Association between blocking folate receptor autoantibodies and subfertility

The association between blocking folate receptor (FR) autoantibodies and subfertility was investigated in a longitudinal study of women attempting to become pregnant. Seventeen women with subfertility (failure to conceive during 12 menstrual cycles) and 25 control women (women who conceived and went on to have normal pregnancy outcomes) were studied. Subfertility risk was 12 times higher in women with blocking FR autoantibodies compared with those without (odds ratio, 12; 95% confidence interval, 1.9–129.6). (Fertil Steril® 2009; 91:1518–21. ©2009 by American Society for Reproductive Medicine.)
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Folate plays an important role in human reproduction (1, 2). It is essential for genomic integrity and DNA methylation (3). Insufficient folate supply during the rapid cell division phase after conception has been associated with neural tube defects (NTDs) (4–6), impaired embryo viability and implantation (7), increased rate of apoptosis in human cytotrophoblastic cells (8), and risk of spontaneous abortion (9). Immunohistochemical evidence of folate receptor (FR) expression in different reproductive tissues of both pregnant and nonpregnant female rats and in different anatomical components of the embryo at different stages of development has been reported, and a specific antiserum capable of blocking the binding of folate to its receptor before conception has been proposed as a cause of infertility (10). Maternal FR autoantibodies capable of blocking folate transport have been reported to be more frequent in women with NTD-affected pregnancies than in controls (11, 12). Blocking FR autoantibodies in children have been associated with cerebral folate deficiency (13, 14), autism (15), and Rett syndrome (16).

Approximately 25% of pregnancies are clinically undetected due to early pregnancy loss (17), and 12% end in spontaneous abortion (18). Subfertility [lower than normal probability of conception during a given menstrual cycle (19)] is another complication associated with the very early stages of conception and human development. The timing and difficulty in detecting these events complicates the investigation of their etiology, especially in the case of a first-time occurrence.

Blockage of FR by maternal FR autoantibodies during the periconception period could alter folate bioavailability during the primary stages of cellular division and embryogenesis. This could lead to subfertility/early pregnancy loss. The aim of this study was to investigate the association between maternal blocking FR autoantibodies and subfertility.

Women planning pregnancy participated in the PREC (PRE Conception) longitudinal study of maternal nutritional status from preconception throughout pregnancy (Unit of Preventive Medicine and Public Health, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili, and Unit of Obstetrics and Gynecology, University Hospital Sant Joan, Reus, Spain [1991–96]) (20–22). Ethics committee approval was obtained, and signed informed consent was provided by the participants in accordance with the Declaration of Helsinki (23). Exclusion criteria were previous diagnosis of infertility or chronic illnesses affecting nutritional status and use of medication interfering with folate metabolism. Data regarding lifestyle, toxic habits, vitamin/mineral supplement, and recent oral contraceptive use were collected, and body mass index BMI) was recorded.

The collection of a fasting blood sample between days 7 and 12 of the menstrual cycle was scheduled on the first day of that cycle. Samples from the antecubetal vein were collected into EDTA-K_3-treated vacutainers, kept at 4°C, and processed within 2 hours of collection. Plasma was stored in aliquots at −20°C. If the participant had not become pregnant during three cycles, another blood sample was collected. Subfertility cases were participants who after providing three preconception blood samples had not become pregnant and who confirmed not being pregnant when contacted to schedule a fourth preconception sample (after 12 menstrual cycles of trying). Seventeen cases of subfertility were detected. Controls were 25 participants

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who became pregnant after providing a preconception blood sample and provided further blood samples at weeks 8, 20, and 32 of gestation and at labor and from the umbilical cord from normal term pregnancies.

None of the participants took folic acid supplements during the periconception phase of the study. The study was carried out before the current recommendation for periconceptional folic acid supplementation was in place in Spain. Flour in Spain is not fortified with folic acid.

Testing for blocking autoantibodies against FR was performed at the State University of New York Downstate Medical Center as described elsewhere (11, 13). Briefly, 200 μL of serum was acidified with 300 μL of 0.1 molar glycine/HCl, pH 2.5/0.5%, Triton X-100/10 mM EDTA and was added to 12.5 mg of dextran-coated charcoal pellets to remove free folate. After centrifugation, the supernatant was collected and the pH was raised to 7.4 with 62 μL of 1 M dibasic sodium phosphate. This sample was incubated overnight at 4°C with 0.34 pmol of apo-FR purified from bovine milk. Tritiated ([^{3}H]) folic acid was added, and the mixture was incubated for 20 minutes at room temperature. Free [^{3}H]folic acid was adsorbed to dextran-coated charcoal, and receptor-bound radioactivity in the supernatant fraction was determined. Blocking autoantibodies prevent the binding of [^{3}H]folic acid to FR, and the autoantibody titer is expressed as picomoles of FR blocked/milliliter of plasma. Total fasting plasma homocysteine (tHcy) was determined by the IMx immunoassay (Abbott Laboratories, Diagnostics Division, Abbott Park, IL).

Statistical analysis was performed using the Statistical Package for the Social Sciences, version 15.0 (SPSS, Chicago). Frequency of blocking FR autoantibody occurrence and smoking habits and use of oral contraceptives (dichotomic variables) between cases and controls was compared using the χ^2-test, and mean age and BMI were compared using analysis of variance. The odds ratio (OR) of having subfertility when positive for blocking FR autoantibodies compared with being negative for them was estimated by logistic regression analysis adjusting for age, smoking habit, BMI, and tHcy. $P<.05$ was considered statistically significant.

The baseline characteristics summarized in Table 1 did not differ significantly between cases and controls. A total of 83 repeated blood samples collected 10–12 weeks apart were available for cases, and 104 were available for controls. A total of 18/83 samples collected from cases had positive FR autoantibody titers compared with 1/104 samples collected from controls ($P<.001$). At least one positive reading for FR autoantibodies was observed in 29.4% (5/17; mean [SD] titer: 0.88 [0.39] pmol FR blocked/mL plasma) of the subfertility cases compared with in 4% (1/25; (titer: 0.19 pmol FR blocked/mL plasma) of the control group ($P<.05$). Autoantibody titers fluctuated to different degrees in the six women who had tested positive in repeated blood samples 12 weeks apart: 2/7 readings over 23 months, 1/10 readings over 31 months, 3/4 readings over 14 months, 5/6 readings over 15 months, 2/3 readings over 12 months, and 1/2 readings over 3 months were below the assay's detection limit (0.1 pmol FR blocked/mL). The remaining 36 women had negative readings for antibodies on all occasions (up to 7 times, each 10–12 weeks apart). The risk of subfertility was 12 times higher in women with autoantibodies compared with those without (OR, 12; 95% confidence interval [CI], 1.9–129.6; $P<.05$). A total of 5/17 cases went on to become pregnant after between 12 and 19 months of trying. Only one case positive for blocking FR autoantibodies became pregnant after 19 months. She went on to have a term pregnancy with the baby's birth weight between P_{25} and P_{50}.

Approximately 30% of cases of subfertility reported in previous studies were not explained by conventional causes (24, 25). In our sample of Spanish women of reproductive age, we observed that having autoantibodies that block folate transport at the FR level is a significant risk factor for subfertility. Folate cell delivery via the FR is especially important in reproductive processes (26). Impairment of this

TABLE 1

Baseline (preconception) characteristics of cases and controls for subfertility.

	Cases	Controls
N	17	25
Age in years, mean (SD)	30.52 (3.06)	29.30 (2.52)
BMI in kg/m^2, mean (SD)	23.78 (3.83)	22.94 (2.39)
Smokers, n (%)	7 (41.0)	7 (28.0)
Cigarettes/day, mean (SD)	10.2 (5.7)	9.4 (10.3)
Oral contraceptive[a] user, previous 6 months, n (%)	4 (28.6)	9 (37.5)
THcy in μmoles/liter, mean (SD) [n]	8.0[b] (3.1) [15]	8.5[b] (2.0) [24]

[a] Oral contraceptive use during the 6 months before starting the study.
[b] Mean of preconception visits.

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mechanism could affect DNA synthesis and repair and apoptosis and trigger oxidative stress mechanisms. This could lead to impaired gamete quality, implantation, fetus development, and pregnancy maintenance as reported in folate deficiency (27, 28). DNA hypomethylation has also been reported to affect the expression of Cyp19, the key gene of estrogen biosynthesis (29).

The importance of FR in cellular folate uptake and the difficulty in successfully conceiving when FR function is inhibited were previously reported in rats (10). Maternal blocking FR autoantibodies have been associated with developmental problems in the embryo during the very early stages of pregnancy (11). Reduced folate is essential in preimplantation embryos (7). Conceivably, in the case of subfertility, insufficient, reduced folate during the rapid cell division phase after conception may prevent embryo viability and implantation or pregnancy maintenance (7, 10). We investigated the presence of blocking FR autoantibodies in repeated samples collected longitudinally and at relatively close time intervals. We detected blocking FR autoantibodies both before and during pregnancy and observed that in some cases titers fluctuated to levels below the detection limit. Such fluctuations in antibody titer especially when the titer is low have been observed in other autoimmune conditions (30, 31). Questions as to what triggers FR antibody response and what factors contribute to fluctuations in the titer are fundamental issues pertaining to all autoimmune disorders. Significant decreases in antibody titer were observed in another study on eliminating cow's milk and related products from the diet of children with positive blocking FR autoantibody titers (14). This led to the hypothesis that the production of blocking FR autoantibodies is caused by exposure to the antigen (cow's milk FR) because of a defect in the immunological barrier of the gastrointestinal tract.

Further investigation is required to understand the nature of the association between FR autoimmunity and subfertility and the potential benefits of using immune suppressants, corticosteroids, and high-dose folic acid in this disorder.

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