

# Evidence that the Expression of Progesterone-Induced Blocking Factor by Maternal T-Lymphocytes Is Positively Correlated with Conception

JEROME H. CHECK, MARIA ARWITZ, JENIFER GROSS, JULIA SZEKERES-BARTHO, AND CHUNG H. WU

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**PROBLEM:** To compare the expression by T-lymphocytes of an immunomodulatory protein known as progesterone-induced blocking factor (PIBF) in conception versus non-conception cycles even when there has been definite fertilization and embryo formation.

**METHOD:** PIBF expression on T lymphocytes was measured using an immunohistochemical method with a PIBF-specific polyclonal antibody. These levels were determined in patients undergoing three types of therapy: non-in vitro fertilization (IVF), IVF-embryo transfer (ET), and frozen ET. Sera were drawn 12 days from ovulation in non-IVF cycles or 9 days after ET and were assayed for PIBF and beta human chorionic gonadotropin. Comparison of the frequency of lymphocyte expression of PIBF in pregnant versus non-pregnant women were made.

**RESULTS:** PIBF was detected in 29.5% of non-pregnant women and 52.5% of pregnant women. There were no differences in PIBF levels by therapy used in non-pregnant cases or in the pregnant group.

**CONCLUSION:** These data are consistent with the hypothesis that maternal expression of PIBF in T-lymphocytes soon after trophoblast invasion may depend on successful implantation.

**Key words:**

Immunomodulatory protein, implantation, luteal phase.

JEROME H. CHECK  
MARIA ARWITZ  
JENIFER GROSS  
CHUNG H. WU

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 and Infertility, Camden, New Jersey

JULIA SZEKERES-BARTHO  
Department of Microbiology,  
University Medical School of Pecs,  
Pecs, Hungary

Address reprint requests to  
Jerome H. Check, M.D.,  
7447 Old York Road,  
Melrose Park, PA 19027.

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## CAPSULE

Expression of a progesterone-induced immunomodulatory protein by T-lymphocytes was observed more often in the late luteal phase when successful pregnancy was achieved, even in patients having embryo transfer with established fertilization.

## INTRODUCTION

There is evidence that the known immunomodulatory effects of progesterone (P) during pregnancy may be, at least in part, related to the induction of a 34-kDa protein called progesterone-induced blocking factor (PIBF).<sup>1,2</sup> Based on some experimental data, one hypothesis for acceptance by the maternal host of the fetal allograft is that the paternal antigens of the embryo-fetus stimulate the development of nuclear P receptors on certain CD8+ T-lymphocytes<sup>3-5</sup>; the interaction of a high con-

centration of P with the receptor leads to the production of PIBF,<sup>6</sup> and the PIBF, in turn, may inhibit immunological destruction of the fetal allograft through its effect on cytokines and influence a shift from T helper (TH)1 to TH2 cytokines with a resulting suppression of CD56+ CD16+ natural killer (NK) cell activity.<sup>1,7</sup>

A lower frequency of lymphocytes expressing PIBF has been found in women threatening to abort when obtained late in the first trimester or in the second trimester and have been associated with a higher rate of pregnancy loss.<sup>8</sup> Recently, PIBF expression in circulating lymphocytes has been found in the late luteal phase, and the frequency of positive lymphocytes had a tendency to be higher at this time than during the early pregnancy period.<sup>9</sup>

There have been no studies to date where the frequency of lymphocyte expression of PIBF has been correlated with the establishment of a pregnancy. The study presented herein was designed to see whether any such correlation could be found.

## MATERIALS AND METHODS

The study group consisted of 234 women presenting with infertility. They were divided into three groups according to their therapy: non-in vitro fertilization (IVF), IVF-embryo transfer (ET), or frozen ET.

PIBF expression on T-lymphocytes was measured 12 days from ovulation or 9 days from ET. Serum beta human chorionic gonadotropin (B-hCG) levels were obtained at the same time.

### PIBF Assay

The measurement of PIBF was determined by immunocytochemistry using a PIBF-specific polyclonal antibody. Mononuclear cells were removed using Isoprep (Robbins Scientific, Sunnyvale, CA) and cold centrifugation and were adjusted to a concentration of  $2 \times 10^6$ /ml; 100 ml aliquots of cell suspension were added to sample chambers and air dried then fixed in cold acetone. The cells were first incubated with protein blocking agent then incubated overnight with anti-PIBF. The cells were washed in phosphate-buffered saline (PBS; GIBCO BRL Life Technolo-

gies, Grand Island, NY) then were covered with anti-rabbit peroxidase. After a second PBS wash, fresh chromogen solution was added and the cells were incubated; the reaction was then stopped with distilled water, and the cells were counter-stained with hematoxylin. The slides were cover-slipped and were read under oil immersion (100 $\times$  objective). A positive reaction was indicated by a reddish precipitate at sites of specific cellular antigen localization; 300 cells were counted and the percentage of the positive cells was then determined. A level of >1% was considered positive.

### Statistical Analysis

The median levels of PIBF-expressing lymphocytes were compared by conception outcome using the Mann-Whitney *U* test. The proportion of patients demonstrating PIBF expression (levels >1%) were compared by conception using chi-square analysis. A *P* value of 0.05 was used.

## RESULTS

Serum B-hCG levels were positive in 139 patients and were negative in 95. Expression of PIBF by T-lymphocytes (i.e., level >1%) was detected in 28 of 95 (29.5%) women with negative B-hCG levels versus 73 of 139 (52.5%) women with positive tests for B-hCG (*P* < 0.05).

PIBF expression according to a positive or negative serum B-hCG in the three groups of non-IVF, IVF-ET, and frozen ET are seen in Table I. Though the group having IVF-ET was the only one showing a significant difference in PIBF expression, comparing those with positive versus negative pregnancy tests, the non-IVF group was extremely close to significance (*P* = 0.054).

There were significant differences (Mann-Whitney *U* Test) between median PIBF expression in pregnant versus non-pregnant patients for non-IVF patients and patients having frozen ETs, and there was a trend toward significance in those having IVF-ET (Table II).

The mean number of embryos transferred was  $3.4 \pm 0.8$  for fresh transfers and  $3.6 \pm 1.0$  for frozen transfers. In both therapies, the number of embryos transferred ranged from 1 to 5.

TABLE I. Progesterone-Induced Blocking Factor (PIBF) Expression According to Conception

	Non-conception cycles		Conception cycles	
	n	Percentage with PIBF > 1%	n	Percentage with PIBF > 1%
Non-IVF <sup>a</sup>	39	38.5% (15) <sup>c</sup>	73	57.5% (42)
IVF-ET <sup>b</sup>	28	17.8% (5)	37	51.3% (19)
Frozen ET	28	28.6% (8)	29	41.4% (12)
All patients <sup>a</sup>	95	29.5% (28)	139	52.5% (73)

<sup>a</sup>*P* = 0.054, chi-square. IVF, in vitro fertilization; ET, embryo transfer.

<sup>b</sup>*P* < 0.05, chi-square, comparing positive beta human chorionic gonadotropin (B-hCG) to negative B-hCG.

<sup>c</sup>Number of cycles in parentheses.

TABLE II. Median Levels of Progesterone-Induced Blocking Factor (PIBF) Expressing Lymphocytes by Conception

Patient group	Median PIBF expression	
	Non-conception cycles	Conception cycles
Non-IVF <sup>a</sup>	0.3	1.3
IVF-ET	0.0	0.7
Frozen ET	0.0	1.5

<sup>a</sup>P < 0.05 using Mann-Whitney U test. IVF, in vitro fertilization.

<sup>b</sup>ET, embryo transfer.

## DISCUSSION

The failure to establish a positive pregnancy test after the exposure of sperm and oocyte to each other may be related to failed fertilization or poor implantation. The secretion of PIBF could possibly occur from some factor released by the embryo shortly after fertilization in the fallopian tubes, by some factor released by the embryo(s) when it reaches the uterine cavity; or finally by implantation and early invasion of the trophoblast that elicits the generation of PIBF by the mother's T-lymphocytes.

The study presented herein was designed to help answer the question of what stage of embryo formation stimulates PIBF expression by T-lymphocytes by comparing pregnancy rates in those conceiving naturally compared to those having ETs. If, in fact, the mere presence of embryos reaching the uterine cavity is the stimulus for PIBF expression, then a higher percentage of women having ETs should demonstrate PIBF expression, because even those with negative B-hCG levels would receive embryos. However, there were no significant differences or even trends for women having ETs to have higher PIBF expression by lymphocytes than in cases not having IVF-ET. The frozen ET group was added to see the effect of controlled ovarian hyperstimulation on PIBF expression, but no differences were found.

These data demonstrate a positive correlation of PIBF expression by maternal T-lymphocytes and positive B-hCG levels, suggesting that implantation may be needed to stimulate a rise in PIBF expression by T-lymphocytes as opposed to the mere presence of embryos in the uterine

cavity. The fact that PIBF was positive in 30% of patients with negative late luteal phase B-hCG levels could be related to early rejection of the embryo after implantation, resulting in negative B-hCG levels. The fact that the antibody used for the assay is polyclonal could also possibly account for some false-positive tests related to non-specific factors.

Thus, one possible use for testing PIBF expression in women not undergoing IVF may be that repeated failure to show expression of PIBF in the luteal phase may be indicative of either failed fertilization or implantation failure. This finding could lead to an earlier trial of IVF-ET to determine whether fertilization is problematic; if normal fertilization is demonstrated, then attempts to improve implantation, e.g., increasing luteal phase support with progesterone, could be tried.

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