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The Impact of Dydrogesterone Supplementation on Hormonal Profile and Progesterone-induced Blocking Factor Concentrations in Women with Threatened Abortion

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PROBLEM: The therapeutic value of progestogens in threatened abortion is still under debate. In the presence of sufficient progesterone levels during pregnancy, lymphocytes synthesize a mediator [progesterone-induced blocking factor (PIBF)] that is anti-abortive in mice. The aim of this study was to evaluate the effect of dydrogesterone on pregnancy outcome of threatened aborters.
METHOD OF STUDY: Twenty-seven threatened aborters were treated for 10 days with dydrogesterone (30–40 mg/day). Sixteen healthy pregnant controls received no treatment. Serum progesterone and estradiol concentrations as well as urine PIBF concentrations were measured by enzyme-linked immunosorbent assay (ELISA).
RESULTS: Pregnancy outcomes in dydrogesterone-treated threatened aborters did not statistically differ from those in healthy controls. Serum progesterone concentrations in control patients, but not those in threatened aborters increased as pregnancy progressed. Following dydrogesterone treatment, initially low PIBF concentrations of threatened aborters significantly increased ($P = 0.001$) to reach the PIBF level found in healthy controls.
CONCLUSIONS: These data suggest that by inducing PIBF production, dydrogesterone might improve pregnancy success rates in threatened aborters.

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INTRODUCTION

Spontaneous abortion is a common problem in everyday practice, affecting 15–20% of all recognized pregnancies. Fifty to sixty per cent of these are due to chromosomal abnormalities, maternal infections, genital tract or endocrine abnormalities, antiphospholipid antibodies, cigarette smoking or environmental factors.

Threatened abortion is manifested by vaginal bleeding and/or uterine cramps while the cervix is closed. This stage may end up in spontaneous abortion or, in alternative cases, pregnancy may proceed normally.

A substantial part of unexplained spontaneous abortions might be attributable to a deleterious immune response of the mother toward the fetus. A growing body of evidence suggests that progesterone might play a significant role in establishing an adequate immune environment during the early stages of pregnancy.[1–3]

In the presence of progesterone, lymphocytes of pregnant women release a protein named the progesterone-induced blocking factor (PIBF)[4] which mediates the immunomodulatory[5] and anti-abortive[6,7] effects of progesterone. Immunologic recognition of pregnancy and subsequent activation of maternal immune system

results in an upregulation of progesterone receptors on activated lymphocytes among placental cells and CD8 + cells.[8,9] In the presence of sufficient progesterone levels, these cells synthesize PIBF.

A significantly increased T-helper (Th)1 cytokine expression may be the underlying immune etiology for reproductive failure.[10,11] Patients at risk of preterm delivery presented increased interleukin (IL)-12 and low PIBF and IL-10 expression on lymphocytes.[12] Data presented by Sacks et al.[13] indicated that circulating monocytes are 'primed' to produce the Th1 cytokine IL-12 in normal pregnancy and Chaouat[14] suggested that the concept that pregnancy is a Th2 phenomenon cannot be generalized to the function of all aspects of maternal cellular immunity.

The immunological pregnancy-protective effect of progesterone is, in part, manifested via controlling cytokine production.[7] PIBF alters the profile of cytokine secretion of activated lymphocytes shifting the balance toward Th2 dominance.[15]

In cases with a history of unexplained pregnancy loss, one of the most promising way of therapeutic intervention is replenishing insufficient intrinsic progesterone levels by dydrogesterone (6-dehydroretroprogesterone).[16] This is an orally active progestogen with high affinity for progesterone receptors, being similar to endogenous progesterone in its molecular structure and pharmacological effect.

Still, there is considerable controversy about the use of progestogens for the treatment of threatened abortion. The question is whether there is a need for progesterone supplementation among women with clinically diagnosed threatened abortion and whether such intervention might help in achieving successful pregnancy among women with threatened abortion.

Therefore, the aim of this prospective study was to compare serum progesterone (P), estradiol (E2) concentrations and urine PIBF concentrations of women with threatened abortion with those of women with normal pregnancy and to evaluate the impact of dydrogesterone supplementation in the former group on the outcome of pregnancy as well as the possible underlying mechanisms.

METHODS

Patients
The study was approved by the Ethical Committee of the Medical University of Lodz, Poland (Decision No. RNN/30/02/KE). Each patient provided a written consent for participation.

The study group comprised 57 pregnant women between the 6th and 12th weeks of gestation, who were enrolled consecutively during a 1-year period. Thirty-six women showed clinical symptoms of threatened abortion (bleeding, spotting and uterine cramps) whereas 21 women had normal, healthy pregnancies (no clinical symptoms of threatened abortion observed either before or at the entry to the study) – reference group. Only singleton pregnancies were qualified for the study. Exclusion criteria were as follows: chronic diseases, e.g. hypertension, diabetes, renal or cardiac diseases or genital tract anomalies of the mother, genetic or anatomical defects of the fetus, and use of other progestogens prior to or during the study, hypersensitivity or medical contradiction to dydrogesterone. Five of 57 women who qualified for the survey did not show up for the second check up or refused to have blood sampling or second ultrasound. Three women used other progestogen-based drugs during the study, thus they were excluded from the survey. For six subjects, complete medical records of the newborns were not available. The final study group comprised 43 women: 27 with threatened abortion and 16 from the reference group.

A standard questionnaire concerning medical history, demographic, constitutional and environmental factors were filled in by each subject with special stress put on clinical signs of threatened abortion, e.g. bleeding, spotting and uterine cramps before and after the treatment.

Threatened aborters were treated with 30–40 mg/day of dydrogesterone (Duphaston, Solvay Pharmaceuticals B.V., Weesp, The Netherlands) for 10 days. Venous blood was drawn before and 10 days after the treatment had started. Control patients did not receive any treatment in between two examinations.

A detailed vaginal ultrasound was performed for all the subjects during the first examination to evaluate gestational age of the fetus (by crown–lump length measurements) and to exclude multiple gestation or fetal anomalies. This was repeated during the second examination. All the subjects were followed up until the termination of pregnancy. Gestational age at delivery, newborns' birth weight, and mode of delivery were recorded in Hospital Medical Database.

Determination of Hormone Concentrations
Serum progesterone and estradiol concentrations were measured by enzyme immunoassay (BioChem Immuno-Systems, Allentown, PA, USA) in both groups.

PIBF determination
Urine samples were collected from all the subjects during the first and second examination and PIBF urine concentration was measured by enzyme-linked immunosorbent assay (ELISA) as described earlier.[17]

Briefly, during overnight incubation at 4 °C, 96-well microtiter plates were coated either with anti-human recombinant PIBF IgG (100 μL/well of 2 μg/mL) in 50 mM carbonate buffer pH 9.6 (plate 1), or with human recombinant PIBF (100 μL of 0.5 μg/mL) in 0.5 M Tris buffer pH 6.5 (plate 2). For generating a standard curve, recombinant PIBF (1000 to 0.1 ng/mL) in logarithmic dilutions in 0.5 M phosphate buffer (pH 7.3–7.4) was incubated with a standard amount of biotin-labeled anti-recombinant PIBF IgG (400 ng) for 60 min at 37 °C. Urine samples were diluted 1/2.5 and 1/5 and incubated with 400 ng biotin-labeled anti-recombinant PIBF IgG in 0.5 M phosphate-buffered saline (PBS) for 60 min at 37 °C before added to ELISA plate 1. During the 1 h incubation at 37 °C, non-specific binding sites on plate 2 (coated with human recombinant PIBF) were blocked with 200 μL of 0.1% bovine serum albumin (BSA), 0.5% gelatin in PBS-Tween. After this incubation step, 100 μL standard solutions or the urine samples were transferred from plate 1 to plate 2 and incubated for 1 h at 37 °C. After washing the plates three times with PBS-Tween, 100 μl of 1:1000 diluted horse radish peroxidase (HRPO)-conjugated streptavidine (AP Hungary Ltd, Budapest, Hungary) in 0.1% BSA PBS-Tween was added and plates were incubated for 30 min at 37 °C. The reaction was developed by adding the substrate orthophenylene-diamine (OPD; Sigma, Budapest, Hungary) and measured at 495 nm.

Statistical Analysis
Student's *t*-test was used to compare the mean values. The distribution of qualitative variables was compared by chi-square test or by Fisher's exact test.

RESULTS

There was no difference between threatened aborters and controls in maternal age or gestational age at the time they entered the study. The mean interval between the first and second check up was also similar: 10.04 days in the threatened abortion group and 10.25 days in normal controls. Active smoking was declared by almost 25% of women in both groups. Women from threatened abortion group were less educated (Table I).

The Effect of Dydrogesterone on PIBF Concentration
Initially, PIBF concentrations in urine samples of patients showing clinical symptoms of threatened abortion were significantly lower than in those of healthy pregnant women (453.3 pg/mL versus 1057.94 pg/mL; $P = 0.008$). After dydrogesterone treatment of threatened aborters, PIBF concentrations significantly increased ($P = 0.001$) to 1291.59 pg/mL. This did not statistically differ from the respective values of healthy pregnant women at the second sampling (1291 pg/mL versus 1831 pg/mL) (Table II).

The Effect of Dydrogesterone Treatment on the Pregnancy Outcomes
There was no significant difference in pregnancy outcomes between threatened aborters and normal pregnancy. The length of gestation and the birth weight of the babies were similar in the two groups.

Three pregnancies with diagnosis of threatened abortion, while only one with a clinically normal pregnancy ended up in missed abortion. Two of threatened aborters delivered before 37th weeks of

	Threatened abortion (n = 27)	Healthy controls (n = 16)	P-value
Maternal age (yr; mean ± S.D.)	27.07 ± 4.20	26.13 ± 3.50	NS
Parity (mean ± S.D.)	1.63 ± 0.84	1.75 ± 1.29	NS
Gestational age at first sampling (weeks; mean ± S.D.)	7.94 ± 2.82	8.79 ± 2.47	NS
Gestational age at second sampling (weeks; mean ± S.D.)	9.48 ± 2.76	10.36 ± 2.37	NS
Interval between first and second samplings (days; mean ± S.D.)	10.04 ± 3.60	10.25 ± 2.46	NS
Smoking (%)	n = 6 (22.2)	n = 4 (25.0)	NS
Educational level [n (%)] with primary education	n = 3 (11.1 (%))	n = 0	NS

TABLE I. Comparison of Selected Maternal Characteristics in Women with Threatened Abortion and Healthy Pregnant Controls

TABLE II. The Effect of Dydrogesterone Treatment on Urine PIBF Concentrations of Healthy Pregnant Women and Patients with Threatened Abortion

PIBF (pg/mL)	Measurement I (mean $\pm$ S.D.)	Measurement II (mean $\pm$ S.D.)	P-value
Threatened abortion ($n = 27$)	453.3 $\pm$ 496.3	1291.6 $\pm$ 1132.9	0.008
Control ($n = 16$)	1057.9 $\pm$ 930.8	1831.6 $\pm$ 1979.2	0.26
P-value	0.001	NS	

TABLE III. The Effect of Dydrogesterone Treatment on Pregnancy Outcome

	Threatened abortion ($n = 27$)	Healthy controls ($n = 16$)	P-value
Missed abortion	3/27	1/16	NS
Preterm delivery	2/27	0/16	NS
Gestational age at delivery (weeks; mean $\pm$ S.D.)	39.2 $\pm$ 2.25	39.5 $\pm$ 1.12	NS
Newborns' birth weight (g; mean $\pm$ S.D.)	3373.57 $\pm$ 789.64	3436.67 $\pm$ 343.11	NS

TABLE IV. The Effect of Dydrogesterone Treatment on Serum Progesterone and Estradiol Concentrations in Healthy Pregnant Women and Patients with Threatened Abortion

	Measurement I (mean $\pm$ S.D.)	Measurement II (mean $\pm$ S.D.)	I/II
Progesterone (ng/mL)			
Threatened abortion ($n = 27$)	24.26 $\pm$ 11.5	22.13 $\pm$ 10.4	1.12
Control ($n = 16$)	21.95 $\pm$ 9.5	28.18 $\pm$ 9.56	0.91
P-value	NS	0.06	
Estradiol (pg/mL)			
Threatened abortion ($n = 27$)	603.21 $\pm$ 532.6	1042.11 $\pm$ 907.7	0.87
Control ($n = 16$)	694.5 $\pm$ 627.3	1092.52 $\pm$ 757.1	0.65
P-value	NS	NS	

gestation, whereas no pre-term delivery was registered in the control group (Table III).

The Effect of Dydrogesterone on Serum Sex Steroid Hormone Levels

Progesterone as well as estradiol concentrations of the first sample were similar in healthy pregnant women and threatened aborters. In normal pregnancy both progesterone and estradiol concentrations increased by the second sampling, whereas progesterone concentrations in the sera of threatened abortion patients failed to increase despite the dydrogesterone treatment. Progesterone concentrations measured in the second samples of the latter group were significantly lower than those of healthy pregnant women (Table IV).

We also compared mean serum progesterone levels and mean urine PIBF concentrations between women who aborted ($n = 4$) and women with subsequent successful pregnancies. Women who later aborted had similar progesterone levels at first sampling when compared with women who did not (19.15 ng/mL versus 24.42 ng/mL, $P = 0.3$). During the second sampling, mean serum P level was significantly lower among women who aborted when compared with women with subsequent successful gestation (13.22 ng/mL versus 26.98 ng/mL, $P = 0.01$). At first sampling, mean urine PIBF concentrations were lower among women who aborted when compared with women who did not (326.25 pg/ml versus 728.18 pg/mL, $P = 0.3$) and remained lower also at second one (656.25 pg/mL versus 1616.53 pg/mL, $P = 0.2$).

DISCUSSION

In this study, we have shown for the first time that dydrogesterone treatment of women with clinical symptoms of threatened abortion was associated with

an increased PIBF production. In threatened aborters undergoing such treatment, the length of gestation did not significantly differ from that of healthy pregnant women, nor did the mean birth weight of the new-borns. The incidence of pre-term delivery was still higher in the threatened abortion group, but the difference between the two groups did not reach statistical significance. For ethical reasons, we had no possibility to include a control group of untreated threatened aborters. However, in our experience, in threatened aborters treated with drotaverine hydro-chloride, promethazine and bed rest, the abortion rate is approximately 25%, which indirectly suggest a beneficial effect of dydrogesterone treatment.

Earlier Polgar et al.[17] showed that PIBF concentration continuously increased during normal pregnancy, but failed to do so in pathological gestation. The presently demonstrated PIBF induction in dydrogesterone-treated threatened aborters suggests that restoring normal PIBF concentrations could be a mechanism by which dydrogesterone supplementation might improve pregnancy rates. The same mechanism has been previously suggested by Joachim et al.[18] in murine model.

The therapeutic value of progesterone supplementation in threatened abortion has not been adequately established. Minimal progesterone support required for pregnancy in mice varies considerably at different stages of pregnancy and is partly modulated by estradiol.[19] Hormonal levels in early pregnancy may have predictive value with regard to the outcome of pregnancy.[20] Estradiol progesterone and total serum testosterone levels in patients with missed abortion or anembryonic pregnancies are significantly lower than in the normal group. The level of progesterone over 12.3 ng/mL was found to be sensitive and specific for detecting a normal pregnancy.[20] Progesterone may be a good marker to identify a putative threat of miscarriage in human and progesterone replacement therapy may abrogate this threat by inducing a Th2-biased immune response from decidua.[21]

A recent clinical review on the evaluation and management of threatened miscarriage[22] revealed that there are no convincing data on the impact of progesterone supplementation in threatened abortion, mainly because of the poor design of studies that have been conducted so far. Recently, a meta-analysis assessed the usefulness of progesterone supplementation on miscarriage rate in various clinical settings; however, it did not provide a separate analysis for progesterone in threatened miscarriage.[23] Furthermore, the question whether progesterone supplementation would be beneficial in women with normal progesterone levels is still open.

Joachim et al.[18] have shown that stressed animals presented lower levels of progesterone and PIBF in plasma and a reduced staining intensity of progesterone receptors at the feto–maternal interface, together with increased abortion rates. Injection of dydrogesterone abrogated the effect of stress on the abortion rate. Further, dydrogesterone increased levels of plasma PIBF in stressed mice, but did not affect progesterone levels. Blois et al.[24] reported that progesterone substitution with dydrogesterone corrected the abortogenic effects of stress exposure by decreasing the frequency of Th1 cytokines via a CD8-dependent pathway.

Progesterone levels in human show a narrow variation in the first trimester. Although in this study progesterone levels in the first samples of threatened aborters were not particularly low, stress associated with the symptoms of threatened abortion might have lowered progesterone concentrations by the time of the second sampling, thus progesterone supplementation might have had a beneficial effect. Despite Duphaston treatment, serum progesterone concentrations of threatened aborters were significantly lower at the time of the second sampling, than those of the controls. At the same time, PIBF concentrations increased following the treatment. This seemingly paradoxical finding is due to the fact that dydrogesterone is not detected by anti-progesterone antibodies, but as it binds to the progesterone receptor it is able to induce PIBF to the same extent as natural progesterone.[25]

These data suggest that by inducing PIBF production, dydrogesterone might improve pregnancy success rates in threatened aborters.

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