Oral dydrogesterone treatment during early pregnancy to prevent recurrent pregnancy loss and its role in modulation of cytokine production: a double-blind, randomized, parallel, placebo-controlled trial

Ashok Kumar, M.D., Ph.D.,[a] Nargis Begum, M.Sc., Ph.D.,[a] Sudha Prasad, M.D.,[a] Sarita Aggarwal, M.D.,[b] and Shashi Sharma, Ph.D.[c]

[a] Department of Obstetrics & Gynecology, Maulana Azad Medical College & Lok Nayak Hospital, New Delhi; [b] Department of Biochemistry, Maulana Azad Medical College, New Delhi; and [c] Institute of Cytology and Preventive Oncology, Noida, India

Objective: To study the impact of administration of dydrogesterone in early pregnancy on pregnancy outcome and its correlation with Th1 and Th2 cytokine levels.
Design: Double-blind, randomized, placebo-controlled study.
Setting: A medical college and its associated hospital.
Patient(s): Women with either: [1] a history of idiopathic recurrent pregnancy loss (RPL), in either a dydrogesterone group or a placebo group, or [2] no history of miscarriage.
Intervention(s): Dydrogesterone 20 mg/day from confirmation of pregnancy to 20 weeks of gestation.
Main Outcome Measure(s): Occurrence of another pregnancy loss and concentrations of T-helper (Th)1 (interferon-γ and tumor necrosis factor-α) and Th2 (interleukin (IL)-4 and IL-10) cytokines in serum at recruitment (4–8 weeks of gestation) and at abortion or 20 weeks of gestation, using commercially available ELISA kits.
Result(s): Occurrence of another abortion after 3 consecutive abortions was significantly higher (29 of 173; 16.76%) in women with RPL compared with healthy pregnant controls (6 of 174; 3.45%). Risk of occurrence of miscarriage after 3 abortions was 2.4 times higher in the placebo group vs. the treatment group (risk ratio = 2.4, 95% CI = 1.3–5.9). Mean gestational age at delivery (excluding those aborted before 20 weeks of gestation) increased significantly in the dydrogesterone group (38.01 $\pm$ 1.96 weeks) compared with the placebo group (37.23 $\pm$ 2.41 weeks). Baby weight was significantly lower in the placebo group (2421.4 $\pm$ 321.6 g) compared with the healthy pregnant controls (2545.3 $\pm$ 554.3 g). At recruitment, serum IL-4 and tumor necrosis factor-α levels were significantly lower in the RPL group compared with the healthy pregnant controls. However, serum interferon-γ level was significantly higher in the RPL group (8.87 $\pm$ 0.72 pg/mL) compared with the healthy pregnant controls (8.08 $\pm$ 1.27 pg/mL).
Conclusion(s): The present study supports the use of dydrogesterone in women with recurrent abortions to improve pregnancy outcome, such as a reduction in abortions and improved gestational age and baby weight at delivery. However, these outcomes were not modulated by Th1 and Th2 cytokine production.
Clinical Trial Registration Number: CTRI/2010/091/000373. (Fertil Steril® 2014;102:1357–63. ©2014 by American Society for Reproductive Medicine.)
Key Words: Recurrent pregnancy loss, dydrogesterone, Th1 cytokines, Th2 cytokines, pregnancy outcome

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/kumara-dydrogesterone-rpl-cytokine-production/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received March 1, 2014; revised July 26, 2014; accepted July 28, 2014; published online September 17, 2014.
A.K. has nothing to disclose. N.B. has nothing to disclose. S.P. has nothing to disclose. S.A. has nothing to disclose. S.S. has nothing to disclose.
Reprint requests: Ashok Kumar, M.D., Ph.D., Department of Obstetrics & Gynecology, Maulana Azad medical College, 13 B, DDA Flats, Ber Sarai, New Delhi 110016, India (E-mail: ashkumar@eth.net or ash64kr@yahoo.com).

Fertility and Sterility® Vol. 102, No. 5, November 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.07.1251

Spontaneous abortion is one of the most common complications of pregnancy, affecting 15% of pregnant women (1). Approximately 0.5%–2% of women experience recurrent miscarriage, defined as 3 or more

consecutive pregnancy losses before 20 weeks of gestation (with the same partner) (2). Despite several well established etiologic factors, the cause of recurrent pregnancy loss (RPL) cannot be determined in almost 50% of cases. It was suggested that these unexplained RPLs might be due to immunologic factors (3). A successful pregnancy depends on tolerance of a semiallogenic embryo/fetus by the maternal immune system. It is believed that this tolerance depends on the interactions of various cytokines excreted by maternal and fetal cells at the site of implantation. A shift from pro-inflammatory T-helper (Th)1 cell-dependent cytokines to Th2-dependent anti-inflammatory cytokines seems to be a characteristic of a successful pregnancy (4).

In women suffering from repeated spontaneous abortion, there is a difference in the Th1/Th2 cytokine ratio compared with that of women who do not have RPLs. Raghupathy et al. (5) found that the ratio of interferon (IFN)-γ/interleukin (IL)-10 produced by mitogen-stimulated peripheral blood mononuclear cells (PBMC) is 0.5 in first-trimester women without RPL as compared to 15 in women with RPL.

Progesterone might play a significant role in establishing an adequate immune environment at early stages of pregnancy (6, 7). The immunological pregnancy-protective effect of progesterone could also be, in part, manifested via controlling cytokine production (8). The progesterone deficiency has been associated with insufficient endometrial maturation and inadequate regulation of inflammatory cytokines. Therefore, support with progesterone may help to establish a sufficient immune response in early pregnancy and prevent miscarriage. Dydrogesterone is an orally active progestogen that is similar to endogenous progesterone in its molecular structure and has a high affinity for progesterone receptors. It has no androgenic side effects in the mother and no masculinizing effect on the female fetus. A study by Raghupathy et al. (9) showed that the levels of Th1 cytokines decreased significantly, whereas Th2 cytokines increased significantly, when the PBMC were exposed to dydrogesterone or progesterone.

Therefore, keeping in view the background that the supplementation of dydrogesterone during early pregnancy may modulate the Th1/Th2 balance, thereby leading to successful pregnancy outcome, the present study was designed to look at the impact of administration of dydrogesterone in early pregnancy on pregnancy outcome and its correlation with Th1 and Th2 cytokine levels (IL-4, IL-10, IFN-γ, and TNF-α).

MATERIALS AND METHODS

The present study was a double-blind, randomized, parallel, placebo-controlled study; therefore, placebo tablets containing 10 mg of lactose were prepared to look identical to the dydrogesterone tablets. The blinding of study participants and investigators (A.K. and S.P.) was done by assigning coded numbers to the packets, each containing 60 tablets of dydrogesterone or placebo, according to a simple randomization sequence developed with the use of computers by S.S. The packets were then distributed to the participants by N.B., using the random numbers in sequence. The randomization code was disclosed after completion of the study. The study was approved by the Institutional Ethics Committee, Maulana Azad Medical College, New Delhi, India.

Assuming RPL rates of 13.4% in the dydrogesterone group, 29% in the placebo group (10), and 3% in the healthy pregnant control group, with alpha 5%, power 80% with 1:1, at least 489 cases in 3 groups (163 in each) are needed. Given these factors and further assuming 10% loss to follow-up, 540 cases (180 in each) were enrolled into the study. The randomization was done in RPL cases (360) using computer-generated random numbers.

A total of 388 consecutive patients with a history of RPL who registered in the antenatal clinic were screened, and 360 patients fulfilling the inclusion criteria were recruited from the department of Obstetrics & Gynaecology, Maulana Azad Medical College and the associated Lok Nayak Hospital, New Delhi, India, between May 2010 and April 2013. A written informed consent form was taken from the recruited subjects. The patients were evaluated on the basis of a predesigned and pretested proforma with respect to history and clinical examination, and ultrasonography was performed to check fetal heart activity.

The inclusion criteria were that patients be age 18–35 years, with a history of ≥ 3 first-trimester pregnancy losses and be currently in the first trimester of a live pregnancy, preferably at 4–8 weeks of gestation (blood levels of human chorionic gonadotropin (hCG) followed by fetal heart activity). The exclusion criteria were patients with ≥ 3 spontaneous abortions, with known causes of recurrent spontaneous miscarriage evaluated as per hospital protocol (anatomical, hormonal, chromosomal, autoimmune, or infection) and who had taken injection of hCG and proluton (hydroxyprogesterone).

The patients were randomly assigned to orally take 2 tablets of 10-mg dydrogesterone or placebo daily from the time of enrollment to 20 weeks of gestation. They were asked to return the packets at each subsequent prenatal visit, when they were given the date of the following visit, regardless of the number of tablets taken. Age-matched healthy pregnant women with no history of miscarriage and at least 1 live birth were recruited as controls. These women with live pregnancy were enrolled at 4–8 weeks of gestation and followed up until 20 weeks of gestation.

A 5-ml venous blood sample was drawn from the subjects and healthy controls at the time of enrollment and then again at 18–20 weeks of gestation or at the time of abortion. The serum levels of cytokines (IL-4, IL-10, IFN-γ, and TNF-α) were estimated using commercially available ELISA kits according to the manufacturer's protocol.

Statistical Analysis

Data were analyzed using SPSS statistical software for Windows, version 20.0. The distribution of qualitative variables was compared by chi-squared test/Fisher's exact test. The Kruskal-Wallis test (one-way analysis of variance for nonparametric) was used, and pairwise comparisons were done by Mann-Whitney U test by adjusting the probability. Wilcoxon's signed rank test was used to see the change in cytokine level after the drug treatment. $P \leq .05$ was considered statistically significant.