

# The Impact of Dydrogesterone Supplementation on Serum Cytokine Profile in Women with Threatened Abortion

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Cytokines, dydrogesterone, early pregnancy, threatened abortion

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## Problem

The role of increased Th1 cytokine expression in pregnancy failure has been questioned recently. The therapeutic value of progestogens in threatened abortion (TA) is still debated. The aim of this prospective study was to compare serum cytokine [tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-12 and IL-10] concentrations in women with TA to those in women with normal pregnancy and to evaluate the impact of dydrogesterone supplementation in the former group on cytokine concentration.

## Methods 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 cytokine concentrations were measured twice in both groups by enzyme-linked immunosorbent assay.

## Results

Mean serum concentrations of Th1- and Th2-type cytokines in women with TA did not differ from those in women with normal pregnancy at first and second sampling. After dydrogesterone supplementation, mean TNF- $\alpha$ /IL-10 ratio changed from 1.08 to 1.75 while IL-12/IL-10 ratio remained almost the same (0.56–0.61) in the threatened aborters group and did not differ from those in healthy women.

## Conclusions

The results of this study indicate that peripheral cytokine production in threatened aborters does not differ from that observed among healthy pregnant women. The protective effect of dydrogesterone supplementation in threatened aborters is manifested via restoring progesterone-induced blocking factor concentration rather than controlling cytokine production.

## Introduction

Spontaneous abortion is one of the most frequent adverse reproductive events in everyday practice, affecting even up to 15% of all recognized pregnancies.<sup>1</sup> Fifty to sixty percent of these are because of

chromosomal abnormalities, maternal infections, genital tract or endocrine abnormalities, antiphospholipid antibodies, cigarette smoking or demographic or environmental factors.<sup>2</sup>

Threatened abortion (TA), one of the most frequently recognized medical problems at early

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

In humans, the specific cellular and molecular mechanisms underlying the maintenance of normal pregnancy and the induction of abortion are still not clearly understood.<sup>3</sup>

A substantial part of unexplained spontaneous abortions might be attributable to a deleterious immune response of the mother towards the fetus. A significantly increased Th1 cytokine expression was often indicated as the immune etiology for reproductive failure.<sup>4,5</sup> According to Raghupathy,<sup>6</sup> cytokines secreted by Th2 cells, including interleukin (IL) 10, seem to be important for the maintenance of pregnancy through down-regulating Th1-type immunity.

Tumor necrosis factor (TNF)- $\alpha$  is an abortogenic cytokine, produced by both Th1 and Th2, cells which mediates cytotoxic activity and inflammatory responses and elicits production of interferon- $\gamma$ . TNF- $\alpha$  levels in Th1 responses are higher than in Th2 responses.<sup>7</sup>

Th1 cytokine-IL-12, plays an important role in the activation of maternal lymphocytes which could possibly result in pregnancy failure syndromes.<sup>8</sup> Szereday et al.<sup>9</sup> observed that patients at risk for pre-term delivery also presented an increased IL-12 and low IL-10 expression on lymphocytes. IL-12 is a macrophage and B cell-derived cytokine which is one of the most sensitive indicators of the Th1-type response and an inhibitor of the Th2-type response *in vivo*. IL-10 is involved in down-regulating Th1-type activity by inhibiting the production of interferon- $\gamma$ .

The role of increased Th1 cytokine expression in pregnancy failure has recently been questioned by Chaouat.<sup>10</sup> Sacks et al.<sup>11</sup> noted that circulating monocytes are 'primed' to produce IL-12, the Th1 cytokine, in normal pregnancy. In contrast to recurrent and spontaneous abortion, the immunological problems in TA have not been well identified.

Progesterone might play a significant role in establishing an adequate immune environment at early stages of pregnancy.<sup>12-15</sup> In the presence of progesterone during pregnancy, lymphocytes release a protein named the progesterone-induced blocking factor (PIBF) which mediates the immunomodulatory and anti-abortive effects of progesterone.<sup>16-18</sup>

As indicated by the findings of the first part of this study,<sup>19</sup> concerning the impact of dydrogesterone on

PIBF concentration in women with TA, dydrogesterone supplementation was associated with an increased PIBF production in humans.

The immunological pregnancy-protective effect of progesterone could also be, in part, manifested via controlling cytokine production.<sup>16</sup> PIBF alters the profile of cytokine secretion of activated lymphocytes, shifting the balance towards Th2 dominance.<sup>20</sup>

The data concerning cytokine profile in women with TA is scarce<sup>21,22</sup> and there are no reports concerning the impact of progestogens supplementation on this profile in humans. Therefore, the aim of the second part of the study was to compare serum cytokine (pro-inflammatory cytotoxic TNF- $\alpha$ , IL-12, and non-inflammatory IL-10) concentrations in women with TA to those in women with normal pregnancy and to evaluate the impact of dydrogesterone supplementation in the former group on cytokine concentration.

## 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 informed consent to participate in the project.

The study group comprised 57 pregnant women between the 6th and 12th week of gestation. Thirty-six women showed clinical symptoms of TA (bleeding, spotting and uterine cramps) whereas 21 women had normal, healthy pregnancies (no clinical symptoms of TA 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, and hypersensitivity or medical contradiction to dydrogesterone.

Five of 57 women qualified for the survey did not show up for the second check-up or refused to have blood sampling or the second ultrasound. Three women used other progestogen-based medications 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 TA and 16 as the reference group.

A standard questionnaire concerning medical history, demographic, constitutional and environmental factors was completed by each subject. Special stress was put on the clinical signs of TA, 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 the examinations.

A detailed vaginal ultrasound was performed for all the subjects during the first examination to evaluate the gestational age of the fetus (by crown-lump length measurement) and to exclude multiple gestation or fetal anomalies. This was repeated during the second examination.

All the subjects were followed until the termination of pregnancy. Gestational age at delivery, newborns' birthweight, and mode of delivery, were recorded in the Hospital Medical Database.

#### Determination of Serum Cytokines

Venous blood was collected and serum was isolated in both groups during the first and second examination. Serum was frozen at  $-70^{\circ}\text{C}$  and stored until the assay. Serum levels of cytokines (TNF $\alpha$ , IL-10 and IL-12) were measured using specific enzyme-linked immunosorbent assay (Tecan, Salzburg, Austria). A monoclonal antibody specific for TNF- $\alpha$ , IL-12 and IL-10 has been coated onto the wells of microtiter strips provided. Samples, including standards of known TNF- $\alpha$ , IL-12 and IL-10 concentrations, control specimens and unknowns were pipetted into these wells. Each sample was tested in duplicate and was blinded to the person conducting the assay.

#### Statistical Analysis of the Data

Student *t*-test was used to compare the mean values. The distribution of qualitative variables was compared by chi-squared test or by Fisher Exact Test. The *P*-value of  $<0.05$  was defined as statistically significant.

#### Results

There was no difference between threatened aborters and controls in maternal age or gestational age at the time they entered the study (Table I). The mean interval between first and second check-up was also similar: 10.04 days in the TA group and 10.25 days in controls.

#### Serum Cytokine Concentrations in Women with Threatened Abortion

In women with TA, mean serum concentrations of the evaluated cytokines did not differ from those in women with normal pregnancy at first sampling (Table II–IV). The second examination showed that TNF- $\alpha$  concentration was non-significantly higher in both groups, while IL-10 and IL-12 concentrations were non-significantly lower when compared with the first measurement, with no significant differences between the groups.

#### The Effect of Dydrogesterone Treatment on Serum Cytokine Concentrations

After dydrogesterone supplementation, the TNF- $\alpha$ /IL-10 ratio changed from 1.08 to 1.75 in threatened aborters and the respective change in controls was from 1.16 to 1.68. The IL-12/IL-10 ratio remained almost the same (0.56–0.61) as compared between two examinations among women in TA group and did not differ from that observed in

**Table I** Comparison of Selected Maternal Characteristics of Women with Threatened Abortion and Healthy Pregnant Controls

	Threatened abortion ( <i>n</i> = 27)	Healthy controls ( <i>n</i> = 16)	<i>P</i> -value
Maternal age (years)	27.07 $\pm$ 4.20	26.13 $\pm$ 3.50	0.45
Parity	1.63 $\pm$ 0.84	1.75 $\pm$ 1.29	0.71
Gestational age at first sampling (weeks)	7.94 $\pm$ 2.82	8.79 $\pm$ 2.47	0.32
Gestational age at second sampling (weeks)	9.48 $\pm$ 2.76	10.36 $\pm$ 2.37	0.29
Interval between first and second sampling (days)	10.04 $\pm$ 3.60	10.25 $\pm$ 2.46	0.84

Values are given as mean  $\pm$  S.D.

**Table II** Mean Serum Concentration of TNF- $\alpha$  in Women with Threatened Abortion and Normal Pregnancy During the First and Second Measurement

pg/mL	Threatened abortion group (n = 27)	Reference group (n = 16)	P-value
First examination	7.97 $\pm$ 1.03	7.88 $\pm$ 0.98	0.78
Second examination	8.96 $\pm$ 2.24	9.05 $\pm$ 1.99	0.89
First/second examination	0.94 $\pm$ 0.20	0.90 $\pm$ 0.16	0.50

Values are given as mean  $\pm$  S.D.

**Table IV** Mean Serum Concentration of Interleukin-10 in Women with Threatened Abortion and Normal Pregnancy at the First and Second Measurement

pg/mL	Threatened abortion group (n = 27)	Reference group (n = 16)	P-value
First examination	7.37 $\pm$ 2.02	6.85 $\pm$ 1.14	0.35
Second examination	5.84 $\pm$ 2.83	5.92 $\pm$ 1.93	0.92
First/second examination	1.44 $\pm$ (0.65)	1.30 $\pm$ (0.55)	0.47

Values are given as mean  $\pm$  S.D.

**Table III** Mean Serum Concentration of IL-12 in Women with Threatened Abortion and Normal Pregnancy at the First and Second Measurement

pg/mL	Threatened abortion group (n = 27)	Reference group (n = 16)	P-value
First examination	4.98 $\pm$ 1.53	4.83 $\pm$ 1.51	0.76
Second examination	3.94 $\pm$ 2.00	4.33 $\pm$ 1.66	0.51
First/second examination	1.29 $\pm$ 0.49	1.20 $\pm$ 0.49	0.56

Values are given as mean  $\pm$  S.D.

healthy women (0.67 versus 0.72, respectively) (Table V).

### The Effect of Dydrogesterone Treatment on Pregnancy Outcomes

No significant difference in pregnancy outcome could be observed between threatened aborters and women with normal pregnancy. The length of gestation and the newborns' birthweight were similar in the two groups.

Three pregnancies with diagnosis of TA and only one with a clinically normal course ended up in missed abortion (11.1% versus 6.25%, respectively). Two of the threatened aborters delivered before 37th week of gestation, whereas no preterm delivery was registered in the control group (Table VI).

We also compared mean serum cytokine concentrations between women who aborted ( $n = 4$ ) and women who had a successful pregnancy. Mean concentrations of the evaluated cytokines at the first sampling did not differ between these groups. After dydrogesterone administration, serum cytokine concentrations also did not differ between both the groups (data not shown). The relatively small number of women who aborted may have influenced these findings.

### Discussion

In this prospective study, we have shown that the Th1- and Th2-type cytokine profiles of women with clinical symptoms of TA did not differ from those of women with normal pregnancies. Mean concentrations of TNF- $\alpha$ , IL-12, IL-10 did not differ significantly between the groups at the beginning of the

**Table V** Interleukin (IL)-12/IL-10 and Tumor Necrosis Factor (TNF)- $\alpha$ /IL-10 Ratios at the First and Second Measurement in both Groups

pg/mL	Threatened abortion group (n = 27)	Reference group (n = 16)	P-value
First examination IL-12/IL-10	0.56 $\pm$ 0.34	0.67 $\pm$ 0.29	0.29
Second examination IL-12/IL-10	0.61 $\pm$ 0.43	0.72 $\pm$ 0.34	0.39
First examination TNF-alpha/IL-10	1.08 $\pm$ 0.32	1.16 $\pm$ 0.21	0.38
Second examination TNF-alpha/IL-10	1.75 $\pm$ 1.00	1.68 $\pm$ 0.81	0.81

Values are given as mean  $\pm$  S.D.

**Table VI** Pregnancy Outcomes in the Threatened Abortion Group Treated with Dydrogesterone and in the Group with Normal Pregnancy

	Threatened abortion ( <i>n</i> = 27)	Healthy controls ( <i>n</i> = 16)	<i>P</i> -value
Missed abortion	3/27 (11.1%)	1/16 (6.25%)	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' birthweight (g), mean $\pm$ S.D.	3373.57 $\pm$ 789.64	3436.67 $\pm$ 343.11	NS

NS, not significant.

study as well as at the second measurement after dydrogesterone supplementation in the TA group.

In contrast to spontaneous abortion, the data concerning cytokine profile in women with TA is relatively scarce.<sup>21,22</sup> Paradisi et al.<sup>21</sup> measured three T-helper 2-type cytokines (IL-6, IL-10 and IL-13) in the sera of women with TA, missed abortion, normal pregnancy and in non-pregnant women. Serum IL-6, IL-10 and IL-13 concentrations in women with TA showed no significant differences between those in women with normal pregnancy and in non-pregnant women. Among threatened aborters, the serum concentrations of the examined cytokines were measured twice, on admission and discharge after 2–3 days. The authors found no significant differences in IL-6, IL-10 and IL-13 serum concentrations between the two evaluations within the TA group. Our data are consistent with these results but also indicate, for the first time, that mean concentrations not only of the Th2- type cytokines but also of the Th1-type did not differ significantly between threatened aborters and healthy pregnant women. Similar cytokine profiles observed in our study at the second measurement confirms the maintenance of a stable, similar Th1/Th2 immune balance in both the groups examined. The data presented by Paradisi et al.<sup>21,22</sup> confirm the existence of a T-helper 2 type pattern deficiency in missed abortion in contrast to TA. Makhseed et al.<sup>23</sup> compared Th1-Th2 cytokine profiles in a subgroup of recurrent aborters who had an abortion with those of recurrent aborters who had a successful pregnancy. A comparison of Th1:Th2 cytokine ratios indicated a higher Th2 bias in recurrent aborters with successful pregnancy. Raghupathy et al.<sup>24</sup> examined the concentrations of Th1 and Th2 cytokines produced by peripheral blood mononuclear cells from women undergoing unexplained recurrent spontaneous abortion (RSA) with those produced during normal pregnancy. Significantly

higher concentrations of Th2 cytokines were found in women with normal pregnancies than in those with RSA while the abortion group had higher concentrations of Th1 cytokines. We also compared mean serum cytokine levels between women who aborted (*n* = 4) and had a successful pregnancy course in subsequent pregnancies. Mean concentration of the evaluated cytokines did not differ between the women who subsequently aborted when compared with women with successful pregnancies but the relatively small group of women who aborted may have influenced these findings.

The data reported by Paradisi et al.<sup>21</sup> indicate that a TA with a good outcome, immunologically resembles the normal pregnancy with a non-enhanced Th1 reactivity. Serum IL-12, IL-8 and soluble IL-2 receptor (SIL-2R) concentrations in women with TA showed no significant differences between those of normal pregnant women. Our data confirm that mean serum concentrations of IL-12 but also of TNF $\alpha$  and IL-10 in women with TA did not differ from those of women with normal pregnancy at first and second sampling with a interval of 10 days between the samplings. Also Gucer et al.<sup>25</sup> found that TNF- $\alpha$  levels did not differ between patients with TA with a good outcome when compared with normal pregnancies (16.1 versus 10.9 pg/mL).

Till date, there is considerable controversy about the use of progestogens for the treatment of TA. The question is whether there is a need for progesterone supplementation among women with clinically diagnosed TA and whether such intervention (to shift the balance of immune reactivity towards Th2 dominance or to change the PIBF concentration) might help in achieving successful pregnancy among women with TA.

Blois et al.<sup>26</sup> reported that progesterone substitution with dydrogesterone corrected the abortogenic effects of stress exposure in mice by decreasing the



frequency of Th1 cytokines via a CD8-dependent pathway. There are no reports, to our knowledge, concerning the impact of progestogens supplementation on cytokine profile among women with TA. We did not find any differences in Th1- and Th2-type mean cytokine concentrations before and after dydrogesterone treatment in women with TA.

A recent clinical review on the evaluation and management of threatened miscarriage<sup>27</sup> revealed that there are no convincing data on the impact of progesterone supplementation in TA, mainly because of the poor design of studies that have been conducted thus 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 TA.<sup>28</sup> In our study, mean gestational age at delivery among threatened aborters undergoing dydrogesterone therapy did not significantly differ from that of healthy pregnant women, nor did the mean birth-weight of the newborns. The incidence of preterm delivery was higher in the TA group, but the difference between the two groups did not reach statistical significance.

As revealed by the previously reported findings of the first part of the study,<sup>19</sup> on the impact of dydrogesterone on PIBF concentration in women with TA, dydrogesterone supplementation was associated with an increased PIBF production in humans. Following dydrogesterone treatment, initially low PIBF concentrations in threatened aborters significantly increased, to reach the PIBF level found in healthy controls.

The results of this study indicate that peripheral cytokine production in threatened aborters does not differ from that observed among healthy pregnant women and support the hypothesis by Chaouat et al.<sup>29</sup> that the concept that pregnancy is a 'Th2 phenomenon' cannot be generalized to all aspects of maternal cellular immunity. The findings of our previous<sup>19</sup> and present study suggest that as no Th1/Th2 cytokine imbalance is observed among women with TA, immunological pregnancy-protective effect of progesterone is manifested via restoring PIBF concentration rather than controlling cytokine production. The previously demonstrated PIBF induction in dydrogesterone-treated threatened aborters suggests that restoring normal PIBF concentrations could be one of the mechanisms by which dydrogesterone supplementation could improve pregnancy outcome rates.

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