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Miscarriage in the first trimester according to the presence or absence of the progesterone-induced blocking factor at three to five weeks from conception in progesterone supplemented women

J.H. Check, E. Levin, A. Bollendorf, J. Locuniak

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine if the failure to detect the immonomodulatory protein progesterone induced blocking factor (PIBF) at three to five weeks of seemingly normal pregnancies in women supplemented by extra progesterone is associated with a higher miscarriage rate.

Methods: Progesterone-induced blocking factor expression by lymphocytes was measured by an immunocytochemistry technique. The serum beta human chorionic gonadotropin (hCG) and/or ultrasound were also deemed appropriate so that by these criteria there was no evidence of a poor pregnancy. The minimum progesterone dosage was 200 mg twice daily vaginal suppositories.

Results: Progesterone-induced blocking factor was detected in 17/39 (43.5%) of pregnant patients at this early time. There were three miscarriages by 12 weeks in this group (17.6%). The miscarriage rate was 6/21 (28.5%) in those where it was not detected.

Conclusions: There was insufficient power to show significance. However there seems to be a trend for higher rates of miscarriage when PIBF is absent so these preliminary data encourage continuation of the study.

Key words: Progesterone; Immonomodulatory protein; Miscarriage.

Introduction

Increased progesterone (P) sensitivity of pregnancy lymphocytes is due to the activation-induced appearance of P binding sites in the lymphocytes [1]. Following recognition of fetally derived antigens, gamma/delta TCR+ cells develop P receptors [1]. Progesterone binding results in the synthesis of a mediator protein named the progesterone-induced blocking factor (PIBF) [1].

Progesterone-induced blocking factor by acting on the phospholipase A1 enzyme interferes with arichodoric acid metabolism, induces a Th2 biased immune response, and by controlling natural killer (NK) cell activity, exerts an anti-abortive effect [1].

Lower levels of PIBF in pregnancy could be related to insufficient P or insufficient induction of P receptors in the gamma/delta TCR+ leukocytes by an insufficient allogeneic stimulus.

The present study evaluated the presence of PIBF in women aggressively supplemented with extra P from the early luteal phase at three to five weeks from conception to see if any association could be found with absence of PIBF expression and spontaneous abortion.

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Materials and Methods

Blood was collected from women three to five weeks from conception. A prerequisite was that all women were receiving at least 200 mg/day supplemental P which was started from the early luteal phase. The date of conception was determined by serial ultrasound and hormonal evaluation (serum estradiol (E2), luteinizing hormone (LH), and P, or in the case of in vitro fertilization (IVF) the day of oocyte retrieval). At the time that the blood for PIBF was obtained the pregnancy appeared viable based on rate of rise in serum beta human chorionic gonadotropin (hCG) level and/or ultrasound criteria.

The PIBF Assay:

Progesterone-induced blocking factor expression was determined by an immunocytochemistry method using a PIBF polyclonal antibody. Whole blood was collected three to five weeks from conception. Mono-nuclear cells were removed using Isoprep and cold centrifugation and were adjusted to a concentration of $2x10^6$/ml; 100 µl aliquots of cell suspension were added to sample chambers and then fixed in cold acetone. The cells were first incubated with protein blocking factor agent and then incubated overnight with anti-PIBF. The white blood cells were then washed in PBS and covered with anti-rabbit peroxidase. After another PBS wash, a fresh chromogen solution was added and the cells incubated. The reaction was then stopped with distilled water. The white blood cells were then counterstained with hematoxylin and the slides read under oil immersion. A positive reaction was indicated by a reddish precipitate at sites of specific cellular antigen localization. Three hundred cells

were counted. A test was considered positive if there were at least four lymphocytes of 300 demonstrating reddish precipitate.

The women were monitored by serial beta hCG tests and ultrasounds. The PIBF tests were only performed and recorded for those women whose pregnancy appeared to be normal by hCG and ultrasound criteria.

Results

Between three to five weeks from conception 17 of 39 women tested (43.5%) expressed PIBF on their lymphocytes (Table 1). Spontaneous abortion occurred in three of 17 (17.6%) of women expressing PIBF (Table 1). Spontaneous abortions occurred in six of 21 (28.5%) women in which PIBF was not detected. Though 50% more women miscarried when PIBF was not detected, the study did not have sufficient power to show a significant difference (p = NS).

Table 1. — *Pregnancy outcome according to the presence or absence of progesterone-induced blocking factor in the early first trimester.*

	PIBF = yes	PIBF = no
# Patients	17	22
# Ongoing (fetal viability at 6 weeks)	12	15
% Ongoing	70.6	68.2
# Fetal demise	3	6
% Fetal demise	17.6	27.3
# Threatened abortion	2	0
% Threatened abortion	11.8	0.0
# Chemical	0	1
% Chemical	0.0	4.5

Discussion

A previous study using a similar assay compared PIBF expression in pregnant women between the 9th and 40th week of gestation (our study was much earlier 5th - 7th week of gestation) [2]. The aforementioned study found that the percentage of PIBF-expressing lymphocytes in the peripheral blood of 96 healthy pregnant women was 67% vs 6.5% in 62 women with pathological pregnancies [2].

There are several differences in study design with the study by Szekeres-Bartho *et al.* and the present one: our testing was much earlier and thus is more suggestive that lack of detection of PIBF expression may be a cause rather than a result of miscarriage. The present study only evaluated women who were still taking supplemental P whereas the patients in the study by Szekeres-Bartho *et al.* were not supplemented by P. The trend for lower PIBF expression in the early first trimester and subsequent miscarriage despite supplementation of P suggests the possibility that a potential etiology for some miscarriages may be the failure to generate sufficient P receptors on gamma/delta TCR+ lymphocytes by the fetal semi-allograft. There are data suggesting that lymphocyte immunotherapy (LIT) increases the expression of PIBF by lymphocytes even in non-pregnant women during the luteal phase [3]. There are data suggesting that women with a history of recurrent miscarriage receiving LIT and P supplementation vs P supplementation alone have lower miscarriage rates [4].

The immunocytochemistry assay is cumbersome and not conducive to obtaining rapid results. The PIBF antigen has been recently purified and an ELISA assay is in the works in Europe by Dr. Szekeres-Bartho and her group. The ELISA assay would allow rapid serial measurements of PIBF.

If the use of such an assay confirms in a larger series that low PIBF expression in the early to mid first trimester is associated with miscarriage despite P therapy, then this would be a target group to determine if immunotherapy could improve outcome.

References

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Address reprint requests to:
J.H. Check, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)