

**Fetal Urine Production Rate in Preterm Premature Rupture of Membranes Is  
Associated with Adverse Neonatal Outcome: A Pilot Study**

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**Running headline:** Fetal Urine Production Rate in PPRM

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**Conflicts of interest statement**

None of the authors have any conflicts of interest to report regarding this study.

## **Abstract**

Introduction: In this study we evaluated the associations between fetal urinary production rate (FUPR), measured by ultrasound, and adverse neonatal outcome in women with preterm premature rupture of membranes (PPROM).

Material and Methods: We conducted a prospective cohort of singleton pregnancies complicated by PPRM occurring at gestational week 24 or later in a single center. Women with PPRM and conservative management until spontaneous labor (after 48 hours of admission), chorioamnionitis, or induction by protocol at 35+0 weeks. FUPR was evaluated by 2D sonography at admission, and corrected for gestational age. Attending physicians were blinded to FUPR results. The main neonatal outcome measures were chorioamnionitis, placental inflammatory grading, first neonatal creatinine value, first neonatal dextrose value, length of neonatal intensive care unit (NICU) stay, necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH) (grades I-IV), blood transfusions, reduced neonatal urine production rate ( $<4\text{mL/kg/h}$ ), and early neonatal sepsis. Samples of maternal (at admission) and umbilical cord blood were analyzed for interleukin-6 (IL-6) level.

Results: The study included 38 women. Low FUPR was associated with chorioamnionitis, longer NICU hospitalization ( $p=0.01$ ), and higher rates of NEC or IVH ( $p=0.008$ ), and blood transfusion ( $p=0.004$ ). There were no significant associations between antenatal FUPR and placental histologic inflammation grading, neonatal creatinine, neonatal dextrose, or early neonatal sepsis. IL-6 levels did not correlate with chorioamnionitis, FUPR, or early sepsis.

Conclusion: A finding of FUPR on *in utero* ultrasound examination in pregnancies complicated by PPRM may be indicative of an inflammatory process and predictive of adverse neonatal outcome.

## **Abbreviations**

FUPR, fetal urine production rate

NICU, neonatal intensive care unit

PPROM, preterm premature rupture of membranes

FIRS, fetal inflammatory response syndrome

IL-6, interleukin-6

IVH, intra-ventricular hemorrhage

NEC, necrotizing enterocolitis

## **Key message**

Low fetal urine production rate *in utero* evaluated by 2D sonography at admission for preterm premature rupture of membranes is associated with increased risk of chorioamnionitis and adverse neonatal outcome.

## **Keywords**

Antenatal diagnosis; Chorioamnionitis; Fetal inflammatory response syndrome; Fetal urine; Preterm birth; Preterm premature rupture of membranes; Ultrasound

## **Introduction**

Preterm premature rupture of membranes (PPROM) continues to be a leading cause of neonatal mortality and remains a major source of short- and long-term neonatal morbidity (1-5). The clinically relevant risk-benefit equation in PPRM focuses on the tradeoff between longer gestation and fetal lung maturation versus the increasing risk of potentially severe complications from neonatal infections, with longer latency (6, 7). The combination of chorioamnionitis and PPRM significantly increases the burden of neonatal prematurity complications (8). Currently, there are no reliable clinical or laboratory markers to predict impending intrauterine infection. Noninvasive models based on serum C-reactive protein (CRP), gestational age, and/or parity have shown low or moderate predictive value for histological chorioamnionitis and were poor predictors of impending intrauterine infection and subsequent neonatal infection (9).

The "syndrome" of preterm birth and PPRM is mediated in part by the initial fetal inflammatory response syndrome (FIRS), which is postulated to be a link between intrauterine infection, generalized and local inflammation, delayed uterine contractions, and birth (10). FIRS implies multi-organ involvement, including the hematopoietic system, thymus, adrenal glands, heart, lung, brain, skin, and kidneys (11, 12). The discrete definition of FIRS is an elevated fetal mean plasma concentration of interleukin-6 (IL-6) (13-15) followed by a fetal paracrine signal towards decidual activation and preterm birth (10).

Among the many organs involved in FIRS are the fetal kidneys, which play a role in the early circulatory redistribution that results in reduced fetal urine production. The objective of the present study was to evaluate the fetal urine production rate (FUPR) in patients with PPRM. We hypothesized that decreases in FUPR might be used as an early indicator of fetal blood redistribution in FIRS, and thus of impending preterm birth and adverse neonatal outcomes.

## **Material and Methods**

This is a prospective blinded observational study of consecutive cases of women with a singleton pregnancy diagnosed with PPROM at gestational age 24+0 to 34+6 weeks, between February 2010 and July 2011. Gestational age was dated using the last menstrual period and confirmed by first trimester ultrasonography. Women who developed spontaneous labor within 48 hours of PPROM, other pregnancy complications, and those whose fetuses had major malformations were excluded.

The diagnosis of PPROM was performed according to departmental protocol, with sterile speculum examination and pH evaluation of fluid collected from the posterior vaginal fornix, in the absence of blood or any other abnormal vaginal discharge. Vaginal and urine cultures were obtained at diagnosis. Amniotic fluid is not routinely sampled at admission in our Department.

Following a diagnosis of PPROM, expectant management was instituted until spontaneous labor, clinical chorioamnionitis, or scheduled delivery at 35+0 weeks (Department policy). According to Departmental protocol, during expectant management, vital signs are taken, a nonstress test is performed twice daily, and intravenous antibiotics are administered in a two-stage process (16). In the first stage, ampicillin (2gr) and erythromycin (250mg) are administered IV every 6 hours for 48 hours; in the second stage oral amoxicillin (250mg) and erythromycin (333mg) are administered every 8 hours for 5 days. A dose of 24 mg intramuscular betamethasone is also administered in two doses, 12 mg at admission and 12 mg at 24 hours.

Clinical chorioamnionitis was diagnosed when two or more of the following criteria were met: maternal fever (temperature  $\geq 37.8^{\circ}\text{C}$  on at least two occasions, one hour apart), maternal tachycardia, fetal tachycardia ( $\geq 160$  beats/minute), uterine tenderness, foul-smelling amniotic fluid, and/or maternal leukocytosis (white blood cell counts  $15,000/\text{mm}^3$  with bands). Placental histology was evaluated by a single experienced perinatal pathologist (L.S.). Placental inflammatory histology was graded according to the Redline guidelines (17, 18).

At admission, all patients underwent ultrasound examination. Fetal presentation, estimated weight, and a cumulative four quadrants amniotic fluid index (AFI) were established. FUPR was determined within 24–48 hours of PPRM and adjusted for gestational age. Assessment of FUPR was performed by ultrasound measurement of change in fetal bladder volume over time (19, 20). The technique (Fig. 1), as updated by Rabinowitz (21) and published with several variations (22), can be described briefly as follows. Ultrasound examination (E8 Voluson, GE Healthcare, Milwaukee, WI, USA) was performed and fetal bladder measurements were obtained every 1–2 minutes for 20–50 minutes until a full bladder cycle (filling and emptying) was detected. Fetal bladder measurements were assessed at admission with FUPR calculations (mL/hour), and FUPR was adjusted (percentile) by calculating the measured FUPR divided by the expected 50% percentile of FUPR for gestational age, as determined according to nomograms published by Rabinowitz (21). Attending physicians caring for the patient were blinded to FUPR results. The biophysical profile score was assessed twice a week. The diagnosis of oligohydramnios was defined when the amniotic fluid index (AFI) was  $<5$  cm (23, 24).

For the purpose of the study, in exception to Department protocol, samples of maternal (at admission) and umbilical blood were sent for IL-6 analyses. Blood samples were analyzed for IL-6 (Human IL-6 ELISA, Bender MedSystems GmbH, Vienna, Austria). The lower and upper detection limits for IL-6 were 1 and 1000 pg/ml, respectively. FIRS was defined as an IL-6 level  $>11$  pg/ml in venous umbilical blood samples at birth (25).

Maternal and neonatal outcomes were recorded as shown in maternal and neonatal medical records. A finding of chorioamnionitis, placental inflammatory grade, neonatal first creatinine value, first dextrose level, length of Neonatal Intensive Care Unit (NICU) stay, diagnosis of necrotizing enterocolitis (NEC) or intraventricular hemorrhage (IVH) grades I–IV, blood transfusion, reduced neonatal urine production rate ( $<4$  mL/kg/h), positive culture, and/or early neonatal sepsis (i.e., positive blood/cerebrospinal fluid culture within first 72 hours of life) (26, 27) were noted.

Institutional Ethics Committee approval (reference number 13/10, dated 17.01.10) was received and informed consent forms were signed by all participants.

### ***Statistical analysis***

Appropriate sample size estimation was made based on the expected correlation between fetal urine output and adverse neonatal outcome. Assuming the correlation would be  $> -0.75$ , the significance is 5% (1-way), and intensity is 80%. Under these assumptions, a sample size of 38 patients was needed to prove significance in this pilot study. Associations between continuous variables were assessed by Pearson's Correlation coefficient ( $r$ ), relations between categorical variables were evaluated by  $\chi^2$  and Fisher's exact tests, and the effect of categorical variables on continuous measurements was tested by the t-test and Mann-Whitney test. For comparison of  $\geq 3$  variables, the Kruskal-Wallis nonparametric test was applied. Choosing parametric or nonparametric testing depended on the distribution of a continuous variable.

Continuous variables are presented as mean  $\pm$  standard deviation (SD) and/or median with interquartile range (IQR), depending on distributions. The p values are 2-sided when  $<0.05$  was considered significant. Analyses were performed using SPSS statistical package version 20 (IBM, Armonk, NY).

### **Results**

During the study period, 21,927 births were recorded. PPROM was diagnosed in 139 (0.6%) women with a singleton pregnancy at 24+0 to 34+6 weeks' gestation. Overall, 101 women were excluded because they had a spontaneous labor onset within 48 hours of admission (71, 51.1%), declined to participate in the study (11, 7.9%), lack of FUPR evaluation within 48 hours of admission (11, 7.9%), intrauterine fetal death (5, 3.6%), or other pregnancy-related complication (3, 2.2%), maternal cardiac disease, cholestasis of pregnancy, HELLP syndrome). A total of 38 women (27.3%) met inclusion criteria for the study (Fig. 2). Maternal demographic characteristics for women in the study group were similar to those for women with PPROM



and singleton pregnancies who were not eligible for the study, including maternal age, education, parity, previous cesarean section, gestational age at admission, mode of delivery, and maternal length of stay after delivery (Table 1).

Maternal and neonatal descriptive characteristics for study participants are depicted in Table 2. Mean gestational age at admission was  $29.6 \pm 3.7$  weeks, and mean latency between admission and delivery was  $19.4 \pm 19.1$  days. There was spontaneous labor onset during the expectant period in 21 women (57.9%), and 24 (63.2%) women delivered vaginally. The mean gestational age at birth was  $32.8 \pm 4.5$  weeks, and the mean birth weight was  $1870 \pm 542$  grams.

The mean FUPR (adjusted for gestational age) was similar for women with oligohydramnios at admission and those with a normal amniotic fluid index ( $85.8\% \pm 45.2\%$  vs.  $115.3\% \pm 57.7\%$ ,  $p=0.16$ ). Two women (16%) had positive urine culture at admission, and eight women (21%) had positive placental cultures. The mean FUPR was significantly lower in women with clinical chorioamnionitis compared to women without signs of clinical chorioamnionitis ( $89.4\% \pm 0.6\%$  vs.  $112.7\% \pm 25.2\%$   $p=0.02$ ). There was no significant difference between low FUPR and histological inflammatory grade of the placenta (Kruskal-Wallis test,  $H(2) = 7.85$ ,  $p=0.17$ ). Four women (10.5%) developed signs of clinical chorioamnionitis. The mean FUPR was significantly lower in women with clinical chorioamnionitis compared to women without signs of clinical chorioamnionitis ( $89.4\% \pm 0.6\%$ , vs.  $112.7\% \pm 25.2\%$   $p=0.02$ ).

Three neonates had a 5 minute Apgar score  $<7$ . The mean length of NICU stay was  $32.3 \pm 40$  days. Neonates with lower *in utero* FUPR had a significantly longer NICU stay ( $r = -0.49$ ,  $p=0.01$ ) (Fig. 3), higher rates of NEC or IVH ( $p=0.008$ ), blood transfusion ( $p=0.004$ ), and reduced neonatal urine production rate during the first 24 hours of life ( $p=0.065$ ), as well as higher rates of early neonatal sepsis ( $p=0.06$ ) (Table 3). However, no significant association was found between low mean FUPR and first neonatal creatinine value ( $r = -0.32$ ;  $p=0.11$ ) or first dextrose level ( $r = -0.34$ ;  $p=0.12$ ) (Table 3).

The mean group maternal IL-6 level was  $2.14 \pm 2.79$  pg/ml (range 0.5–10.0 pg/ml). Maternal IL-6 levels showed no correlation with the diagnosis of chorioamnionitis; the median (and interquartile range) of IL-6 was 5.26 pg/ml (0.39–7.62) as compared with 1.20 pg/ml (0.66–2.10) in women who did not have a clinical diagnosis of chorioamnionitis ( $p=0.833$ ). Mean umbilical cord venous IL-6 levels were  $6.46 \pm 8.9$  pg/ml. Mean fetal IL-6 values were not correlated with FUPR ( $R_{\text{spearman}}=0.267$ ,  $p=0.427$ ) or neonatal urine production ( $p=0.235$ ). The mean umbilical sample (fetal) IL-6 was  $4.63 \pm 3.0$  in neonates without early sepsis and  $8.45 \pm 3.1$  in neonates with an early diagnosis of sepsis ( $p=0.071$ ).

## Discussion

This study is among the first to show an association between an organ involved in intrauterine FIRS in mothers with PPROM, and subsequent neonatal outcome. Using a single, noninvasive, easy-to-perform ultrasound examination based on rapid, unsophisticated techniques to evaluate FUPR at admission in mothers with PPROM, we found that gestational age lower range FUPR values were an early sentinel sign for chorioamnionitis and adverse neonatal outcome.

Previous studies have shown that FIRS in PPROM is associated with significant oligohydramnios at admission, and therefore, presumably, reduced renal blood flow as the underlying pathophysiologic mechanism (28). Additional possible explanations of the link between oligohydramnios and FIRS include the reduced antimicrobial properties of amniotic fluid and/or redistribution of blood flow away from the kidneys, inducing oligohydramnios (29).

Techniques for measuring fetal urine production by real-time ultrasonography were first published four decades ago (20), and an improved technique has been available for a decade and a half (21). Mean fetal urine production rates usually increase from 5 mL/hour at 20 weeks' gestational age to 51 mL/hour in a full-term 40-week fetus. The improved method was described and validated, with technical variations, in two studies by Fägerquist et al. (30) and Lee et al. (22). The Lee team (22) had similar outcomes with two-to-four serial measurements obtained

using a 3D ultrasound machine; however, the Lee technique is time-consuming and it requires sophisticated ultrasound machines and highly-trained medical personnel. In the current study, serial measurements of the fetal bladder volume were performed in real-time, using a 2D imaging technique, until a full cycle of bladder filling and emptying was detected. This method has the advantages of simplicity and thus an expected good interobserver reproducibility in prenatal diagnostic centers, including those with fewer resources and limited staff. Other techniques to assess fetal blood redistribution, such as Doppler ultrasound (30, 31), have the advantage that they enable quantification of renal blood flow, but they are comparatively costly to perform since they require more sophisticated equipment. The noninvasive nature of sonography was a crucial feature of the protocol for this study, which began with the hypothesis that FUPR is an early sign of fetal renal blood flow reduction in women with PPROM.

Admittedly, fetal urine production is complex, involving both diuretic and antidiuretic factors, and none of these features are considered in this pilot study of renal blood flow and FUPR. The present study aimed for a simple feature evaluable at admission that would be predictive of adverse neonatal outcome; thus, continued assessment of FUPR during hospitalization was beyond its scope. FUPR was measured and compared to expected urine output according to the gestational age (21, 32). FUPR was adjusted by gestational age rather than estimated fetal weight, based on the assumption that gestational age is as a more stable and accurate determinant that is also less influenced by a potentially extant FIRS.

Despite the small numbers, we found that reduced *in utero* FUPR in PPROM is associated with markedly increased risk of maternal chorioamnionitis and neonatal morbidity, including increased rates of NEC or IVH, blood transfusions, early neonatal sepsis, and longer NICU stays. Interestingly, reduced FUPR *in utero* was associated with a reduced neonatal urine production rate during the 24 first hours of life, despite the fact that there was no apparent alteration in creatinine levels of these neonates. This reflects the robustness of *in utero* measurements with our technique, and may point towards the time required to correct the redistribution of the FIRS. In our study, renal function as estimated by neonatal creatinine was

not altered. We may postulate that the time frame for *in utero* vascular redistribution was short and thus did not affect the renal structure. Due to the well-known plasticity of the neonatal kidney, neonatal renal function is best assessed with markers that are stable over time and are not affected by muscle mass or tubular reabsorption and secretion (33).

We did not find a significant positive correlation between FUPR, placental pathology, and neonatal outcome. The prevalence of chorioamnionitis is a function of gestational age at birth, ranging from 94% at 21–24 weeks' gestation to only 3–5% of placentas in births that are close to term and term. Funisitis and chorionic vasculitis are characteristic of FIRS and associated with impending preterm birth and neonatal morbidity (13). The finding of overt inflammation in the cases of chorioamnionitis has the highest predictive value for chorioamnionitis and a high agreement between the pathologist as compared to the agreement in the assessment of grading and staging of placenta findings (34). Thus the use we made of grading and staging might have caused an underestimation of the characteristic findings for FIRS.

The microbiological patient and laboratory milieu in our medical center may have caused a underestimation towards the diagnosis of infectious complications. The reported prevalence of amniotic fluid cultures that are positive for microorganisms in women with preterm labor and intact membranes is approximately 13% (35); in PPROM, the prevalence is approximately 32% (36). In the present study, we did not have amniotic fluid samples available; however, a surrogate in the form of placental cultures showed of 21% women with positive cultures. This rate may have been lower than expected due to the lack of cultures for atypical microorganisms (37). The low rate of antenatal infectious complications may be attributed in part to a relative short latency between diagnosis and delivery (mean = 19 days), which averted infection in some women. It is also possible that FIRS occurred without microbial invasion of the amniotic cavity in some cases (38). The lack of correlation between maternal IL-6 levels and a diagnosis of chorioamnionitis in the present study may be due to a combination of the relative rarity of overt antenatal chorioamnionitis and the limited number of subjects in the study.

Additionally, as reported previously (39, 40), we found a positive correlation between mean umbilical IL-6 levels and early neonatal sepsis. This correlation was not evident for *in utero* FUPR or neonatal urine production rate. FUPR was determined after 24 hours of PPRM, while FIRS is a complex entity. No predictable time frame has been established for the development of fetal circulatory redistribution and reduced fetal renal blood flow in PPRM, especially under maternal antibiotic protocols (15). Besides the impediment of the small sample number of samples analyzed in the present report, the samples used also reflect the possible changes that may occur in cytokine level due to the birth process itself. Previous studies that present high IL-6 levels in cases of FIRS are based on antepartum amniotic or fetal blood samples, thus reaching a different cutoff to predict FIRS. Due to the limited sample size we were unable to build a model to establish diagnostic values for umbilical samples at birth.

Limitations of our study include, first, the relatively small sample size, despite adequate statistical power calculations. Second, the maternal population was socioeconomically stable, and the women were all married, with consistent and frequent antenatal care, including antibiotic treatment.

The strength of this study are the strict inclusion criteria, the relatively simple method of FUPR evaluation, which can be easily and reliably reproduced, adherence of all patients to a single Departmental protocol, and ability of neonatal follow-up in a single-center NICU.

In conclusion, the present study is among the few available suggesting that fetal pathophysiological processes *in utero* in women with PPRM are amenable to noninvasive evaluation and correlation with post-natal events. Future studies are required to substantiate our hypothesis. In the interim, FUPR may provide additional insight into subtle intrauterine processes that may adversely impact neonatal outcome.

## **Acknowledgments**

We would like to thank Shifra Fraifeld, a hospital-based medical writer and editor, for her editorial assistance during manuscript preparation.

**Funding statement**

The authors did not receive special funding for this study.

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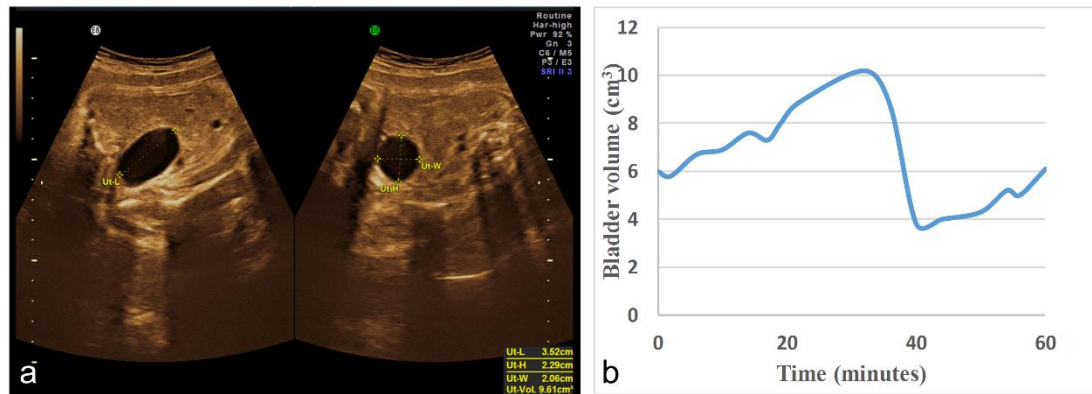
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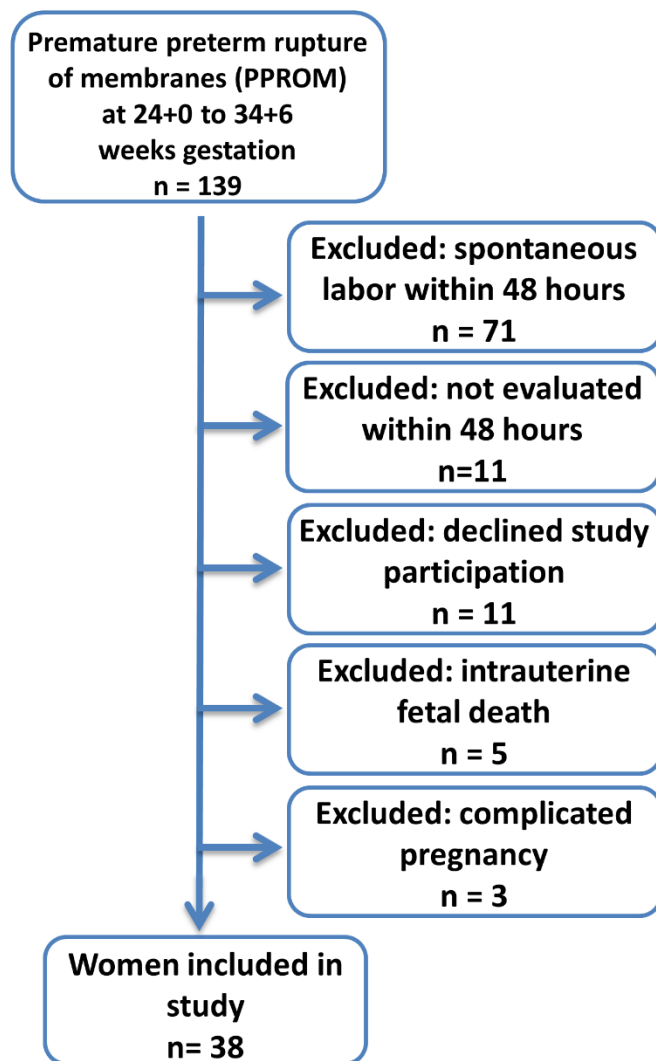
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## Legends of figures

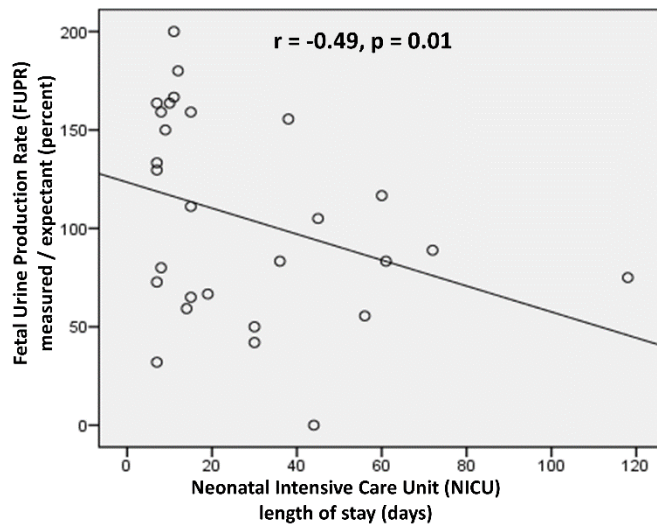


**Figure 1:** Fetal urine output assessment.

Serial fetal bladder measurements were performed every 1–2 minutes over 20–60 minutes, until a complete bladder cycle (filling and emptying) could be detected. **(a)** Transversal and longitudinal fetal bladder sections. **(b)** Fetal urine volumes (cm<sup>3</sup>) over time (minutes)



**Figure 2:** Women included in the study.



**Figure 3:** The correlation between percent of expected fetal urine production rate (FUPR) and neonatal intensive care unit (NICU) length of stay (days)

**Table 1:** Comparison of maternal characteristics and outcomes for women in the FUPR group and those with PPROM and singleton births who were excluded from the study

	<b>FUPR study group (n=38)</b>	<b>Singleton PPROM excluded (n=101)</b>	<b>p-value</b>
Maternal age (years)	29.6 ± 5.6	29.2 ± 6.3	0.74
Maternal education (years)	14 [12–16]	14 [12–16]	0.834
Parity	2.5 [2–4]	2 [1–5]	0.997
Previous cesarean delivery	5 (13.2)	22 (0.22)	0.45
Gestational age (weeks)	34 [29–35]	34 [32–35]	0.26
Spontaneous onset of labor	22 (57.9)	60 (59.4)	0.39
Mode of delivery			
Vaginal	23 (60.5)	65 (64.3)	0.37
Assisted vaginal	1 (2.6)	3 (3.0)	
Cesarean delivery	14 (36.8)	33 (32.7)	
Hospital length of stay (days)	4.6 [3.1–6.7]	3.9 [3.1–5.6]	0.092

Data are mean ± standard deviation; median [interquartile range]; number (%)



**Table 2:** Maternal and neonatal descriptive characteristics of women included in the study (n=38)

Characteristic	
Gestational age at admission (weeks)	29.6 ± 3.7
Time from admission to delivery (days)	19.4 ± 19.1
Gestational age at birth (weeks)	32.8 ± 4.5
Spontaneous onset of labor	21 (57.9%)
Vaginal delivery	24 (63.2%)
Birthweight (grams)	1870 ± 542
Male gender	22 (57.9%)

Data are mean ± standard deviation; number (%)

**Table 3:** Adverse neonatal outcome and fetal urinary production rate (FUPR), adjusted for gestational age

Adverse outcome	Number of neonates with adverse outcome	Adjusted FUPR in neonates with adverse outcome (%)	Adjusted FUPR in of neonates without adverse outcome (%)	p value
Reduced urine output in first 24 hours	8 (21.0)	75.9% $\pm$ 17.9%	107.8% $\pm$ 53.4%	0.065
Positive cultures (urine, blood, CSF)	4 (10.5)	95.7% $\pm$ 52.5%	105.5% $\pm$ 55.7%	0.64
Early sepsis	8 (21.0)	67.7% $\pm$ 79.2%	115.51% $\pm$ 50.2%	0.06
NEC or IVH	13 (34.2)	75.9% $\pm$ 17.6%	108.8% $\pm$ 53.4%	<b>0.008</b>
Blood products transfusion	11 (28.9)	72.2% $\pm$ 35.3%	120.9% $\pm$ 51.4%	<b>0.004</b>

Data are number (%); mean  $\pm$  standard deviation; FUPR – Fetal urinary production rate; CSF- Cerebrospinal fluid; NEC – Necrotizing Enterocolitis; IVH – Intraventricular hemorrhage