An increased proportion of progesterone receptor A in peripheral B cells from women who ultimately underwent spontaneous preterm birth

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Abstract

The enormous challenge in unraveling the etiology of preterm birth (PTB) is to understand the complex interactions between gestational hormones, the immune system and reproductive tissues. PTB can be divided into spontaneous PTB (sPTB) and medically indicated PTB, for example due to pre-eclampsia (PE) or HELLP syndrome. Progesterone (P4) is important for establishment and maintenance of pregnancy and exerts important anti-inflammatory effects. Since the impact of P4 on B cells, and how this interplay supports the maintenance of maternal-fetal tolerance, is widely unexplored, we aimed to determine whether B cells express the progesterone receptor (PR) and to dissect a possible role of PR+ B cells in PTB.

We found enhanced IL-6, IL-21 and TNF-α concentrations in maternal plasma in 14 patients with sPTB and PE/HELLP compared to term delivery (TD), which was accompanied with enhanced PR-A expression by CD19+ B cells. In a second phase of the study, we recruited patients with imminent PTB (iPTB) and controls and collected samples at hospital admission and to a later time point, and divided them in iPTB patients who delivered pre-term and patients whose PTB could be prevented. Interestingly, within our group of iPTB patients there were very clear differences in cytokines and B cell frequency depending upon the fact of whether they delivered pre-term or not. Enhanced levels of pro-inflammatory cytokines and increased percentages of PR-A+CD19+ B cells were found in iPTB patients that delivered preterm compared to patients who did not deliver preterm, the latter having comparable values to term control women.

We conclude that PTB is associated with the activation of an inflammatory pathway leading to the induction of PR-A by B cells. This might further trigger inflammation, result in the break of maternal-fetal tolerance and induce delivery.

Keywords

Progesterone, progesterone receptor, B cells, imminent preterm birth, preterm birth

1. Introduction

Progesterone (P4) is involved in the establishment and maintenance of pregnancy [1] and supports immune tolerance towards to the semiallogeneic fetus [2]. The P4 level in plasma rises gradually until about 32 weeks of gestation [3], followed by a "functional P4 withdrawal" in uterine tissues. One important mechanism therefore is mediated by altering the expression levels of P4 receptor (PR) isoforms, PR-A and PR-B [4-7]. In pregnancy, myometrial quiescence is mediated by PR-B, labor is associated with an increased PR-A to PR-B ratio that results in an increased expression of pro-labor genes [8].

PRs are also expressed by T cells, which enable them to respond to P4 [9-11]. Ligation of P4 to its receptor on T cells might suppress their activation during pregnancy [12], proliferation and secretion of inflammatory cytokines [13].

B cells express PR, predominantly PR-A [14]. Stimulation of B cells and B regulatory cells (Breg) with P4 induced IL-10 [15, 16] and the production of asymmetric, protective antibodies [17]. We have shown that the Breg cell population expands in normal pregnant women [18]. Interestingly, in the occurrence of preterm birth (PTB), their frequency was reduced and instead a shift towards inflammatory B cells was observed [19, 20]. However, the expression of PR in these cells has not been studied.

PTB, the delivery of a living baby before 37 weeks of gestation, might be spontaneous or medically indicated. Each year, about 15 million babies are born premature worldwide [21]. Therefore, prematurity is one of the biggest problems in obstetrics and a common cause of newborn death and long-term morbidity in children [22].

Currently, there is no test that reliably predicts PTB for women diagnosed for imminent PTB. In most women that were admitted to hospital with preterm labor (PTL), labor can be stopped and delivery averted. Since these women are still at a high risk for PTB, a maintenance tocolytic agent such as vaginal P4 might help to further prevent PTB [23, 24], although the exact mode of action is unknown. Therefore, there is an urgent need to identify mechanisms underlying the treatment with P4.

In this study, we investigated the PR expression by B cells in PTB compared to term birth. Moreover, we addressed the question whether PR expression by B cells could

serve as a marker to identify women that will deliver preterm from women that will not give birth preterm following admission to hospital with signs of PTL.

2. Material and methods

2.1 Human subjects

The study was carried out in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Otto-von-Guericke University Medical Faculty (EK28/08). All patients were informed properly about the purpose of the study and gave written consent before participating. Patients included in this study were recruited between December 2017 and February 2019 at the University Hospital for Gynecology, Obstetrics, and Reproductive medicine. The demographic data of the patients are summarized in Table 1A and B. Venous EDTA blood was taken from each pregnant woman and immediately stored on ice. Blood was processed within one hour. Plasma was obtained and stored at -80°C.

2.2 Cell staining and flow cytometry

$3x10^5$ PBMCs were stained for cell surface markers for 30min. at 4°C. The following anti-human antibodies were used: FITC-labeled CD19 (clone HIB19) and eFluor506-labeled CD45 (clone H30). Afterwards, cells were fixed for 30min. with Fix and Perm (Invitrogen/ ebioscience, #00-5123-43 and #00-5223-56) and stained with eFluor 660-labeled PR (clone KMC912; staining PR-A and PR-B; all reagents ebioscience) for 30min. at 4°C. Measurements were performed on Attune NxT flow cytometer (Thermo Fisher Scientific, Waltham, USA). Data were analyzed with FlowJo software (Ashland, USA).

2.3 Cytokine detection in maternal plasma samples

Cytokines in maternal plasma samples were quantified by the Biolegend Legendplex Human Th Cytokine panel (sensitivity IL-6: 1.0pg/ml; IL-21: 6.9pg/ml; TNF-α: 0.7pg/ml) according to the supplier's recommendation.

2.4 Progesterone detection in maternal plasma samples

The level of P4 in plasma was determined by Progesterone ELISA kit (DRG Diagnostics, Marburg, Germany; sensitivity: 0.14ng/ml) according to the supplier's recommendation.

2.5 Western Blot analysis

B cells from pregnant healthy volunteers were isolated using the human B cell isolation kit II (Miltenyi Biotech, Bergisch Gladbach, Germany). MCF-7 (HTB-22) cells and HEK 293 cells were obtained from ATCC (Manassas, Virginia, USA). Proteins from B cells and MCF-7 cells was extracted by incubation with lysis buffer (10% NP-40, 0.1 mg/ml n-Dodecil-β-D-maltoside, 500 mM sodium fluoride, 10 mM sodium metavanadate, 100 mM PMSF, 1 M Tris, 0.5 M EDTA and 5 M NaCl) for 60min. on ice. Following sonication and centrifugation, supernatants were collected and stored at −80°C. The protein concentration was determined by Bradford assay (Bio-Rad, Feldkirchen, Germany). Proteins were resolved on a 10% SDS-PAGE and transferred onto a 0.2 µm nitrocellulose membrane. Non-specific binding sites were blocked with 5% (w/v) BSA in TBS for 1h at RT. Blots were incubated with the Progesterone Receptor A/B (D8Q2J) XP Rabbit mAb (#8757; cell signaling, Frankfurt, Germany) overnight at 4°C. Following incubation with a biotinylated goat anti-rabbit secondary Ab (E0432; Dako, Waldbronn, Germany) for 1h at RT, blots were developed using Immobilon chemiluminescent HRP substrate (Merck, Darmstadt, Germany). Then, blots were incubated with β-actin Ab (AC-15, Sigma-Aldrich, St. Louis, USA) for 1h at RT and developed.

2.6 Data analysis and statistics

Statistical analysis was performed using GraphPad Prism 8.0 software. Normality of distribution was determined by Shapiro-Wilk test. Data were analyzed by Kruskal-Wallis test followed by Dunn´s multiple comparisons test or Two-way ANOVA followed by Tukey´s multiple comparisons test.

3. Results

3.1 PR-A expression in B cells is enhanced in the event of inflammation-associated PTB

We have recently shown [19, 20] that PTB is associated with an enhanced level of pro-inflammatory cytokines. Since PTB can be distinguished into spontaneous and medically induced, we analyzed the expression of pro-inflammatory cytokines in maternal plasma immediately before delivering via cesarean section either at term (TD; N=15 patients), spontaneous preterm (sPTB; N=14 patients) or medically induced due to an underlying preeclampsia (PE)/HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count; N=17 patients).

We found that, compared to TD, the levels of IL-6 (p=0.0002), IL-21 (p=0.0008), and TNF-α (p=0.0030) were increased in maternal plasma of sPTB patients. In patients suffering from PE/HELLP, enhanced concentrations of IL-6 (p=0.0008), IL-21 (p=0.0008) and TNF-α (p=0.0432) were detected in maternal plasma (Figures 1A, B, C) as well. The concentration of progesterone (P4) was diminished in serum of patients delivering preterm either spontaneous (p=0.0045) or because of PE/HELLP (p=0.0005; Figure 1D) when compared to term controls. Next, we investigated the expression of PR-A in peripheral blood B cells from women immediately before delivering term or preterm via caesarian section and controls. We found that a small proportion of B cells express PR-A in term birth (mean 0.71%), while an enhanced number of PR-A-positive B cells were detected in sPTB (mean 2.688%, p=0.0001) and in PE/HELLP (mean 1.302%; p=0.0557; Figure 1E). The flow cytometry gating strategy is shown in Figure 1F, examples are presented in Figure 1G. We also performed Western Blot analysis of isolated B cells from pregnant women showing the expression of PR-A (Figure S1). As controls, we used MCF-7 breast cancer cells known to express PR-A and PR-B [25] and PR-negative HEK293 cells. Having found diminished levels of serum progesterone and enhanced expression of PR-A in B cells, we now sought to understand the correlation among these players for PTB outcome.

3.2 Expression of PR-A by B cells from patients suffering from imminent PTB

Next, to address the question whether the expression of PR-A by B cells raises before the onset of PTB, we recruited patients that were hospitalized with imminent

PTB (iPTB) before onset of therapeutic treatment. Control women included in this group were pregnant at the second and third trimester and gestational age-matched to the women delivering preterm. Besides, women at the end of pregnancy (GA 39 weeks) were included. We found an enhanced secretion of IL-6 (p=0.0124) and TNF-α (p=0.0476), but not IL-21, in women with iPTB compared to the controls (Figures 2A, B, C). However, neither the concentration of P4 (Figure 2D) nor the frequency of PR-A+ CD19+ B cells were altered in patients with iPTB compared to control women (Figure 2D).

The results state that despite enhanced inflammatory cytokines in iPTB, the PR-A expression is not changed.

3.3 Induction of PR-A expression by B cells of women with imminent PTB that indeed delivered preterm compared to women that did not

It is known that only a small percentage of women admitted to a hospital with imminent PTB do deliver preterm. Therefore, we recruited patients that were hospitalized with iPTB before onset of therapeutic treatment (time point 1, TP1). A second time point (TP2) was chosen in dependence whether the women delivered or not: either after 7 days, when PTB could be prevented or immediate before delivery (usually 3-5 days after admission to hospital). Control healthy pregnant women between gestational weeks 38 and 40 were first analyzed 3-7 days before planned delivery (TP1) and again immediate before delivery (TP2).

We detected no differences in the expression of IL-6, IL-21 or TNF-α in maternal plasma at TP1 between iPTB and controls. However, at TP2 patients delivering preterm had enhanced level of IL-6 (p=0.0003), IL-21 (p=0.0004) or TNF-α (p=0.0350) compared to women delivering term (Figures 3A, B, C). While we detected no changes in P4 concentration (Figure 3D), we found an enhanced frequency of B cells expressing PR-A at TP2 in women delivering preterm compared to term-delivering women (p= 0.0063, Figure 3E). This was also true compared to patients whose preterm birth was adverted. Patients who delivered preterm had had enhanced plasma level of IL-6 (p=0.0003), IL-21 (p=0.0002) and TNF-α (p=0.0052) at TP2. P4 levels were comparable: however more PR-A-expressing B cells were detected (p=0.0238; Figures 3A-E).

These data indicate that the expression or secretion of pro-inflammatory cytokines and the frequency of PR-A+CD19+ B cells is different between women admitted with iPTB that will deliver preterm from those who will not.

4. Discussion

In the present study, we report that B cells express PR-A. Furthermore, we observed that PR-A expression in B cells was increased in patients suffering from spontaneous or medically induced PTB compared to controls; both are accompanied by an enhanced level of pro-inflammatory markers. A higher percentage of PR-expressing B cells were also detectable in patients with imminent PTB who later delivered preterm compared to women admitted at the hospital with threatened PTB but later delivered at term because PTB could be prevented. These changes could not be attributed to alterations in plasma P4 level.

P4 affects the maternal-fetal immunological relationship at several levels, but less is known about the effects of P4 on B cells. Stimulation of murine splenic B cells with P4 reduced the expression of CD80 and CD86; thereby inhibiting the capacity for antigen presentation of B cells [26], which might serve as an important mechanism to modulate the immune system in pregnancy. Stimulation of PBMCs with P4 induced B-cell activating factor (BAFF), a cytokine promoting survival and maturation of B cells.

The reproductive phenotypes of PR-A and PR-B deficient mice suggest that both receptors provide distinct functions and regulate the expression of distinct genes: Ligation of P4 to PR-A is required for implantation and decidualization, but a PR-B deficiency has no uterine phenotype [8, 27]. PR-A represses the activity of PR-B and the transcriptional activities of the glucocorticoid and the estrogen receptor. At the end of pregnancy, the PR expression by endometrial cells changes from PR-B towards PR-A. Thereby, the functions of P4 changes towards inflammation and enabling delivery [5].

In addition to its impact on endometrial and myometrial cells, PR expression was linked to different outcomes on immune cells. Human peripheral NK cells, which express predominantly PR-A, are susceptible to P4-induced apoptosis [28]. T cells, which mainly express membrane-bound PRs, showed reduced secretion of IFN-γ, TNF-α, IL-5 and IL-10 following P4 treatment, but enhanced IL-4 [13]. Thereby, P4 promotes maternal-fetal tolerance and B cells might be affected by P4 in a similar manner. In general, the role of B cells in healthy pregnancy, parturition and pregnancy disorders such as PTB, where P4 levels might play an important role, is not well understood.

It was demonstrated that mediators such as prostaglandins or pro-inflammatory cytokines, mainly TNF-α, are responsible for the different expression of PR-A and PR-B in the myometrium [29, 30]. We have shown that B cells exhibit important functions in PTB: PTB because of PE was associated with increased CD19+CD5+ B cell numbers that were able to secrete autoantibodies involved in the pathogenesis of PE [31]. The presence of autoantibodies in PE was accompanied by enhanced level of IL-6 and TNF-α [32]. Additionally, in sPTB we found pro-inflammatory, IL-6 secreting B cells, accompanied with a reduction of IL-10 producing Breg cells [19, 20]. In the present study, we found elevated level of IL-6, IL-21 and TNF-α in sPTB and PE/HELLP patients, supporting previous findings. Compared to patients at term, P4 level was lower in maternal blood of sPTB and PE/HELLP patients. This is most likely due to the differences in gestational age at delivery since we found no differences compared to gestational age-matched healthy pregnant women. Further, it was shown that the P4 level in blood progressively increases throughout gestation until term [33, 34]. However, the level of PR-A was enhanced in sPTB and PE/HELLP compared to TD women in our study. Several data suggest that PRs are involved in B cell functions. By using intracellular PR-deficient mice it was shown that low physiologic P4 levels may be sufficient to activate membrane-bound PR and suppress T-dependent antibody responses [35]. The iPR agonist medroxyprogesterone was shown to enhance the IgG1 release from B cells maximally at a low physiologic concentration [36].

Our study is the first to show a correlation between PR-A-expressing B cells and the onset of PTB. However, at this point, we did not know whether the up-regulation of PR-A by B cells is cause or consequence of the inflammation. Therefore, further measurements were performed at different time points upon admission to the hospital with iPTB.

Taken together, we found that pro-inflammatory pathways are activated in both spontaneous and medically induced preterm delivery due to PE/HELLP diagnosis. This is accompanied by an increased frequency of PR-A expressing CD19+ B cells. Patients admitted to hospital with imminent PTB, which later delivered preterm, presented an increased plasma level of pro-inflammatory cytokines and PR-A+CD19+ B cells, which was not the case in women who did not deliver preterm. This indicates that the B cell-specific expression of PR-A might serve as a biomarker

Fto differentiate both patient groups. This is particularly useful as it could be tested hours after hospital admission.

It is important to mention that only some of the women who were admitted to hospital with imminent PTB, delivered preterm. These patients did not differ from non-delivering iPTB women in term of cytokines, P4 level or percentage of PR-A expressing B cells upon admission, but their cytokine profile and PR-expression changed afterwards. These patients were characterized by enhanced IL-6, IL-21 and TNF-α as well as increased PR-A+CD19+ B cells before delivery, which occurred within the following days. This might indicate that the subsequent events after symptoms and usually admission to hospital drive inflammation and increase the frequency of PR-A-expressing B cells.

Due to its immunoregulatory effects, P4 is administered to protect against imminent PTB [24, 37]. P4 suppresses the release of pro-inflammatory mediators in uterine tissues [38, 39], but also act directly on immune cells by suppressing Th1 and supporting Th2 cell differentiation [40]. In PTB, the T cell activation was shown to induce maternal pro-inflammatory responses and to activate uterine contractility prior to PTB, which could be prevented by P4 treatment [41]. The PTB-related activation of T cells was associated with a subsequent activation and differentiation of IFN-γ-producing B cells [41]. Despite differences in the cytokine production, we found no alterations in the P4 level in patients with imminent PTB that subsequently delivered (or not). This is not surprisingly since in human parturition is rather associated with a functional P4 withdrawal rather than with a decrease in P4 level. Despite, the P4 level increases constantly throughout pregnancy, a dramatic change within the few days between TP1 and TP2 was accordingly not to be expected. Nevertheless, in a rat model of preeclampsia, P4 treatment attenuated hypertension and decreased the level of autoantibodies [42] and P4 treatment was also used in human pre-eclampsia [43].

Although P4 signaling is primarily mediated via its PR, the hormone can also bind weakly to the glucocorticoid receptor (GR). Glucocorticoids exhibit important anti-inflammatory effects. Treatment of B cells from healthy donors with glucocorticoids reduced their activation and could induce apoptosis, but these effects were only transient and mostly gone 48h after treatment [44, 45]. Further experiments with samples from PTB patients would help shedding light into the possible GR involvement. The interaction between GR and P4 might contribute to P4-mediated

anti-inflammatory effects on GR-expressing immune cells like B cells [46]. Antenatal corticosteroids were administered to women at risk of preterm birth to accelerate fetal lung maturation [47]. We might speculate that treatment with betamethasone decreases the availability of GR for the anti-inflammatory effects of P4 on B cells, inducing the production of PR-A.

In summary, we show that PTB is associated with the activation of an inflammatory pathway leading to the induction of PR-A by B cells. This may happen shortly before birth but measuring PR-A in peripheral B cells might be a powerful tool to dissect whose patients are at imminent risk for delivering pre-term.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Supplementary Material

Figure S1

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Table/ Figure Legends

Table 1:

Study cohort

A) Fifteen women delivering at term (term delivery, TD), 14 women delivering preterm spontaneous (sPTB) and 17 patients delivering following PE/HELLP diagnosis were included in the study. Maternal characteristics included age of the mother, gestational age (GA; weeks), number of pregnancies and parities. Neonatal features included birth weight (grams, g), head circumference (cm), body length (cm), APGAR scores at 1min., 5 min. and 10min. after birth, the cord blood pH value and base excess.

B) Six healthy pregnant women at the second and third trimester (controls), twelve women delivered via planned caesarean section (TD/TD), seven patients admitted to our university hospital with imminent PTB that delivered at term (iPTB/TD) and five patients with imminent PTB that delivered preterm (iPTB/PTB) were included. Maternal characteristics included age of the mother, gestational age (GA; weeks) at participating in the study and at delivery, number of pregnancies and parities. Neonatal features included birth weight (grams, g).

Table 2:

Overview about the patients groups and the time points of blood tests

Figure 1:

Cytokine and progesterone level in maternal plasma and PR-A expression by B cells immediate before delivery

(A)- (D) Cytokine and progesterone levels in maternal plasma immediate before delivery. Maternal blood from patients delivering at term (term delivery; TD; black squares), spontaneous preterm birth (sPTB; black triangles) and induced preterm birth following PE/HELLP diagnosis (black hexagons) was obtained immediate before delivery. The plasma levels of IL-6 (A), IL-21 (B) and TNF-α (C) were determined using a Th panel multiplex bead-based assay, the concentration of progesterone was determined by ELISA (D).

(E)- (G) Determination of PR-A expressing B cells in maternal blood immediate before delivery. The frequency of PR-A expressing B cells within maternal PBMCs was determined by flow cytometry (E). The flow cytometry gating strategy is shown in

(F): following lymphocyte gating, first CD45-positive cells and then CD19-expressing cells were pre-selected. Representative results from women delivering at term (TD), as a consequence of spontaneous preterm birth (sPTB) and following PE/HELLP diagnosis were shown in (G). Presented are the individual values and the mean. Data were analyzed by Kruskal-Wallis test, followed by Dunn´s multiple comparisons test; *p<0.05; **p<0.005; ***p<0.001.

Figure 2:

Cytokine and progesterone level in maternal plasma and PR-A expression by B cells in imminent PTB

(A)- (D) Cytokine and progesterone levels in maternal blood from non-laboring women and patients with imminent preterm birth. Plasma from healthy pregnant women in the second and third trimester (control; open circles), healthy pregnant women within one week before delivering via planned caesarean section (TD; open squares) and from patients immediate following admission to hospital for imminent preterm birth (iPTB; open rhombus) were determined. The level of IL-6 (A), IL-21 (B) and TNF-α (C) were determined using a Th panel multiplex bead-based assay. The concentration of progesterone was determined by ELISA (D).

(E) Determination of PR-A expressing B cells in maternal blood from non-laboring women and patients with imminent preterm birth. The frequency of PR-A+CD19+ B cells within peripheral PBMCs from control, TD and iPTB patients was analysed by flow cytometry according to the gating strategy shown in Figure 1F (E); Shown are the individual values and the mean. Data were analyzed by Kruskal-Wallis test, followed by Dunn´s multiple comparisons test; *p<0.05.

Figure 3:

Cytokine and progesterone level in maternal plasma and PR-A expression by B cells in imminent PTB in dependence whether delivery was preterm or PTB was adverted

(A)- (D) Cytokine and progesterone levels in maternal plasma from iPTB patients that either delivered or not. Time point (TP) 1: Maternal blood was obtained from healthy pregnant women within one week before planned caesarean section at term (term delivery; TD; open squares) or immediate following admission to hospital for imminent preterm birth (iPTB; open triangles). The second time (TP2) of blood draw was immediate before delivery via caesarean section at term (term delivery; TD/TD;

550 black squares), about one week following admission when PTB was adverted and
551 delivery was at term (iPTB/TD; triangle pointing downwards) or immediate before
552 delivering preterm (iPTB/PTB; triangle pointing up) or. The level of IL-6 (A), IL-21 (B)
553 and TNF-α (C) were determined using a Th panel multiplex bead-based assay. The
554 concentration of progesterone in maternal plasma was determined by ELISA (D).
555 *(E) Determination of PR-A expressing B cells in maternal blood from iPTB patients*
556 *that either delivered or not.* The frequency of PR-A+ CD19+ B cells was determined
557 by flow cytometry according to the gating strategy shown in Figure 1F (E). Shown are
558 the individual values and the mean. Data were analyzed by Two-way ANOVA,
559 followed by Tukey´s multiple comparisons test; *p<0.05; **p<0.005; ***p<0.001.
560
561 **Figure S1:**
562 Western Blot analysis of PR-A and PR-B expression by MCF-7 cells (positive
563 control), B cells from two patients and HEK293 cells (negative control).

564 **Table 1 A)**

	TD	sPTB	PE/HELLP	
characteristics	**N=15**	**N=14**	**N=17**	**p**
maternal				
age (years)	31.3 ± 5.0	29.2 ± 3.9	31.0 ± 6.6	0.0910
GA (weeks)	39.7 ± 1.2	32.4 ± 3.2	31.2 ± 3.6	<0.0001
pregnancy	2.5 ± 2.0	2.0 ± 2.1	1.8 ± 1.3	0.1947
parity	1.9 ± 0.9	1.5 ± 1.1	1.5 ± 0.9	0.1059
neonatal				
birth weight (g)	3607 ± 368	1906 ± 604	1569 ± 811	<0.0001
head circumference (cm)	35.2 ± 1.2	31.3 ± 3.1	29.5 ± 5.8	0.0003
body length (cm)	52.1 ± 2.6	45.0 ± 4.4	44.2 ± 6.9	0.0002
APGAR 1min.	9.1 ± 0.5	7.7 ± 2.5	7.5 ± 2.0	0.0066
APGAR 5min.	9.9 ± 0.4	8.4 ± 2.7	8.8 ± 0.8	0.0017
APGAR 10min.	10 ± 0	8.9 ± 2.4	9.1 ± 0.7	0.0070
pH (cord blood)	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	0.0363
base excess	-3.3 ± 4.5	-1.5 ± 3.5	-1.7 ± 3.4	0.6824

565

566 **Table 1 B)**

	TD	iPTB/TD	iPTB/PTB	
characteristics	**N=12**	**N=7**	**N=5**	**p**
maternal				
age (years)	31.1 ± 5.4	29.9 ± 3.4	29.8 ± 4.3	0.8511
GA (weeks) study	39.0 ± 1.0	29.3 ± 3.4	28.8 ± 3.6	<0.0001
GA (weeks) delivery	39.8 ± 1.1	39.8 ± 1.1	29.5 ± 1.6	0.0026
pregnancy	2.3 ± 1.1	1.5 ± 0.8	2.0 ± 1.4	0.2898
parity	1.8 ± 0.9	1.2 ± 0.4	2.0 ± 1.4	0.3489
neonatal				
birth weight (g)	3391 ± 450	3354 ± 450	1346 ± 653	0.0039

567

568

Table 2

Patient group	Time point 1 (TP1)	Time point 2 (TP2)
TD/TD	3-7 days before planned delivery	immediate before delivery
iPTB/ TD	Diagnosis: imminent PTB, before onset of therapeutic treatment	7 days after admission to hospital when PTB was prevented
iPTB/ PTB	Diagnosis: imminent PTB, before onset of therapeutic treatment	immediate before delivery