set, analyses were performed as described above. Results were then combined using Rubin's rule (44). Adjustment could not be made for maternal pre-pregnancy body mass index and education level because information was missing in too many cases to allow for imputation.

Heterogeneity of maternal characteristics among

Table 2. Maternal characteristics by cohort*

Maternal characteristics	MTX-exposed		Disease-matched comparison (n = 459)	Non–autoimmune disease comparison (n = 1,107)
	Pre-conception (n = 136)	Post-conception (n = 188)		
Age				
No. (%) with available data	131 (96.3)	186 (98.9)	446 (97.2)	1,051 (94.9)
Median (IQR) years	30 (27, 35)	32 (28, 36)	32 (28, 35)	31 (28, 35)
BMI				
No. (%) with available data	54 (39.7)	55 (29.3)	201 (43.8)	432 (39.0)
Median (IQR)	25.2 (20.6, 27.4)	23.9 (21.7, 29.5)	23.0 (20.5, 25.2)	22.6 (20.6, 25.1)
Smoking history				
No. (%) with available data	96 (70.6)	127 (67.6)	338 (73.6)	900 (81.3)
≤5 cigarettes/day, no. (%)	7 (7.3)	10 (7.9)	14 (4.1)	41 (4.6)
>5 cigarettes/day, no. (%)	13 (13.5)	19 (15.0)	20 (5.9)	56 (6.2)
Alcohol consumption				
No. (%) with available data	96 (70.6)	128 (68.1)	335 (73)	909 (82.1)
≤1 drink/day, no. (%)	10 (10.4)	12 (9.4)	32 (9.6)	72 (7.9)
>1 drink/day, no. (%)	0 (0)	1 (0.8)	2 (0.6)	9 (1.0)
Previous parity				
No. (%) with available data	122 (89.7)	179 (95.2)	426 (92.8)	966 (87.3)
1 birth, no. (%)	41 (33.6)	41 (22.9)	116 (27.2)	300 (31.1)
2 births, no. (%)	27 (22.1)	39 (21.8)	52 (12.2)	155 (16.1)
≥3 births, no. (%)	15 (12.3)	30 (16.8)	27 (6.3)	62 (6.4)
Previous SAB				
No. (%) with available data	120 (88.2)	170 (90.4)	422 (91.9)	926 (83.6)
1 SAB, no. (%)	19 (15.8)	20 (11.8)	49 (11.6)	128 (13.8)
≥2 SAB, no. (%)	7 (5.8)	12 (7.1)	19 (4.5)	40 (4.3)
Previous children with birth defects				
No. (%) with available data	88 (64.7)	157 (83.5)	346 (75.4)	616 (55.6)
1 child, no. (%)	4 (4.6)	3 (1.9)	3 (0.9)	15 (2.4)
≥2 children, no. (%)	0 (0)	1 (0.6)	1 (0.3)	0 (0)
Education level				
No. (%) with available data	48 (35.3)	54 (28.7)	187 (40.7)	418 (37.8)
≤9 years, no. (%)	5 (10.4)	12 (22.2)	12 (6.4)	34 (8.1)
>9 and ≤13.5 years, no. (%)	29 (60.4)	32 (59.3)	90 (48.1)	188 (45.0)
Academic degree obtained, no. (%)	14 (29.2)	10 (18.5)	85 (45.5)	196 (46.9)
Systemic steroid use, no. (%)†	54 (39.7)	77 (40.6)	242 (52.7)	15 (1.4)
Systemic DMARD use, no. (%)†	52 (38.2)	60 (31.9)	270 (58.8)	0
Gestational week at first TIS contact				
No. (%) with available data	136 (100)	188 (100)	459 (100)	1,107 (100)
Median (IQR)	8.2 (6.1, 11.1)	7.4 (5.9, 10.3)	9.0 (6.1, 15.0)	9.0 (6.6, 13.6)

* Data were not available for all women because medical records may have been missing, questions may not have been asked during the telephone interview, the physician may not have known the answer, or the patient may not have been willing to answer. Body mass index (BMI) was not routinely recorded by most teratology information service (TIS) centers until 2005. Disease-modifying antirheumatic drugs (DMARDs) were drugs other than methotrexate (MTX). IQR = interquartile range; SAB = spontaneous abortion.
† All numbers are per pregnancy, e.g., 2 DMARDs taken during 1 pregnancy was counted as 1 DMARD-exposed pregnancy.

groups was tested using an analysis of variance (ANOVA) F test for continuous data and Pearson's chi-square test for categorical data. *P* values for these 2-sided comparisons were corrected for multiple comparisons using the Bonferroni-Holm procedure as implemented in R software (R Development Core Team). All other comparisons used 1-sided testing to assess the embryotoxic potential of MTX. Significance was determined using a common alpha level of 5%. No correction for multiple comparisons was performed. Heterogeneity among contributing teratology information service centers was tested using the Breslow-Day test of homogeneity for dichotomous outcomes and the ANOVA F test for continuous outcomes. All data analysis was performed at the Berlin Institute using R version 2.15.

RESULTS

In total, data on 1,890 pregnancies (in women from 9 countries) were included in this study. Of these pregnancies, 188 were in women exposed to weekly doses of MTX during the post-conception period (exposure only after the first trimester in 2); doses of MTX were consistent with those used for the treatment of rheumatic diseases. For 136 pregnancies, MTX exposure occurred only during the pre-conception period. A total of 459 pregnancies were included in the disease-matched cohort, and 1,107 pregnancies were included in the cohort

of women without autoimmune diseases. The median weekly MTX dosage was 10 mg/week (range 1.9, 30.0 mg; interquartile range [IQR] 8.8, 15.0) in the MTX-exposed post-conception cohort and 15 mg/week (range 2.5, 30.0 mg; IQR 10.0, 17.5) in the pre-conception cohort. The maximum dosage of 30 mg/week was administered in only 2 patients (1 in each MTX cohort) (data available upon request from the corresponding author). Both of these patients had rheumatoid arthritis. In the post-conception cohort, the median duration of MTX administration was 4.3 weeks after the last menstrual period (range 0.1, 28.0 weeks; IQR 3.0, 6.0); 50% of pregnant women were treated at least until week 5 and 14.4% (27 of 188) were still taking their medication between weeks 8 and 10 (data available upon request from the corresponding author). In the pre-conception cohort, 50% of the women discontinued MTX no later than 2 weeks prior to their last menstrual period (range –10.0, 1.9 weeks; IQR –5.0, 0.6) and 50% stopped taking the drug sometime between 2 weeks prior to the last menstrual period and conception. Of 298 women with known route of administration, 236 took MTX in tablet form.

Rheumatoid arthritis was the most frequent underlying condition in the exposed and disease-matched groups (Table 1). Systemic lupus erythematosus was more prevalent in the disease-matched comparison cohort (9.8%) than in the MTX-exposed post-conception cohort (6.9%). More than half of the MTX-treated women were concomitantly exposed to systemic glucocorticoids and/or another immunomodulatory drug (Table 2). Among the latter group, leflunomide exposure occurred during 6 pregnancies (4.4%), 4 pregnancies (2.1%), and 20 pregnancies (4.4%) in the pre-conception, post-conception, and disease-matched comparison cohorts, respectively (data available upon request from the corresponding author). No birth defects were observed in the offspring that resulted from these 30 pregnancies. The exposed and comparison groups differed by smoking status ($P = 0.003$), parity ($P < 0.001$), education level ($P < 0.001$), and gestational age at enrollment ($P < 0.001$) (Table 2). After propensity score stratification, we did not find a substantial imbalance of covariates used for the propensity score estimation.

Spontaneous abortions occurred more frequently in the post-conception cohort, with a cumulative incidence of 42.5% (95% CI 29.2–58.7) compared to 14.4%, 22.5%, and 17.3% in the pre-conception, disease-matched, and non–autoimmune disease comparison cohorts, respectively (Figure 1). The adjusted HR for post-conception exposure was 2.1 (95% CI 1.3–3.2) relative to the disease-matched comparison group and 2.5 (95% CI 1.4–4.3) relative to the comparison group of women without autoimmune diseases. The effect of disease-modifying drugs or systemic glucocorticoids on the spontaneous abortion rate was not significant (adjusted HR 1.3 [95% CI 0.7–2.5]). Crude rates of pregnancy outcomes are shown in Table 3.

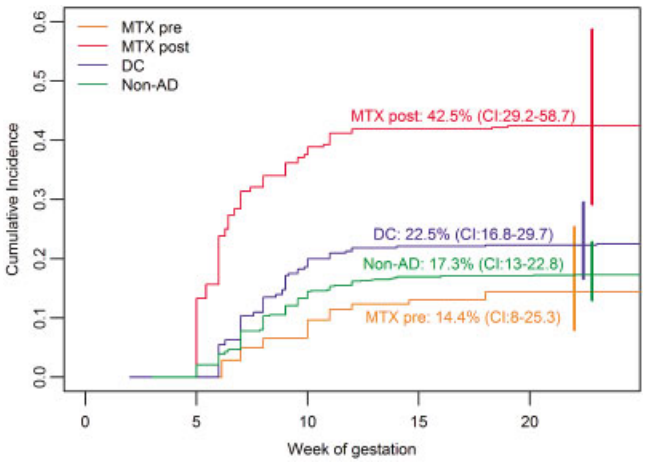

Figure 1. Cumulative incidence rates of spontaneous abortion stratified by cohort. Cumulative incidences of spontaneous abortion are plotted for the cohort of women exposed to methotrexate (MTX) before conception (MTX pre), the cohort of women exposed to MTX after conception (MTX post), the disease-matched cohort (DC), and the cohort of women without autoimmune diseases (non-AD). The adjusted hazard ratio (HR) for post-conception exposure was 2.1 (95% confidence interval [95% CI] 1.3–3.2) relative to the disease-matched cohort and 2.5 (95% CI 1.4–4.3) relative to the cohort of women without autoimmune diseases. The HR for pre-conception exposure was 0.8 (95% CI 0.4–1.4) relative to the disease-matched cohort and 1.3 (95% CI 0.6–3.0) relative to the cohort of women without autoimmune disease. Vertical lines show the 95% CI for each incidence rate.

The proportion of infants with birth defects in each group is shown in Table 4. There was a significantly increased risk of major birth defects (7 of 106 [6.6%]) in the MTX post-conception group compared to the non–autoimmune disease cohort (29 of 1,001 [2.9%]) (adjusted odds ratio [OR] 3.1 [95% CI 1.03–9.5]). Therapy with DMARDs and/or systemic glucocorticoids did not affect the risk significantly (adjusted OR 1.2 [95% CI 0.4–4.1]). The adjusted OR was elevated, but the 95% CI overlapped 1 when compared to the disease-matched comparison group (14 of 393 [3.6%]) (adjusted OR 1.8 [95% CI 0.6–5.7]). There were no differences between the pre-conception cohort and the 2 comparison groups after adjustment (for the pre-conception cohort versus the disease-matched comparison cohort, adjusted OR 1.1 [95 % CI 0.3–4.2] and for the pre-conception cohort

Table 3. Pregnancy outcome by cohort*

	MTX-exposed		Disease-matched comparison	Non–autoimmune disease comparison
	Pre-conception	Post-conception		
No. of pregnancies	136	188	459	1,107
SAB, no. (%)	12 (8.8)	39 (20.7)	44 (9.6)	79 (7.1)
Stillbirth, no. (%)	1 (0.7)	2 (1.1)†	2 (0.4)	1 (0.1)
Elective termination, no. (%)	13 (9.6)	49 (26.1)	33 (7.2)	49 (4.4)‡
Voluntary, no.	10	40	25	34
Maternal disease, no.	1	5	4	3
Fetal complications, no.	2	4	4§	10¶
Live birth, no. (%)	110 (80.9)	99 (52.7)†	380 (82.8)	978 (88.4)

* Live births are defined as pregnancy resulting in at least 1 live birth. MTX = methotrexate; SAB = spontaneous abortion.
† Including 1 twin pregnancy that resulted in 1 live birth and 1 stillborn child and was counted as 2 outcomes.
‡ Reasons for elective termination of pregnancy were unknown in 2 cases.
§ One elective termination at week 29 because of insufficient development of the fetus, but no malformation was noted on prenatal ultrasound.
¶ Malformations were present in 9 cases and fetal cytomegalovirus infection resulting in microcephaly and encephalitis was present in 1 case.

versus the non–autoimmune disease comparison cohort, adjusted OR 1.6 [5 % CI 0.4–7.3]). No infant in either of the MTX-exposed groups exhibited defects clearly consistent with the MTX embryopathy (Table 5) (further data available upon request from the corresponding author).

A cumulative incidence of 33.1% was reported for elective termination of pregnancy in the MTX-exposed post-conception cohort (adjusted HR 7.4 [95% CI 4.5–12.2] relative to women without autoimmune diseases). Four of the 49 incidences of elective termination in this group were due to fetal malformations; the remaining pregnancies were terminated due to fear of malformations or for other maternal or social reasons (Table 3) (further data upon request from the corresponding author). In the pre-conception cohort, the cumulative incidence of elective termination was 14.1% (adjusted HR 2.4 [95% CI 1.1–5.6] relative to the non–autoimmune disease cohort) (data available upon request from the corresponding author).

After adjustment for the propensity score, mean gestational age and birth weight in the liveborn infants did not differ significantly between groups (data available upon request from the corresponding author). There were no significant differences between teratology information service centers with regard to any of the study end points. Data from the Finnish, Dutch, and Swiss teratology information services centers were excluded from heterogeneity analysis due to small numbers.

Table 4. Birth defects by cohort

	MTX-exposed		Disease-matched comparison (n = 459)	Non–autoimmune disease comparison (n = 1,107)
	Pre-conception (n = 136)	Post-conception (n = 188)		
No. of live births, including twins	113	103	392	997
Major birth defects, no./total assessed (%)	4/114 (3.5)*	7/106 (6.6)†	14/393 (3.6)‡	29/1,001 (2.9)§
Minor birth defects, no./total assessed (%)	5/113 (4.4)	2/104 (1.9)¶	22/392 (5.6)	23/997 (2.3)
Genetic birth defects, no./total assessed (%)	1/114 (0.9)*	0	2/394 (0.5)#	7/1,003 (0.7)**

* One hundred thirteen liveborn infants and 1 elective termination of pregnancy.
† One hundred three liveborn infants (excluding 2 cases with methotrexate [MTX] exposure started after the first trimester) and 5 elective terminations.
‡ Three hundred ninety-two liveborn infants and 1 elective termination.
§ Nine hundred ninety-seven liveborn infants, 3 elective terminations, and 1 stillbirth.
¶ One hundred three liveborn infants and 1 elective termination.
# Three hundred ninety-two liveborn infants and 2 elective terminations.
** Nine-hundred ninety-seven liveborn infants and 6 elective terminations.

Table 5. Description of major birth defects in the MTX cohorts*

	Treatment indication and MTX dosage	Comedication, smoking, alcohol intake by trimester	Gestational age at birth or ETOP, weeks after LMP	Birth defects
Post-conception cohort				
MTX-exposed from 4 weeks plus 5 days after LMP until 8 weeks plus 5 days after LMP	RA, 20 mg/week	Methylprednisolone and ethinyl estradiol plus dienogest, >5 cigarettes/day, trimester 1; >1 drink/day, trimesters 1–2	ETOP at week 18	Autopsy: gastroschisis with complete small bowel protruding, scoliosis, no anomalies of the limbs, no facial dysmorphia
MTX-exposed from before pregnancy until 2 weeks plus 2 days after LMP	RA, 15 mg/week	Omeprazole and prednisolone, trimesters 1–3; ibuprofen, trimesters 1 and 2; etanercept and adalimumab, trimester 1	Birth at week 40 plus 2 days; male; 2,520 gm (<3rd percentile)	Cystic adenomatoid malformation of the right lung dorsobasal (multiple cysts of different sizes totaling 2.3 cm × 4.2 cm, surgery performed), incomplete right bundle branch block, pericardial effusion, PFO and PDA, slight persistent pulmonary hypertension
MTX-exposed from before pregnancy until 4 weeks after LMP	RA, 10 mg/week	None	Birth at week 39; male; 2,550 gm (<3rd percentile)	Pelvic ectopic kidney (behind bladder)
MTX-exposed from 4 weeks plus 1 day after LMP until 5 weeks plus 1 day after LMP	Psoriasis, 10 mg/week in 4 weeks and 1 day and 7.5 mg/week in 5 weeks and 1 day	Diflucortolone topical and 20 cigarettes/day, trimesters 1–2	ETOP at week 14	Limb defects (no upper or lower limbs), no autopsy
MTX-exposed from 4 weeks after LMP until 5 weeks after LMP	RA, 10 mg/week	None	ETOP at week 19 plus 3 days	Atrioventricular canal, dextroposition of the heart, left diaphragmatic hernia, karyotype 46, XX
MTX-exposed from 2 weeks plus 1 day after LMP until 3 weeks plus 1 day after LMP	RA, 30 mg/week	None despite Hashimoto thyroiditis; 20 cigarettes/day, trimesters 1–2	ETOP at week 15	Holoprosencephaly, megabladder
MTX-exposed from 4 weeks after LMP until 8 weeks after LMP	RA, 10 mg/week	Prednisolone, trimester 1	1 stillborn twin at week 34 plus 5 days; female; 860 gm (<3rd percentile); other twin healthy	Renal agenesis, cardiomegaly
Pre-conception cohort				
MTX-exposed up to 4 weeks before LMP	PsA, 15 mg/week	None	ETOP at week 12	Caudal regression syndrome, omphalocele
MTX-exposed up to 9 weeks before LMP	RA, 12.5 mg/week	None	Birth at week 39; male; 3,310 gm	Left kidney in sigmoid position, grade III bilateral uretero vesical reflux right, preauricular tag
MTX-exposed up to 8 weeks before LMP	RA, 12.5 mg/week	Prednisone, trimesters 1–3; vitamin D, calcium, ibuprofen, antacids, acetaminophen, trimester unspecified; etanercept, trimester 1	Birth at week 40 plus 6 days; female; 3,462 gm	Cataract in the right eye
MTX-exposed up to 6 days after LMP	RA, 7.5 mg/week	Methylprednisolone, trimesters 1–3; unspecified corticosteroid, trimester 1; fluticasone nasal, acetaminophen, chlorpheniramine, hydrocodone, propoxyphene, ondansetron, influenza vaccine, trimester unspecified; adalimumab, trimesters 2 and 3; prednisolone and infliximab, trimester 3	Birth at week 38 plus 6 days; female; 3,584 gm	Atrial septal defect, peripheral pulmonic stenosis

* MTX = methotrexate; ETOP = elective termination of pregnancy; LMP = last menstrual period; RA = rheumatoid arthritis; PFO = persistent foramen ovale; PDA = persistent ductus arteriosus; PsA = psoriatic arthritis.

DISCUSSION

The objective of our study was to assess the embryotoxic potential of MTX treatment at dosages typically used in the treatment of rheumatic diseases. The most striking result was the increased cumulative incidence of spontaneous abortion among those exposed to MTX after conception, a result that was not explained by underlying maternal disease or the other factors that we measured. Although it has not previously been established that administration of MTX at dosages typically used in the treatment of rheumatic diseases is associated with pregnancy loss, it is well known that a single high dose of 1 mg/kg or 50 mg/m^2 can induce abortion by inhibiting DNA synthesis in rapidly dividing cells (such as trophoblasts). This abortifacient property is used intentionally for elective termination or medical treatment of ectopic pregnancies. Since gross structural defects might lead to miscarriage, the observed high proportion of spontaneous abortion may also reflect MTX-related teratogenicity. Other teratogens associated with an increased risk of spontaneous abortion include thalidomide (45), mycophenolate (46), vitamin K antagonists (47), and isotretinoin (48).

The rate of major birth defects associated with post-conception MTX exposure (6.6%) was significantly increased in comparison to the cohort of women without autoimmune diseases (2.9%) (adjusted OR 3.1 [95% CI 1.03–9.5]). It was also increased compared to the disease-matched cohort (3.6%) (adjusted OR 1.8 [95% CI 0.6–5.7]); however, statistical significance was not reached. It is important to note that the sample size for the comparison between the MTX-exposed post-conception cohort and the disease-matched cohort was only sufficient to detect a 3-fold increase in risk of major birth defects, given a baseline risk of 3% with a power of ~80%.

Of note, none of the fetuses/infants clearly exhibited the typical MTX embryopathy. However, there were 2 fetuses with anomalies that merit further discussion. One fetus exposed to MTX until week 5 plus 1 day had serious limb reduction defects (a detailed ultrasound report was not available), and 1 fetus exposed to MTX until week 3 plus 1 day had holoprosencephaly and megabladder without skeletal or limb defects on prenatal ultrasound. Autopsy of these fetuses was not performed. Previously published reports of holoprosencephaly (13,27) suggest that this malformation might require additional attention.

An international panel of rheumatologists has recommended discontinuing MTX at least 3 months before conception (33). This recommendation is based on the observation that MTX bound as glutamate conjugates can remain in the body for several months, particularly in liver cells. Interestingly, except for the rate of elective termination, we found no increased risk of any study end point among the women whose only exposure was within the 3-month window prior to conception. Furthermore, the observed birth defects in this group were not at all indicative of MTX embryopathy. Although our study suggests that a 3-month MTX-free interval prior to conception may not be clinically relevant, recommendations should not yet be changed, considering the limited power of our study (with 136 pregnancies exposed to MTX before conception).

The strengths and limitations of prospective observational studies of pregnancy outcomes have recently been discussed in detail (34). Although this is the largest study published to date on administration of MTX, at dosages typically used in the treatment of rheumatic diseases, before or during pregnancy, the sample size is still limited in power to address all objectives. Furthermore, only a few women received MTX during the entire period of organogenesis, meaning there was an even smaller informative subsample for all sensitive organ-specific periods. In addition, we cannot provide information on disease activity; however, RA itself does not seem to pose a risk of birth defects.

It is possible that some birth defects were not detected, especially given the high rate of elective termination in the MTX-exposed cohorts. For personal reasons, autopsy of the fetus is not regularly performed when a subject elects to terminate a pregnancy. However, it does not seem plausible that aborted fetuses among MTX-exposed women would have been less likely to be examined than aborted fetuses among comparison cohorts.

With respect to the risk of spontaneous abortion, in cohort studies in which enrollment takes place after recognition of pregnancy, it is not possible to evaluate the risks of very early spontaneous pregnancy losses. Although the survival methods we used corrected for delayed study entry, our data do not allow estimates of miscarriage risks before the earliest gestational age when subjects enrolled (roughly before week 5).

Finally, although we cannot completely rule out any bias, the prospective approach of our study with similar procedures of ascertainment across cohorts and contributing centers makes substantial bias in the ascertainment of exposure and the outcome data unlikely.

In conclusion, our study did not demonstrate an increased risk for the typical MTX embryopathy in

pregnancies exposed to low-dose MTX after conception. This does not exclude teratogenicity of weekly low doses, but makes high risk unlikely. Furthermore, the overall moderate but significant increase in the number of major birth defects in our MTX-exposed post-conception cohort compared to our comparison group of women without autoimmune diseases, together with the substantial increase in spontaneous abortion, suggests embryotoxicity and warrants caution. In the case of inadvertent exposure during early pregnancy, treatment should be stopped immediately and a level II ultrasound should be offered in order to examine fetal development. Although our study results suggest that a 3-month MTX-free interval prior to conception may not be necessary, further studies are needed to substantiate or refute the need for this measure of precaution.

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All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Weber-Schoendorfer had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Weber-Schoendorfer, Chambers, Shechtman, Clementi, Malm, Koren, Vial, Ornoy, Meister, Schaefer.

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