# Early Pregnancy Loss - the role of Progesterone-Induced Blocking Factor (PIBF)

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**Abstract**

**Background:** Early Pregnancy loss (EPL) is one of the significant issues of reproductology. It seems to be a purely immunological phenomenon, which concerns especially the first weeks of gestation. Embrioprotective factors are activated from the first day of pregnancy. One is progesterone-produced blocking factor (PIBF), which maintains the pregnancy.
**Aim:** To assess the diagnostic value of PIBF in early pregnancy loss, including the preclinical stage and find the possible correlation between PIBF and Progesterone (PG), PIBF and β human Chorionic Gonadotropin (βhCG).
**Methods:** The prospective and retrospective study included 31 women, aged 18-35 (28.77±6.41), with one or more early pregnancy losses in anamnesis. The inclusion criteria were βhCG >25 ng/ml on the 12-14th day after ovulation, and the exclusion criteria - all causes of EPL. Retrospectively, women were divided into three groups: Group I – patients with progressive pregnancy (n=11); Group II- patients with early pregnancy loss (EPL) (n=10); Group III – patients with biochemical pregnancy (preclinical stage pregnancy) (n=10). PIBF and PG were assessed on the 12-14th day after ovulation. Data analysis was performed using SPSS software package version 26.0 for Windows.
**Results:** The mean PIBF level was significantly higher in group I (15.4 ± 4.6 ng/ml) than in group II (10.3 ± 4.7 ng/ml) $P<0.05$, group III (10.1 ± 5.5 ng/ml) $P<0.05$, but there was no significant difference in PIBF level between groups II and III ($P>0.05$). PG was statistically higher in the patients with progressive pregnancy than in the women with EPL ($P<0.05$) and biochemical pregnancy ($P<0.05$). Also, a statistically significant difference was found in the mean PG level between groups II and III ($P<0.001$). βhCG was significantly high in groups I and II compared to group III, $P<0.05$. There was no significant correlation between PIBF and PG levels, also between PIBF and βhCG in all groups.
**Conclusions:** PIBF may be considered as a possible diagnostic marker of EPL, including the preclinical stage.
**(TCM-GMJ June 2024; 9 (1):P7-P10)**

**Keywords**: Early Pregnancy Loss (EPL); Progesterone-induced blocking factor (PIBF); preclinical stage of pregnnancy, Progesterone (PG), biochemical pregnancy.

## Introduction

Prevalence of infertility worldwide reaches 17.5%, in favour of primary infertility (10.5%). Due to this fact Pregnancy Loss (PL) is one of the most important issues of Reproductology. Its frequency is 13.5%, but in the case of 3 consecutive miscarriages - is rated at 55%. 90% of miscarriages occur in the first trimester (1,2). The causes of spontaneous miscarriages are multiple: genetic and immunological causes, infectious factors, hormonal disturbances, anatomical defects, etc. 50% is caused by feto-maternal factors: fetal - chromosomal anomalies, maternal-anatomical anomalies, endometrial synechias, fibroids, advanced age, chronic diseases, immunological diseases, endocrine disturbances, genetical diseases. The reason for pregnancy loss often is unknown as 60% occurs in the first two weeks of gestation – before the delay of period and the first blood beta

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chorionic gonadotropin (βhCG) determination (3). Additionally, it must be noted, that the incidence of EPL increases after advanced maternal age (4,5).

During pregnancy complex neuro-endocrinological and immunological mechanisms are activated, which contributes to the normal development of pregnancy. In these processes, one of the main roles plays Progesterone Induced Blocking Factor (PIBF). Properly, PIBF suppresses constriction of the myometrium, impairing pro-inflammatory cytokines production; suppresses the activation of pro-inflammatory cytokines, thus increasing the differentiation and proliferation of T helpers; blocks the natural killer (NK) cells degranulation and thus reduces their cytolytic function (6).

The scientists' attention to the PIBF was increased during the last several decades. PIBF consists of 757 amino acids and the molecular mass is 89 kDa (7,8). There are also shorter forms – 30, 43, and 57 kDa, which are localized in the cytoplasm. They are associated with cell-specific intra and extracellular expression (9). It is thought that the short forms act as PIBF's receptor ligands (10). PIBF is produced in the γδ T lymphocytes at the preclinical stage of pregnancy (soon after conception) (11). It must be noted that inhibiting an immune response is a reliable sign for maintaining pregnancy but also may contribute to other pathologies, such as tumour growth, due to local immunosuppression (12). Szekeres-Bartho et al. first demonstrated that in the lymphocytes of women, who take PG, PIBF is produced, which blocks the cytotoxic activity and synthesis of prostaglandin F2α (PGF2α). Thus, in women with threatening preterm delivery, PIBF synthesis was reduced (13). In other studies, a considerable reduction of PIBF and an increase of pro-inflammatory cytokines – IL-6 and γ interferon (γIFN) - was demonstrated in the urine and plasma of women with threatening preterm delivery (14,15). Pro-inflammatory cytokines, also, are associated with Recurrent Pregnancy Loss (RPL) and preterm delivery. Besides, the PIBF level in urine and plasma is significantly lower in women with threatening miscarriages (14). Hereby, Szekeres-Bartho et al. in their study have noted that PIBF maintains the normal tonus of the uterus (16). Thus, it turned out, that PIBF is very important in the maintenance of pregnancy because it participates in the modulation of the immune response. PIBF and PG have immunomodulatory effects on the membrane progesterone receptors (mPR) of CD4+ (Cluster differentiation) T cells. In one study it was concluded that PIBF was able to significantly increase mPR expression on the surface of peripheral CD4+ T cells. Thus, a decrease in PIBF concentration during abnormal pregnancy can modulate mPR expression and regulatory performance of PG on T cells. Hence, Rafiee M. et al. have concluded that the research must be continued to open up a new understanding of the etiology of pregnancy loss (17).

PIBF has become more popular after its determination in different tissues of the reproductive system and meanwhile, in tumour tissues (9), (18). In one of the studies, the impact of dydrogesterone on the hormonal profile and PIBF concentration in women with threatening miscarriage has been evaluated. The results have revealed, that the induction of PIBF by the dydrogesterone may improve the outcome of pregnancy (19). Low PIBF level is the predictor of preterm delivery at 24-28 weeks of gestation (20). PIBF, also, is expressed on the surface of the trophoblast and participates actively in its invasion. Miko E, Halasz M. Et al. have described that PIBF is expressed by the normal placenta, and also by the hydatidiform moles. Still, its expression is considerably decreased during the complete mole and is not expressed at all during the choriocarcinoma (21). PIBF increases from the first days of conception and progresses with the pregnancy (22). The role of PIBF is very important in in vitro fertilization (IVF). During IVF determining PIBF level at the early stage of pregnancy may be used as the predictive value for the pregnancy outcome (23).

All these mechanisms maintain the pregnancy progression. However, regarding the absence of diagnostic markers for the preimplantation and early implantation stage, the rate of undiagnosed pregnancy and thus, the rate of Early Pregnancy Loss still remains very high.

Therefore, the objective of our research became the assessment of the diagnostic value of PIBF in early pregnancy loss, including the preclinical stage and finding the possible correlation between PIBF and PG, PIBF and βhCG.

## Methods

The prospective and retrospective study included 31 patients and was conducted on the basis of "Prof. Zhordania and Prof. Khomasuridze Institute of Reproductology", Tbilisi, Georgia. The study was approved by the local ethical committee. The informed consent was obtained from the patients. The inclusion criteria were: one or more EPL in anamnesis, normal ovulation, and positive βhCG (>25 mIU/ml) level in the blood on the 12-14$^{th}$ day after ovulation. The exclusion criteria contained all causal factors of EPL: tubal, endocrine disorders, ovarian dysfunction, endometriosis, congenital and acquired anomalies of the pelvic organs, confirmed genetical disorders, congenital and acquired thrombophilia, sexually transmitted diseases, acute and chronic inflammatory diseases of pelvic organs, uterine fibroids and polyps, abnormal uterine bleedings, infertility caused by male factor.

In 31 patients, aged 18-35 (28.77±6.41), biochemical pregnancy was diagnosed (βhCG>25 mIU/ml) but in ten women menstruation started timely, which, in our belief, probably indicates that in those cases the pregnancy was lost on the preclinical stage. Other 21 women had delayed menstruation and pregnancy was diagnosed clinically. However, pregnancy loss was occurred in 10 women at different weeks of gestation (3-9 weeks). 11 patients had progressive pregnancies, which lasted in the term delivery. Retrospectively, 31 patients were divided into three groups according to the course of pregnancy: Group I – patients with progressive pregnancy (n=11); Group II- patients, with early pregnancy loss (n=10); Group III – patients with biochemical pregnancy (n=10). The blood was collected on the 12-14$^{th}$ day after ovulation. PIBF, PG, and

βhCG were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Data analysis was performed using SPSS software package version 26.0 for Windows.

## Results and discussion

The study has revealed quite interesting results: The mean level of PIBF (15.4 ± 4.6 ng/ml) was significantly high in patients with progressive pregnancy, compared to patients with EPL (10.3 ± 4.7 ng/ml) $P<0,05$; Also, the difference in PIBF level was significant between the patients with progressive pregnancy and patients with biochemical pregnancy (10.1 ± 5.5 ng/ml), $P<0.05$, but no significant difference was found between women with EPL (10.3 ± 4.7 ng/ml) and biochemical pregnancy (10.1 ± 5.5 ng/ml) $P>0.05$.

The mean PG level also was significantly higher in the patients with progressive pregnancy (21.9 ± 5.8 ng/ml) compared to women with EPL (15.1 ± 4.1 ng/ml) $P<0.05$; The difference was significant between patients of group I and women of group III (biochemical pregnancy group) (6.3 ± 4.4 ng/ml) $P<0.05$. A significant difference was found in PG level between patients with EPL and women with biochemical pregnancy $P<0.001$.

βhCG level was the highest in women with progressive pregnancy (82 ± 20.70 ng/ml). Still, there was no statistical difference between the women with progressive pregnancy and patients with EPL (67.24 ± 16.34 ng/ml) $P>0.05$. But, a statistically significant difference was found between groups I (82 ± 20.70 ng/ml) and III (46.90 ± 8.70 ng/ml) $P<0.05$. βhCG, also, was statistically higher in women with EPL compared to women with biochemical pregnancy ($P<0.05$) (Additional file 1). Besides, there was no significant correlation between PIBF and PG in the groups (I group – $r = -0.15$ ($P=0.5$), II group – $r = -0.35$ ($P=0.16$), III group – $r = -0.48$ ($P=0.1$)), no significant correlation was revealed between PIBF and βhCG in all groups, as well (I group – $r = -0.08$ ($P=0.7$), II group – $r = -0.21$ ($P=0.4$), III group – $r = 0.38$ ($P=0.2$)).

The prevalence of infertility worldwide is rather high – 17.5%, which means that every 6th adult faces to infertility problem (24). According to several studies incidence of primary infertility is higher in different countries compared to secondary infertility – 6-16% (average 10.5%) vs. 2% (25,26). However, from 1990-2010 the rate of secondary infertility was higher than primary – 8.7-32.6% vs 0.6-3.4%, respectively (27). The rates mentioned above, are confusing and it must be considered, that in developing countries, they may be much higher. All data concern clinically approved pregnancies, but considering the number of pregnancies we may lose before delaying menstruation, the rate will be increased significantly, which is already alarming. Obviously, the reason for infertility, at least in half of the cases, is undetectable, which is called "unexplained infertility" (28). Theoretically, maybe those patients even get pregnant, but these pregnancies are lost in the first two weeks of pregnancy, and actually, remain undiagnosed. Due to this reason, we decided to assess the possible early diagnostic markers of pregnancy: PIBF, PG, and βhCG, and evaluate the diagnostic value of PIBF in early pregnancy loss, including the preclinical stage and finding the possible correlation between PIBF and PG, PIBF and βhCG.

According to our study, all variables were the highest in the women with progressive pregnancy (group I) compared to women with EPL and biochemical pregnancy. Quite interesting was the result concerning PIBF, which was significantly lower in women with EPL, including the biochemical pregnancy compared to patients with progressive pregnancy. Our result coincides with the study results of Polgar et al. according to which PIBF was the one most important associated risk factor, as its concentrations in urine and plasma are increased with the advancing pregnancy, while in women with miscarriage or preterm delivery, the high level of PIBF is not noted (29). However, it must be mentioned, that in our study, PIBF level in women with EPL was as low as in the biochemical pregnancy group – there was no statistically significant difference, which supposed, that low PIBF level may be the marker for threatened pregnancy loss as at early, so at preclinical stage. This may be one reason for infertility, which is, also, confirmed in a study by Sahin ME et al., where a significantly low PIBF level was found in women with unexplained infertility compared to the fertile control group (30). Besides, Ku C. et al. revealed that low PG and PIBF concentrations in blood predict spontaneous miscarriage among women with threatened miscarriages between 6-10 weeks of gestation (31). The similar results we get in our study concerning the PG level.

PG rate was the highest in women with progressive pregnancy similar to PIBF. However, in contrast to PIBF, PG was statistically higher in women with EPL compared to biochemical pregnancy. These results coincide with Ku et al. who revealed that serum PG level is increased linearly during 5-13 weeks of gestation and a low level of PG is associated with a threatened miscarriage and a complete miscarriage at 16 weeks of gestation (32). However, in our study, despite the relatively high PG level, compared to women with biochemical pregnancy, early pregnancy loss occurred at 5-8 weeks of gestation. Besides, considering the fact, that PIBF is released by the lymphocytes in the presence of PG, also, the percentage of these lymphocytes increases in the luteal phase (33), both – PG and PIBF are promising biomarkers for predicting pregnancy viability (8), however, in our research high level of PG did not indicate a high PIBF level and no linear correlation was found between those biomarkers in all groups. Similar results were found in another study, where the corpus luteum was not a reliable sign for producing PIBF (34).

Not least important was the βhCG concentration in the serum. βhCG was the lowest in women with biochemical pregnancy on the 12-14th after ovulation, and it was the highest in women with progressive pregnancy, however, there was no statistically significant difference in women with progressive pregnancy and patients with EPL. Albeit, βhCG level was statistically higher in women with progressive pregnancy and EPL than in patients with biochemical pregnancy. Our results coincide with one study, where

βhCG was significantly higher in the uncomplicated pregnant women group compared to the women with a miscarriage (35). Besides, in our study, similar to PIBF and PG, there was no significant correlation between PIBF and βhCG in all groups, which may be related to the small sample size.

Thus, βhCG is less informative in the prognosis of maintenance of pregnancy. PG is more informative than PIBF in progressive pregnancy and its favourable outcome. PIBF is more informative than PG and βhCG in the prediction of early pregnancy loss, including biochemical pregnancy.

The importance of all those markers is undoubtedly high and more large-scale studies must be continued in this connection.

Considering all the above, in patients with low PIBF levels, the prescription of PG in the preclinical stage may reduce the index of EPL, and thus, the rate of infertility.

## Conclusion

PIBF may be considered as a possible diagnostic marker for EPL, including the preclinical stage.

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