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Keywords:	p-PROM, preterm delivery, membranes, latency
Abstract:	Objective The early identification of women with p-PROM who are at higher risk of imminent delivery remains challenging. The aim of our study was to evaluate if an increased echogenicity of the amniotic membranes may represent a sonographic marker of impending delivery in women with p-PROM. Study design This was a prospective study including women with singleton pregnancies and diagnosis of p-PROM between 22-37 gestational weeks. A sonographic examination was performed within 24 hours from the hospital admission and the appearance of the amniotic membranes close to the internal os was specifically evaluated. The membranes were defined as hyperechoic when their echogenicity was similar to that of the fetal bones or normoechoic in the other cases. The primary aim of the study was to compare the admission to spontaneous onset of labor interval and the pregnancy outcome between the cases of p-PROM with and without hyperechoic membranes. Results Overall, 45 women fulfilled the inclusion criteria with similar characteristics at admission. In women with hyperechoic membranes the admission to spontaneous onset of labor interval was significantly shorter (3.9□3.5 vs 19.9□21.9 p=0.04) compared to women with normo-echoic membranes. At binomial logistic regression after adjustment for GA at hospital admission the presence of hyperechoic membranes was found as the only independent predictor of spontaneous onset of labor □72 hours (aOR: 6.1; 95% CI: 1.0-36.9) Conclusion

	The presence of hyperechoic membranes is associated with a 6-fold higher incidence of spontaneous onset of labor within 72 hours independently from the gestational age at p-PROM.

Reviewer 1

This was a prospective study including singleton pregnancies between 22-37 gestational weeks with specific inclusion criteria. The membranes were defined as hyperechoic when their echogenicity was similar to that of the fetal bones and were evaluated by transvaginal ultrasound.

The aim of the study was to evaluate if an increased echogenicity of the amniotic membranes may represent a sonographic marker of impending delivery in p-PROM patients.

The authors concluded that there was a shorter interval between the diagnosis of pPROM and admission to delivery. Moreover, the presence of hyperechoic membranes was found as the only independent predictor of delivery within 72 hours.

Although this use of the ultrasound marker is fast, simple and usable in any hospital, there are several main limitations of the study, which represent the most important critical issues.

 The aim is different in the introduction and in the discussion: does the neonatal outcome represent an aim or not?

> *R. Thank you for this comment. The neonatal outcome was not the primary outcome of our study As stated in Materials and Methods section, the primary aim of our study was to compare the admission to delivery interval and the pregnancy outcome between the cases of p-PROM with and without hyperechoic membranes. The discussion has been mostly focused on the possible explanation of the biochemical process which may cause the increased echogenicity of the amniotic membranes and eventually lead to a shorter interval between premature rupture of membranes and onset of labor . However, although the neonatal outcome was not the primary aim of our study, we feel appropriate to point out that the prediction of the latency time in women presenting with p-PROM allows the optimization of antenatal care thus improving the neonatal outcome itself.*

 From a statistical point of view:

- Given the low patients' number, was it convenient to use the median and the ranges rather than the mean and standard deviation?

R. We thank the reviewer for this smart comment. We have changed the tables and the text using median and ranges.

- On several occasions, the SD is greater than the average itself: this seems to be the expression of a non-homogeneous sample.

R. Thank you for this observation. Following appropriate tests for normality and homogeneity, between-groups comparison of continuous variables has been carried out using T-test and the Mann-Whitney non parametric equivalent test.

In accordance with the reviewer's observation we have provided a more detailed statistical description in the appropriate section of the manuscript

- Given the low size of the sample with hyperechoic membranes, the results obtained could be secondary to chance, not to a real statistical difference.

R: we are aware that our sample size is small (this is actually acknowledged as a study limitation) and that these observations need to be confirmed by larger studies; however, appropriate statistical tests seem to confirm that our results are statistically significant and not secondary to chance.

The methodological description relating to the interpretation of the image is missing:

- if the three experts were blinded to each other
- if the ultrasound machine was the same for all the experts and if not, which technical difference was present
- the correct setting for displaying the membranes
- above all, the inter-sonographer coefficient of variability, which can express the agreement among the expert and the ease of interpretation.

R. we thank the reviewer for these methodological observations. As explained in the text, ultrasound images were reviewed in consensus, at the same time, by three expert sonographers (all with more than 10 years of experience in prenatal ultrasound) to qualitatively evaluate the presence/absence of hyperechoic membranes; they were not blinded to each other. All transvaginal examinations were performed using a 5.0-7.0 MHz transvaginal transducers (General Electric Voluson E6; Samsung HS60) using the standard factory settings for 2nd-3rd trimester evaluation and the bone (eg. fetal skull) as reference. This has been more clearly specified in the text.

The study was not designed to assess inter-sonographer variability, as images were reviewed in consensus. The need for further studies to evaluate the reproducibility has been mentioned among the conclusions.

 The different gestational week at the entrance could explain the lower latency in the group of hyperechoic membranes.

R: thank you for this comment that gives us the opportunity to better clarify some points. As reported on table 2, the more advanced gestational age at admission in the hyperechoic membranes group was not significantly different compared with the controls (p 0.06). Moreover, as shown on table 3, at multiple logistic regression (adjusted for all potential confounders including gestational age at admission) hyperechoic membranes were confirmed as the only significant and independent predictor of the risk of delivery <72 h.

 The absence of placenta and membranes histological investigation in most patients does not allow the inclusion of this variable in the descriptive table. It also represents one of the major limitations of the study, since the verification of the presence of an inflammatory / infectious state would have supported the explanation that the authors give in the discussion about the hyper-echogenicity of the membranes (the authors do not consider other explanations

about it). Moreover, the neonatal outcome, here absent, it is also influenced by a condition of chorioamnionitis, as well as by the gestational age and neonatal co-morbidities.

R: Thank you for this smart observation. We agree with the reviewer on the fact that due to the lack of histological investigations, this variable should be removed from the descriptive table and we have amended Table 2 accordingly. However, we respectfully disagree with the reviewer about the fact that we did not considered other explanations except the inflammatory state. As stated in the discussion (Interpretation Section), we have acknowledged other factors including mechanical stress and bleeding among the factors which may initiate the inflammatory cascade responsible for the biochemical and histological changes of the "weak zone".

 The tables lack of any measurement units and the metric reference.

R. Thank you. We have amended the manuscript adding the measurement units in the tables.

 The references they are not homogeneous each other.

R. Thank you, the references have been changed according to the journal style.

 The second image does not allow a clear visualization of the membrane. As in the previous point, this technical aspect becomes crucial.

R. We agree with the reviewer about this point. However, this is the only available image of a pPROM with a fetus in breech presentation where the iliac bones are visible and can be used as a reference to confirm the increased echogenicity of the amniotic membranes. We therefore submitted it even if its quality is suboptimal.

If required, we can delete this image or add another with a fetus in cephalic presentation.

This is a promising study, but it requires an increase in the sample size and a greater methodological definition.

R. we thank the reviewer for his encouraging evaluation. We are aware that further studies are needed in order to evaluate the reproducibility of this sign, and to elucidate whether its introduction in the ultrasound assessment of women with p-PROM may improve the identification of those at higher risk of spontaneous labor or infection/inflammation in the short term. Furthermore, thanks to the Reviewer's suggestions, the methodological definition has been improved.

Reviewer: 2

Comments to the Author

Volpe and colleagues performed a very interesting study on a new ultrasound sign to predict the interval to spontaneous delivery in 45 women with diagnosis of pPROM. The methodology of the study is overall good and well explained in the methods section. However, there are some major points that need to be addressed.

1. The main conclusion of the study is that "the presence of hyperechoic membranes is associated with a 6-fold higher incidence of spontaneous onset of labor within 72 hours independently from the gestational age at p-PROM". This conclusion cannot be stated since the logistic regression analysis shows that hyperechoic membranes are associated to a spontaneous delivery <72 hours with an adjusted OR 6.1; 95% CI: 1.0-36.9 (Table 3). Since the CI includes 1.0 this result is not statistically significant (Hyperechoic membranes cannot be considered either protective nor harmful for the outcome). Therefore, Discussion and Conclusion sections should be corrected accordingly.

R: We thank the reviewer for this comment that gives us the opportunity to better clarify some points. The exact value of CI is 1.04-36.88 (we had previously rounded to only a

decimal throughout the whole text) and the p-Value for this association is in fact 0.04; we have specified p-values in the table.

For this reason, we retain that discussion and conclusion do not require to be changed.

The univariate analysis in Table 3 includes 37 women grouped according to spontaneous delivery > or < 72 hours (excluding 7 women with IOL and 1 elective CS). However, in the Results section authors state that only 24/37 (64.9%) women with spontaneous onset of labor delivered with vaginal delivery (page 8 line 3). Authors should clarify whether the univariate analysis includes also women with spontaneous onset of labor that delivered by urgent CS, which should be 13/37 (35.1%) according to the Results section. If that is the case, authors should point out the major indications for urgent CS in the included cases and include the delivery by urgent CS in the univariate analysis and evaluate whether this variable could affect the relationship between the presence of hyperechoic membranes and the interval to delivery in the included women.

R. We thank the reviewer for this smart observation. Of course, in the univariate analysis also women with spontaneous onset of labor that delivered by urgent CS in labor have been included. By mistake the header of 2 columns of table 3 in the original version was ***spontaneous delivery*** *> or <72 hours but in fact the correct header is* ***spontaneous onset of labor (and not delivery) before or after 72 hours*** *from the admission. This error has been amended in the revised version of table 3, and we apologize for this inaccuracy which may have caused confusion. The indications to urgent CS in labor have been now specified in the text: breech presentation in 3/13 cases, CTG anomalies in 5/13 cases, intrapartum hemorrhage in 1 case and labor arrest in 4/13 cases. Urgent CS done in labor had been originally included in the univariate analysis (comparing women with spontaneous onset of labor before or after 72 hours from pPROM). Of course since all cases of CS but one have been performed during labor after its spontaneous onset, the relationship between the*

presence of hyperechoic membranes and the admission to delivery interval was not affected by the mode of delivery itself (CS vs vaginal delivery).

3. Minor revision of English language should be performed

Page 4 Line 49: "analysed" instead of "sent"

Page 4 Line 39: "avoided" instead of "prohibited"

R: thank you. The text has been amended accordingly.

Page 5 Line 3 Clarify "At transabdominal ultrasound following the confirmation of fetal viability and the scarcity or lack of amniotic fluid the estimated fetal weight were determined"

R: We thank the reviewer for the comment. As suggested, this sentence has been made more clear in the revised version of the manuscript

Page 6 Line 5 "fetal adnexa" instead of "adnexa"; "histopathological examination" instead of *"pathology"*.

R: The text has been amended accordingly.

References:

Please follow Journal style, see attachment

R: The text has been amended accordingly.

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ABSTRACT

Objective

The early identification of women with p-PROM who are at higher risk of imminent delivery remains challenging. The aim of our study was to evaluate if an increased echogenicity of the amniotic membranes may represent a sonographic marker of impending delivery in women with p-PROM.

Study design

This was a prospective study including women with singleton pregnancies and diagnosis of p-PROM between 22-37 gestational weeks. A sonographic examination was performed within 24 hours from the hospital admission and the appearance of the amniotic membranes close to the internal os was specifically evaluated. The membranes were defined as hyperechoic when their echogenicity was similar to that of the fetal bones or normoechoic in the other cases. The primary aim of the study was to compare the admission to spontaneous onset of labor~~delivery~~ interval and the pregnancy outcome between the cases of p-PROM with and without hyperechoic membranes.

Results

Overall, 45 women fulfilled the inclusion criteria with similar characteristics at admission. In women with hyperechoic membranes the admission to spontaneous onset of labor ~~delivery~~ interval was significantly shorter (3.9±3.5 vs 19.9±21.9 p=0.04) compared to women with normo-echoic membranes. At binomial logistic regression after adjustment for GA at hospital admission the presence of hyperechoic membranes was found as the only independent predictor of spontaneous onset of labor ~~delivery~~ ≤72 hours (aOR: 6.1; 95% CI: 1.0-36.9)

Conclusion

The presence of hyperechoic membranes is associated with a 6-fold higher incidence of spontaneous onset of labor within 72 hours independently from the gestational age at p-PROM.

Keywords: p-PROM, membranes, latency, preterm delivery

List of abbreviation

p-PROM: preterm premature rupture of membranes

CPR: C-reactive protein

WBC: white blood cell

CTG: CardioTocoGraphy

BMI: Body Mass Index

INTRODUCTION

Premature rupture of membranes (PROM) occurring before 37 weeks of gestation is defined preterm PROM (p-PROM) and complicates up to 3% of pregnancies accounting for about one-third of preterm births [1].

In clinically stable cases with no signs of maternal infections or uterine contractions expectant management is currently recommended up to 34 or even 36 weeks of gestation while elective delivery is considered thereafter since the risk of intrauterine infection outweighs the benefit of prolonging the pregnancy [2].

Hospital admission is usually offered to all asymptomatic women with p-PROM from 24 weeks onward with the aim of providing antepartum surveillance and appropriate intrapartum and neonatal care in case of preterm birth [3,4]. However, the temporal interval between the pPROM and the spontaneous onset of labor is extremely variable and may range from a few hours to several weeks [5,6].

Accurate and timely identification of those women with p-PROM who are at risk of impending preterm labor or chorioamnionitis would be desirable [7-9]. In these cases, strict clinical monitoring, steroids with or without magnesium sulphate administration and transfer to a center with neonatal intensive care facilities, are warranted to improve the neonatal outcome [3,4,10,11].

Corticosteroids in particular have been shown to improve the outcome of preterm neonates if administered within 2-7 days from birth while this benefit is uncertain if delivery occurs at more than a week from their administration [12-14].

To date, the early identification of women with p-PROM who are at higher risk of imminent delivery remains challenging [15]. Clinical and laboratory findings as the cervical length at transvaginal ultrasound have been shown to be poor predictors of the actual risk of impending delivery, while the intraamniotic assessment of inflammatory markers although promising is not widely available and easy to perform [16-21].

An increased thickness of the membranes as a marker of impending delivery has been also evaluated by some with not encouraging results [22,23]. As suggested by earlier studies [24,25], it is plausible to hypothesize that the inflammatory milieu which characterizes the choriodecidual interface before the clinical onset of preterm labor may alter the sonographic features of the amniotic membranes [26]. The aim of our study was to evaluate if an increased echogenicity of the amniotic membranes may represent a sonographic marker of impending delivery in women with p-PROM.

MATERIALS AND METHODS

Study design and population

This was a prospective study conducted at the University Hospital of Parma between February 2019 and March 2020. Women with singleton pregnancies and diagnosis of p-PROM between 22-37 gestational weeks were enrolled based on the availability of one of the investigators.

Gestational age was calculated from crown-rump length measure at 11+0-13+6 weeks' gestation [27]. Exclusion criteria were abnormalities of placentation, previous diagnosis of uterine malformations, cervical cerclage, fetal abnormalities and congenital infections, presence of uterine contractions and delivery within 12h from the p-PROM. The criteria for the diagnosis of p-PROM included clinical diagnosis of rupture based on the presence of amniotic fluid leakage from the cervical os during sterile speculum examination and confirmation by biochemical tests [28]. Active labor was defined by a fully effaced, >6 cm dilatated cervix coupled with >3 contractions in 10 minutes recorded at tocography.

Upon admission, cervical and vaginal swabs and mid-stream urine culture were collected and a blood sample including white blood count (WBC) and C-reactive protein (CRP) was ~~analysed~~analyzed~~sent~~. Further digital examinations were avoided~~prohibited~~ in absence of signs of active labor.

Management

In the study group a targeted sonographic examination was performed transabdominally and transvaginally within 24 hours from the hospital admission by an expert examiner who was in charge of this research project and not involved in the clinical management of the patients~~. At transabdominal ultrasound following the confirmation of fetal viability and the scarcity or lack of amniotic fluid the estimated fetal weight were determined~~. At transabdominal ultrasound, following the confirmation of fetal viability, the estimated fetal weight was determined.

~~All transvaginal examinations were performed using multifrequency real-time transducers. Two ultrasound machines have been used for this study, a Samsung HS60 (2.0-11.0 MHz transvaginal probe, Samsung Medison Co Ltd, Seoul, South Korea), and a General Electric Voluson E6 (3.0-10.0 MHz, GE Medical Systems, Zipf, Austria). The standard factory settings for 2nd-3rd trimester evaluation have been used for the transvaginal examinations, and the fetal bone (e.g. skull) has been used as reference to define membranes echogenicity.~~

The cervical length was measured transvaginally along its longitudinal axis with empty bladder, from the internal to the external os. During the transvaginal examination, the presence of a caput succedaneum was noted and the sonographic appearance of the amniotic membranes close to the internal os was specifically evaluated. ~~The sonographic pictures were stored on the ultrasound machine and assessed in consensus by the three main study investigators.~~

-The membranes were defined as hyperechoic when their echogenicity was similar to that of the fetal bones (either skull, femur or ~~sacrum~~ pelvic bones depending upon the fetal presentation) (Figure 1, Figure 2), or normoechoic in the other cases (Figure 3).

All transvaginal examinations were performed using multifrequency real-time transducers. Two ultrasound machines have been used for this study, a Samsung HS60 (2.0-11.0 MHz transvaginal probe, Samsung Medison Co Ltd, Seoul, South Korea), and a General Electric Voluson E6 (3.0-10.0 MHz, GE Medical Systems, Zipf, Austria). The standard factory settings for 2nd-3rd trimester evaluation have been used for the transvaginal examinations, and the fetal bone (e.g. skull) has been used as reference to define membranes echogenicity.~~The transvaginal ultrasound findings were not~~

~~revealed to the clinicians whose clinical management was blinded to the appearance of the membranes.~~

The sonographic pictures were stored on the ultrasound machine and ~~assessed in consensus by the three main study investigators. Ultrasound images were~~ reviewed in consensus by the three main study investigators ~~expert sonographers~~ to qualitatively evaluate the presence/absence of hyperechoic membranes. The transvaginal ultrasound findings were not revealed to the clinicians whose clinical management was blinded to the appearance of the membranes.

All women received antibiotics (ampicillin 2 g intravenously every 6 hours and azithromycin 500 mg intravenously daily for 2 days, followed by oral ampicillin 250 mg every 8 hours and azithromycin 500 mg daily for 5 day) and antenatal steroids from 24 weeks of gestation. ~~Blood samples for white blood cells (WBC) and were collected at admission and on alternate days [3,4].~~

Maternal surveillance was based on daily monitoring of clinical signs of chorioamnionitis including temperature, heart rate, uterine tenderness, vaginal discharges with laboratory tests (white blood cells and C-Reactive Protein) collected at admission and on alternate days. Fetal surveillance was carried out by means of daily CardioTocoGraphy (CTG) and biweekly transabdominal ultrasound with umbilical Doppler. The residual amount of amniotic fluid was not sonographically assessed.

Delivery was expedited in cases of suspected chorioamnionitis or fetal compromise while in clinically stable cases a policy of expectant management was adopted until 37^{+0} weeks of gestation with the exception of those cases with a documented Group B streptococcus infection, ins whom elective delivery was carried out at 34^{+0} weeks of gestation[2]. Chorioamnionitis was suspected in accordance with the Triple I criteria [29]. Spontaneous preterm labor was not inhibited by tocolytic and in case of delivery $<32^{+0}$ weeks magnesium sulphate for neuroprotection was administrated at least 2 hours before delivery. Based on obstetric indications elective delivery was carried by means of induction

of labor (IOL) or CS. Placental and fetal adnexa specimen was not sent for histopathological examinationpathology on a routine basis.

Demographic and clinical details of each pregnancy were retrieved from medical records.

Outcome

The primary aim of the study was to compare the admission to delivery spontaneous onset of labordelivery interval and the pregnancy outcome between the cases of p-PROM with and without hyperechoic membranes.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) v. 22 (IBM Inc., Armonk, NY, USA). Data were shown as median [Interquartile Range]mean±standard deviation (SD) or as number (IQRpercentage). Between-group comparison of continuous variables was undertaken using T-test and the Mann-Whitney nonparametric equivalent test while cCategorical variables were compared using the Chi-square or Fisher exact test. Binomial logistic regression was used to assess the strengths of the variables significantly different at the univariate analysis (gestational age at p-PROM, presence of hyperechoic membranes) and spontaneous onset of labor delivery within 72 hours; data were expressed as Odd Ratio (OR) and 95% Confidence Interval (CI). A Kaplan-Meier analysis was used to assess the latency time in women with and without hyperechoic membranes. Two-sided p-values were calculated and p-values <0.05 were considered as statistically significant. The study was performed following the STROBE guidelines [30].

The study was approved by the local Ethics Committee (1007/2019).

RESULTS

Over the study period 89 women were admitted with a diagnosis of p-PROM at our Maternity Hospital and 75 were evaluated for the study purpose; hyperechoic membranes were described in 14/75 cases (18.6 %). Of them, 45 women fulfilled the inclusion criteria and were included in the

study; the medianan cervical length at admission was 26.0[18.0-32.0]25.5±10.1 mm and 11 women presented hyperechoic membranes (Figure 4).

Medianan gestational age at admission was 30.6[26.6-34.3]0±4.37 weeks with a median gestational age at delivery of an admission to delivery 34.0[29.7-35.4] weeks interval of 16.8±19.8 days (Table 1). Overall, vaginal delivery was achieved in 31/45 (68.9%) patients, following spontaneous onset of preterm labor in 24/37(64.9%) and IOL in 7/7 (100%) cases; Urgent CS during labor was performed in 13 cases with the following indications: breech presentation in 3/13 cases, CTG anomalies in 5/13 cases, abruptio placentaintrapartum hemorrhagee in 1 case and labor dystociaarrest in 4/ 13 cases. elective Scheduled Caesarean Section (CS) was performed in 1/45(2.2%) case due to for breech presentation. Overall One one case of suspected chorioamnionitis was observed. Urgent CS during labor was performed in 13 cases with the following indications: breech presentation in 3/13 cases, CTG anomalies in 5/13 cases, abruptio placentae in 1 case and labor dystocia in 4/13 cases.

Maternal and pregnancy characteristics of the included women with normo-echoic and hyper-echoic membranes women are reported in Table 2. Gestational age (29.9[25.8-33.2]32.2±4.1 *vs.* 34.6[29.4-35.1]29.3±4.3 p=0.06) and cervical length at admission (27.6[18.8-33.8] *vs.* 24.0[17.0-27.5] p=0.1826.7±10.7 vs. 21.9±7.4; p=0.18) were comparable not significantly different between the two groups. In women with hyperechoic membranes the admission to spontaneous onset of labor deliverydelivery interval was significantly shorter (11.5[5.3-25.0] vs. 3.0[1.5-9.0]3.9±3.5 vs 19.9±21.9 p=0.04). At binomial logistic regression after adjustment for gestational age at hospital admission the presence of hyperechoic membranes was found as the only independent predictor of spontaneous onset of labordeliverydelivery ≤72 hours (aOR: 6.1; 95% CI: 1.0-36.9) (Table 3).aOR: 6.1; 95% CI: 1.0-36.9; p=0.04) (Table 3).

At Figure 5 the proportion of undelivered patients with and without hyperechoic membranes according to latency time is displayed.

DISCUSSION

Main findings

Among women with p-PROM the presence of hyper-echoic amniotic membranes seems associated with a shorter latency from p-PROM to ~~spontaneous onset of labor~~delivery. More importantly, our results demonstrate that the presence of hyperechoic membranes is associated with a 6-fold higher incidence of spontaneous onset of labor within 72 hours independently from the gestational age at p-PROM. Interestingly, women with and without hyper-echoic amniotic membranes had similar characteristics at admission with no difference in parity, maternal age and BMI, inflammatory indices (CPR, WBC count) and incidence of genito-urinary infections. Of note, a shorter cervical length was found in women with hyper-echoic membranes compared with women with normo-echoic membrane. However, this difference was not statistically different and may also depend on the non significantly more advanced gestational age at admission among the women with hyperechoic membranes.

Interpretation

The portion of the amniotic membranes overlying the cervix and the lower uterine segment (LUS) has been previously identified as the "weakest zone" of the fetal membranes whose biochemical and histological properties are different from those of the remaining amnion. make this zone more susceptible to rupture in presence of a repetitive stretching process, as contractions [31].

The mechanisms leading to the development of this weak zone with its biochemical peculiarities is still not well understood. Infection, inflammation, mechanical stress, bleeding are among the acknowledged factors which may initiate the inflammatory cascade responsible for the biochemical and histological changes of the "weak zone". In women with preterm PROM an increased echogenicity of the amniotic membranes overriding the cervix may reflect the early inflammatory changes which characterize the weakest zone when the biochemical cascade of labor has been triggered[30]. Ultrasound features of other known fetal inflammatory conditions such as echogenic bowel or meconium peritonitis or myocarditis or encephalitis are characterized by a higher

echogenicity. Independently from the causal agent, in case of inflammatory response, the common pathway is represented by exudation and tissue edema. This can show up as bright areas on ultrasound as bright areas [32,33]. Similarly, overall loss of water, collagen degradation, over-production of inflammatory proteins may all be responsible for the increased echogenicity and thickness of the amniotic membranes overlying the cervix and these sonographic signs may possibly reflect an advanced stage of the inflammatory process leading to preterm labor.

Previous studies

A few studies have previously investigated some ultrasound markers which might predict the time to delivery interval among women with premature rupture of membranes. Some authors [34] hypothesized that a thickened myometrium at the time of p-PROM may be predictor of a longer latency before the spontaneous onset of labor and demonstrated that a fundal myometrial thickness <8.1 mm had a sensitivity of 55.6% and a specificity of 88.9% in predicting labor onset <48h.

Given the evidence that the activation of the biochemical cascade leading to preterm labor is expected to involve at once the amniotic membranes and the cervix, further studies evaluated the role of cervical length in predicting latency time. In 2015, Mehra et al. [21] demonstrated that a shorter cervical length and an Amniotic Fluid Index (AFI) <5 cm independently predict delivery within 7 days in women presenting with p-PROM. In addition, they found that the combination of an AFI >5 cm and cervical length >2 cm greatly improved the potential to remain undelivered at 7 days with a negative predicting value (NPV) of 93%. However, these same ultrasound findings yielded a poor positive Predictive Value (PPV) in heralding an impending delivery and their diagnostic accuracy was dependent from gestational age at p-PROM.

In the last 25 years increased levels of cytokines or Metalloproteinases (MMP) in the amniotic fluid have been extensively investigated in women with p-PROM and proposed as a reliable marker of intraamniotic infection or inflammation [35-37] and ultimately of impending delivery alone or in combination with a short cervical length [16]. Unfortunately, the use and the availability of these tests in clinical practice has been limited by the necessity to retrieve amniotic fluid by amniocentesis in

women with p-PROM. Under these circumstances amniotic fluid sampling may be challenging due to the limited amount of amniotic fluid and may ~~iatrogenically~~ increase the risk of iatrogenic infections. The recent development of a bedside test based on the IL-8 dosage in the amniotic fluid retrieved from the cervical canal is expected to offer promising results in the timely identification of cases at higher risk of impending preterm delivery. A preliminary study demonstrated that cervical fluid IL-8 concentrations ≥9.5 ng/ml had significantly higher sensitivity than a transabdominally obtained amniotic fluid WBC count in the identification of intra-amniotic inflammation/infection using as gold standard a positive culture for bacteria or an MMP-8 >23ng/mL (91% vs 75%; $p<0.05$) [38].

Clinical implication

The prediction of the latency time in women presenting with p-PROM is a crucial issue for both clinicians and patients. More specifically, the use of a simple ultrasound marker which may predict ~~spontaneous onset of labor~~delivery within 72 hours may be valuable in improving the clinical management of these cases. In particular, identifying those women at higher risk of impending preterm delivery allows to optimize the timing of antenatal steroids administration and to select those women with p-PROM who require hospitalization in a tertiary care center.

Strengths and limitation

The prospective design of the study, its originality, the standardization of the clinical management and the sonographic assessment performed by expert examiners are among the main strengths of this work. On the other hand, the small sample size, the subjective assessment of amniotic membranes echogenicity and the lack of ~~longitudinal and~~ follow-up data on neonates represent its major limitations. Although the echogenicity of fetal bones is used as a reference, the assessment and the definition of hyper-echoic membranes remains a qualitative and subjective evaluation.

Finally, the histology of fetal adnexa was not requested in all cases and this did not allow us to investigate the correlation between the ultrasound findings and the specimen appearance; however, we do not think that this represents a major issue as our primary study aim was to evaluate the

occurrence of a clinical outcome such as the onset of spontaneous labor in women rather than the incidence of histological chorioamnionitis which is commonly described in more than 1/3 of p-PROM without relevant clinical consequences [39,40].

Conclusion

In conclusion, the presence of hyperechoic amniotic membranes overriding the cervix may anticipate an imminent preterm delivery in women presenting with p-PROM. Further studies are needed in order to evaluate the reproducibility of this sign, and to elucidate whether its introduction in the ultrasound assessment of women with p-PROM may improve the identification of those at higher risk of spontaneous labor or infection/inflammation in the short term for whom a closer clinical surveillance and transfer to tertiary care facilities is warranted.

Acknowledgments: none to declare

Conflict of interest: none to declare

REFERENCES

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371:75-84. doi:10.1016/S0140-6736(08)60074-4
2. Morris JM, Roberts CL, Bowen JR, Patterson JA, Bond DM, Algert CS et al. Immediate delivery compared with expectant management after preterm pre-labour rupture of the membranes close to term (PPROMT trial): a randomised controlled trial. Lancet. 2016;387:444-452. doi:10.1016/S0140-6736(15)00724-2
3. Thomson AJ. Royal College of Obstetricians and Gynaecologists. Care of Women Presenting with Suspected Preterm Prelabour Rupture of Membranes from 24(+0) Weeks of Gestation: Green-top Guideline No. 73. BJOG. 2019 Aug;126:e152-e166. doi: 10.1111/1471-0528.15803. Epub 2019 Jun 17. PubMed PMID: 31207667

4. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics. Practice Bulletin No. 172: Premature Rupture of Membranes. Obstet Gynecol. 2016 Oct;128:e165-77. doi: 10.1097/AOG.0000000000001712. Review. PubMed PMID: 27661655

5. Dagklis T, Petousis S, Margioula-Siarkou C, Mavromatidis G, Kalogiannidis I, Prapas N et al. Parameters affecting latency period in PPROM cases: a 10-year experience of a single institution. J Matern Fetal Neonatal Med. 2013;26:1455-1458. doi:10.3109/14767058.2013.784257

6. Frenette P, Dodds L, Armson BA, Jangaard K. Preterm prelabour rupture of membranes: effect of latency on neonatal and maternal outcomes. J Obstet Gynaecol Can. 2013;35:710-717. doi:10.1016/S1701-2163(15)30861-6

7. Drassinower D, Friedman AM, Običan SG, Levin H, Gyamfi-Bannerman C. Prolonged latency of preterm premature rupture of membranes and risk of neonatal sepsis. Am J Obstet Gynecol. 2016;214:743.e1-743.e7436. doi:10.1016/j.ajog.2015.12.031

8. Nayot D, Penava D, Da Silva O, Richardson BS, de Vrijer B. Neonatal outcomes are associated with latency after preterm premature rupture of membranes. J Perinatol. 2012;32:970-977. doi:10.1038/jp.2012.15

9. Ramsey PS, Lieman JM, Brumfield CG, Carlo W. Chorioamnionitis increases neonatal morbidity in pregnancies complicated by preterm premature rupture of membranes. Am J Obstet Gynecol. 2005;192:1162-1166. doi:10.1016/j.ajog.2004.11.035

10. Simhan HN, Canavan TP. Preterm premature rupture of membranes: diagnosis, evaluation and management strategies. BJOG. 2005;112 (Suppl 1):32-37. doi:10.1111/j.1471-0528.2005.00582.x

11. Rouse DJ, Hirtz DG, Thom E, Warner MW, Spong CY, Mercer BM et al. A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy. N Engl J Med. 2008;359:895-905. doi:10.1056/NEJMoa0801187

12. Vidaeff AC, Ramin SM. Antenatal corticosteroids after preterm premature rupture of membranes. Clin Obstet Gynecol. 2011;54:337-343. doi:10.1097/GRF.0b013e318217d85b

13. Jobe AH, Goldenberg RL. Antenatal corticosteroids: an assessment of anticipated benefits and potential risks. Am J Obstet Gynecol. 2018 Jul;219:62-74

14. di Pasquo E, Saccone G, Angeli L,Dall'Asta A, Borghi E, Fieni S et al. Determinants of neonatal hypoglycemia after antenatal administration of corticosteroids (ACS) for lung maturation: Data from two referral centers and review of the literature. Early Hum Dev. 2020; 143:104984. doi:10.1016/j.earlhumdev.2020.104984

15. Ville Y, Rozenberg P. Predictors of preterm birth. Best Pract Res Clin Obstet Gynaecol. 2018; 52:23-32. doi:10.1016/j.bpobgyn.2018.05.002

16. Lee SM, Park KH, Jung EY, Jang JA, Yoo HN. Frequency and clinical significance of short cervix in patients with preterm premature rupture of membranes. PLoS One. 2017; 12:e0174657. Published 2017 Mar 30. doi:10.1371/journal.pone.0174657

17. Park KH, Lee SY, Kim SN, Jeong EH, Oh KJ, Ryu A. Prediction of imminent preterm delivery in women with preterm premature rupture of membranes. J Perinat Med. 2011;40:151-157. Published 2011 Nov 16. doi:10.1515/JPM.2011.124

18. Yagur Y, Weitzner O, Ravid E, Biron-Shental T. Can we predict preterm delivery in patients with premature rupture of membranes?. Arch Gynecol Obstet. 2019;300:615-621. doi:10.1007/s00404-019-05196-8

19. Kayem G, Batteux F, Girard N, Schmitz T, Willaime M, Maillard F et al. Predictive value of vaginal IL-6 and TNFα bedside tests repeated until delivery for the prediction of maternal-fetal infection in cases of premature rupture of membranes. Eur J Obstet Gynecol Reprod Biol. 2017; 211:8-14. doi:10.1016/j.ejogrb.2017.01.013

20. Trochez-Martinez RD, Smith P, Lamont RF. Use of C-reactive protein as a predictor of chorioamnionitis in preterm prelabour rupture of membranes: a systematic review. BJOG. 2007;114:796-801. doi:10.1111/j.1471-0528.2007.01385.x

21. Mehra S, Amon E, Hopkins S, Gavard JA, Shyken J. Transvaginal cervical length and amniotic fluid index: can it predict delivery latency following preterm premature rupture of membranes?. Am J Obstet Gynecol. 2015;212(3): 400.e1-400.e4009. doi:10.1016/j.ajog.2015.01.022

22. Aviram A, Quaglietta P, Warshafsky C, Zaltz A, Weiner E, Melamed N et al. The utility of ultrasound assessment in the management of preterm prelabor rupture of the membranes. Ultrasound Obstet Gynecol. 2019;10.1002/uog.20403. doi:10.1002/uog.20403 [Epub ahead of print, 2019 Jul 23].

23. Nune V, Cross J, Speich JE, Morgan DR, Strauss JF 3rd, Ramus RM. Fetal membrane imaging and the prediction of preterm birth: a systematic review, current issues, and future directions. BMC Pregnancy Childbirth 2016; 16, 387 . https://doi.org/10.1186/s12884-016-1176-5

24. Frigo P, Lang C, Sator M, Ulrich R, Husslein P. Membrane thickness and PROM--high-frequency ultrasound measurements. Prenat Diagn. 1998;18:333-337.

25. Severi FM, Bocchi C, Voltolini C, Borges LE, Florio P, Petraglia F. Thickness of fetal membranes: a possible ultrasound marker for preterm delivery. Ultrasound Obstet Gynecol. 2008;32:205-209. doi:10.1002/uog.5406

26. Dinglas C, Chavez M, Vintzileos A. Resolution of intra-amniotic sludge after antibiotic administration in a patient with short cervix and recurrent mid-trimester loss. Am J Obstet Gynecol. 2019;221:159. doi:10.1016/j.ajog.2019.01.003

27. Salomon LJ, Alfirevic Z, Bilardo CM, Chalouhi GE, Ghi T, Kogan KO et al. ISUOG practice guidelines: performance of first-trimester fetal ultrasound scan [published correction appears in Ultrasound Obstet Gynecol. 2013 Feb;41(2):240]. Ultrasound Obstet Gynecol. 2013;41:102-113. doi:10.1002/uog.12342

28. Lockwood CJ, Wein R, Chien D, Ghidini A, Alvarez M, Berkowitz RL. Fetal membrane rupture is associated with the presence of insulin-like growth factor-binding protein-1 in

vaginal secretions. Am J Obstet Gynecol. 1994;171:146-150. doi:10.1016/0002-9378(94)90461-8

29. Higgins RD, Saade G, Polin RA, Grobman WA, Buhimschi IA, Watterberg K et al. Evaluation and Management of Women and Newborns With a Maternal Diagnosis of Chorioamnionitis: Summary of a Workshop. Obstet Gynecol. 2016;127:426-436. doi:10.1097/AOG.0000000000001246
30. Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP for the STROBE Initiative. The strengthening the reporting of the observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet 2007; 370:1453-7
31. Kumar D, Moore RM, Mercer BM, Mansour JM, Redline RW, Moore JJ. The physiology of fetal membrane weakening and rupture: Insights gained from the determination of physical properties revisited. Placenta. 2016; 42:59-73. doi:10.1016/j.placenta.2016.03.015
32. Hogge WA, Hogge JS, Boehm CD, Sanders RC. Increased echogenicity in the fetal abdomen: use of DNA analysis to establish a diagnosis of cystic fibrosis. J Ultrasound Med. 1993;12:451-454. doi:10.7863/jum.1993.12.8.451
33. Foster MA, Nyberg DA, Mahony BS, Mack LA, Marks WM, Raabe RD. Meconium peritonitis: prenatal sonographic findings and their clinical significance. Radiology. 1987;165:661-665. doi:10.1148/radiology.165.3.3317498
34. Buhimschi CS, Buhimschi IA, Norwitz ER, Sfakianaki AK, Hamar B, Copel JA et al. Sonographic myometrial thickness predicts the latency interval of women with preterm premature rupture of the membranes and oligohydramnios. Am J Obstet Gynecol. 2005;193:762-770. doi:10.1016/j.ajog.2005.01.053
35. Athayde N, Edwin SS, Romero R, Gomez R, Maymon E, Pacora P et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. Am J Obstet Gynecol. 1998;179:1248-1253. doi:10.1016/s0002-9378(98)70141-3

36. Maymon E, Romero R, Chaiworapongsa T, Kim JC, Berman S, Gomez R et al. Value of amniotic fluid neutrophil collagenase concentrations in preterm premature rupture of membranes. Am J Obstet Gynecol. 2001;185:1143-1148. doi:10.1067/mob.2001.118166

37. Shim SS, Romero R, Hong JS, Park CW, Jun JK, Kim BI et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. Am J Obstet Gynecol. 2004;191:1339-1345. doi:10.1016/j.ajog.2004.06.085

38. Oh KJ, Lee JH, Romero R, Park HS, Joon-Seok H, Yoon BH. A new rapid bedside test to diagnose and monitor intra-amniotic inflammation in preterm PROM using transcervically collected fluid . Am J Obstet Gynecol. 2020; S0002-9378(20)30225-8. [Epub ahead of print, 2020 Feb 27].

39. Ghidini A, Salafia CM, Minior VK. Lack of relationship between histologic chorioamnionitis and duration of the latency period in preterm rupture of membranes. J Matern Fetal Med. 1998;7(5):238-242

40. Armstrong-Wells J, Post MD, Donnelly M, Manco-Johnson MJ, Fisher BM, Winn VD. Patterns of placental pathology in preterm premature rupture of membranes. J Dev Orig Health Dis. 2013;4:249-255. doi:10.1017/S2040174413000056

~~1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84. doi:10.1016/S0140-6736(08)60074-4~~

~~2. Morris JM, Roberts CL, Bowen JR, et al. Immediate delivery compared with expectant management after preterm pre-labour rupture of the membranes close to term (PPROMT trial): a randomised controlled trial. Lancet. 2016;387(10017):444-452. doi:10.1016/S0140-6736(15)00724-2~~

~~3. Thomson AJ; Royal College of Obstetricians and Gynaecologists. Care of Women Presenting with Suspected Preterm Prelabour Rupture of Membranes from 24(+0) Weeks of Gestation:~~

Green-top Guideline No. 73. BJOG. 2019 Aug;126(9):e152-e166. doi: 10.1111/1471-0528.15803. Epub 2019 Jun 17. PubMed PMID: 31207667

4. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics. Practice Bulletin No. 172: Premature Rupture of Membranes. Obstet Gynecol. 2016 Oct;128(4):e165-77. doi: 10.1097/AOG.0000000000001712. Review. PubMed PMID: 27661655
5. Dagklis T, Petousis S, Margioula-Siarkou C, et al. Parameters affecting latency period in PPROM cases: a 10-year experience of a single institution. J Matern Fetal Neonatal Med. 2013;26(14):1455-1458. doi:10.3109/14767058.2013.784257
6. Frenette P, Dodds L, Armson BA, Jangaard K. Preterm prelabour rupture of membranes: effect of latency on neonatal and maternal outcomes. J Obstet Gynaecol Can. 2013;35(8):710-717. doi:10.1016/S1701-2163(15)30861-6
7. Drassinower D, Friedman AM, Običan SG, Levin H, Gyamfi-Bannerman C. Prolonged latency of preterm premature rupture of membranes and risk of neonatal sepsis. Am J Obstet Gynecol. 2016;214(6):743.e1-743.e7436. doi:10.1016/j.ajog.2015.12.031
8. Nayot D, Penava D, Da Silva O, Richardson BS, de Vrijer B. Neonatal outcomes are associated with latency after preterm premature rupture of membranes. J Perinatol. 2012;32(12):970-977. doi:10.1038/jp.2012.15
9. Ramsey PS, Lieman JM, Brumfield CG, Carlo W. Chorioamnionitis increases neonatal morbidity in pregnancies complicated by preterm premature rupture of membranes. Am J Obstet Gynecol. 2005;192(4):1162-1166. doi:10.1016/j.ajog.2004.11.035
10. Simhan HN, Canavan TP. Preterm premature rupture of membranes: diagnosis, evaluation and management strategies. BJOG. 2005;112 Suppl 1:32-37. doi:10.1111/j.1471-0528.2005.00582.x

11. Rouse DJ, Hirtz DG, Thom E, et al. A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy. N Engl J Med. 2008;359(9):895-905. doi:10.1056/NEJMoa0801187

12. Vidaeff AC, Ramin SM. Antenatal corticosteroids after preterm premature rupture of membranes. Clin Obstet Gynecol. 2011;54(2):337-343. doi:10.1097/GRF.0b013e318217d85b

13. Jobe AH, Goldenberg RL. Antenatal corticosteroids: an assessment of anticipated benefits and potential risks. Am J Obstet Gynecol. 2018 Jul;219(1):62-74

14. di Pasquo E, Saccone G, Angeli L, et al. Determinants of neonatal hypoglycemia after antenatal administration of corticosteroids (ACS) for lung maturation: Data from two referral centers and review of the literature. Early Hum Dev. 2020;143:104984. doi:10.1016/j.earlhumdev.2020.104984

15. Ville Y, Rozenberg P. Predictors of preterm birth. Best Pract Res Clin Obstet Gynaecol. 2018;52:23-32. doi:10.1016/j.bpobgyn.2018.05.002

16. Lee SM, Park KH, Jung EY, Jang JA, Yoo HN. Frequency and clinical significance of short cervix in patients with preterm premature rupture of membranes. PLoS One. 2017;12(3):e0174657. Published 2017 Mar 30. doi:10.1371/journal.pone.0174657

17. Park KH, Lee SY, Kim SN, Jeong EH, Oh KJ, Ryu A. Prediction of imminent preterm delivery in women with preterm premature rupture of membranes. J Perinat Med. 2011;40(2):151-157. Published 2011 Nov 16. doi:10.1515/JPM.2011.124

18. Yagur Y, Weitzner O, Ravid E, Biron-Shental T. Can we predict preterm delivery in patients with premature rupture of membranes?. Arch Gynecol Obstet. 2019;300(3):615-621. doi:10.1007/s00404-019-05196-8

19. Kayem G, Batteux F, Girard N, et al. Predictive value of vaginal IL-6 and TNFα bedside tests repeated until delivery for the prediction of maternal-fetal infection in cases of premature rupture of membranes. Eur J Obstet Gynecol Reprod Biol. 2017;211:8-14. doi:10.1016/j.ejogrb.2017.01.013

20. Trochez-Martinez RD, Smith P, Lamont RF. Use of C-reactive protein as a predictor of chorioamnionitis in preterm prelabour rupture of membranes: a systematic review. BJOG. 2007;114(7):796-801. doi:10.1111/j.1471-0528.2007.01385.x

21. Mehra S, Amon E, Hopkins S, Gavard JA, Shyken J. Transvaginal cervical length and amniotic fluid index: can it predict delivery latency following preterm premature rupture of membranes?. Am J Obstet Gynecol. 2015;212(3):400.e1-400.e4009. doi:10.1016/j.ajog.2015.01.022

22. Aviram A, Quaglietta P, Warshafsky C, et al. The utility of ultrasound assessment in the management of preterm prelabor rupture of the membranes [published online ahead of print, 2019 Jul 23]. Ultrasound Obstet Gynecol. 2019;10.1002/uog.20403. doi:10.1002/uog.20403

23. Nunes, V., Cross, J., Speich, J.E. et al. Fetal membrane imaging and the prediction of preterm birth: a systematic review, current issues, and future directions. BMC Pregnancy Childbirth 16, 387 (2016). https://doi.org/10.1186/s12884-016-1176-5

24. Frigo P, Lang C, Sator M, Ulrich R, Husslein P. Membrane thickness and PROM--high-frequency ultrasound measurements. Prenat Diagn. 1998;18(4):333-337.

25. Severi FM, Bocchi C, Voltolini C, Borges LE, Florio P, Petraglia F. Thickness of fetal membranes: a possible ultrasound marker for preterm delivery. Ultrasound Obstet Gynecol. 2008;32(2):205-209. doi:10.1002/uog.5406

26. Dinglas C, Chavez M, Vintzileos A. Resolution of intra-amniotic sludge after antibiotic administration in a patient with short cervix and recurrent mid-trimester loss. Am J Obstet Gynecol. 2019;221(2):159. doi:10.1016/j.ajog.2019.01.003

27. Salomon LJ, Alfirevic Z, Bilardo CM, et al. ISUOG practice guidelines: performance of first-trimester fetal ultrasound scan [published correction appears in Ultrasound Obstet Gynecol. 2013 Feb;41(2):240]. Ultrasound Obstet Gynecol. 2013;41(1):102-113. doi:10.1002/uog.12342

28. Lockwood CJ, Wein R, Chien D, Ghidini A, Alvarez M, Berkowitz RL. Fetal membrane rupture is associated with the presence of insulin-like growth factor-binding protein-1 in vaginal secretions. Am J Obstet Gynecol. 1994;171(1):146-150. doi:10.1016/0002-9378(94)90461-8

29. Higgins RD, Saade G, Polin RA, et al. Evaluation and Management of Women and Newborns With a Maternal Diagnosis of Chorioamnionitis: Summary of a Workshop. Obstet Gynecol. 2016;127(3):426-436. doi:10.1097/AOG.0000000000001246

30. Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP for the STROBE Initiative. The strengthening the reporting of the observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet 2007; 370:1453-7

31. Kumar D, Moore RM, Mercer BM, Mansour JM, Redline RW, Moore JJ. The physiology of fetal membrane weakening and rupture: Insights gained from the determination of physical properties revisited. Placenta. 2016;42:59-73. doi:10.1016/j.placenta.2016.03.015

32. Hogge WA, Hogge JS, Boehm CD, Sanders RC. Increased echogenicity in the fetal abdomen: use of DNA analysis to establish a diagnosis of cystic fibrosis. J Ultrasound Med. 1993;12(8):451-454. doi:10.7863/jum.1993.12.8.451

33. Foster MA, Nyberg DA, Mahony BS, Mack LA, Marks WM, Raabe RD. Meconium peritonitis: prenatal sonographic findings and their clinical significance. Radiology. 1987;165(3):661-665. doi:10.1148/radiology.165.3.3317498

34. Buhimschi CS, Buhimschi IA, Norwitz ER, et al. Sonographic myometrial thickness predicts the latency interval of women with preterm premature rupture of the membranes and oligohydramnios. Am J Obstet Gynecol. 2005;193(3 Pt 1):762-770. doi:10.1016/j.ajog.2005.01.053

35. Athayde N, Edwin SS, Romero R, et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. Am J Obstet Gynecol. 1998;179(5):1248-1253. doi:10.1016/s0002-9378(98)70141-3

36. Maymon E, Romero R, Chaiworapongsa T, et al. Value of amniotic fluid neutrophil collagenase concentrations in preterm premature rupture of membranes. Am J Obstet Gynecol. 2001;185(5):1143-1148. doi:10.1067/mob.2001.118166

37. Shim SS, Romero R, Hong JS, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. Am J Obstet Gynecol. 2004;191(4):1339-1345. doi:10.1016/j.ajog.2004.06.085

38. Oh KJ, Lee JH, Romero R, Park HS, Joon-Seok H, Yoon BH. A new rapid bedside test to diagnose and monitor intra-amniotic inflammation in preterm PROM using transcervically collected fluid [published online ahead of print, 2020 Feb 27]. Am J Obstet Gynecol. 2020;S0002-9378(20)30225-8.

39. Ghidini A, Salafia CM, Minior VK. Lack of relationship between histologic chorioamnionitis and duration of the latency period in preterm rupture of membranes. J Matern Fetal Med. 1998;7(5):238-242. doi:10.1002/(SICI)1520-6661(199809/10)7:5<238::AID-MFM6>3.0.CO;2-5

Armstrong-Wells J, Post MD, Donnelly M, Manco-Johnson MJ, Fisher BM, Winn VD. Patterns of placental pathology in preterm premature rupture of membranes. J Dev Orig Health Dis. 2013;4(3):249-255. doi:10.1017/S2040174413000056

Table 1. General characteristic of included population

	n=45
Maternal age (years)	33.0[29.0-35.0]~~2.3±5.4~~
BMI (Kg/m2)	24.0[22.0-27.0]~~24.5±4.5~~

Nulliparous	21(46.7)
Caucasian	33(73.3)
Smoke	4(8.9)
Gestational Age at admission (weeks)	30.6[26.6-34.3]~~0.0±4.37~~
Gestational Age at delivery (weeks)	34.0[29.7-35.4]~~2.5±3.7~~
Genito-urinary infections	31(68.9)
Cervical lenght -at admission (mm)	26.0[18.0-32.0]~~5.5±10.1~~
Hyperechoic membranes	11(24.4)
Spontaneous onset of labor	37(82.2)

Table 2. Maternal, pregnancy and neonatal characteristics of women with normo-echoic and hyper-echoic membranes

	Normo-echoic membranes (n=34)	Hyper-echoic membranes (n=11)	*p* value
Maternal Age (years)	33.0[28.3-35.0]~~32.4±5.4~~	32.0[29.5-35.0]~~31.9±5.6~~	0.83
Caucasian	25(73.5)	8(72.7)	0.96
Pre-pregnant BMI (Kg/m²)	25.0[22.0-27.0]~~24.9±4.7~~	22.0[21.5-25.0]~~23.0±3.1~~	0.21
Smoke	3(8.8)	1(9.1)	0.90
Nulliparous	16(47.1)	5(45.5)	0.93
WBC at diagnosis (n x10⁹/L)	11.4[8.8-13.2]~~11.6±3.1~~	11.2[10.0-12.6]~~10.9±2.7~~	0.56
CRP at diagnosis	6.0[3.2-13.0]~~13.3±21.2~~	5.2[3.3-7.8]~~6.4±5.0~~	0.31

(mg/L)			
WBC maximum value (n x10^9/L)	13.0[10.7-15.5]~~14.0±4.6~~	13.1[10.0-14.5]~~12.7±3.1~~	0.41
PCR maximum value (mg/L)	11.6[6.1-18.8]~~22.8±30.8~~	6.2[4.8-17.7]~~17.4±25.9~~	0.63
Genito-urinary infections	23(67.6)	8(72.7)	0.75
Cervical Length at admission (mm)	27.0[18.8-33.8]~~26.7±10.7~~	24.0[17.0-27.5]~~21.9±7.4~~	0.18
Gestational Age at admission (weeks)	29.9[25.8-33.2]~~29.3±4.3~~	34.6[29.4-35.1]~~32.2±4.1~~	0.06
Latency time from p-PROM to spontaneous onset of labor (days)	11.5[5.3-25.0]~~19.9±21.9~~	3.0[1.5-9.0]~~.9±3.5~~	0.04
Hyperpyrexia during latency period	5(14.7)	1(9.1)	0.63
Birthweight (grams)	1953.0 [1283.0-2338.0]~~838.2±652.0~~	2350.0 [1763.0-2338.0]~~2119.6±625.6~~	0.22
Oligohydramnios	13(38.2)	3(27.3)	0.63
Spontaneous onset of labor	28(82.3)	9(81.8)	0.97
Arterial pH	7.3[7.2-7.4]~~30±0.1~~	7.3[7.2-7.3]~~27±0.04~~	0.51
~~**Hystological diagnosis of chorioamniositis**~~	~~12/22 (54.5)~~	~~2/6 (33.3)~~	~~0.36~~

WBC: White Blood Cells; CPR: C-Reactive Protein

Table 3. Univariate analysis and association of independent variables with the risk of spontaneous onset of labor ~~delivery~~ ≤72 hours by multiple logistic regression

	Spontaneous onset of labor ~~delivery~~ >72 hours (n=26)	Spontaneous onset of labor~~delivery~~ ≤72 hours (n=11)	*p*-value	*Crude OR 95%CI*	*Adjusted* OR 95%CI*
Maternal age	33.0[29.3-34.8]~~32.5±5.5~~	31.0[27.5-36.5]~~2.3±5.4~~	0.92	-	-
BMI (Kg/m²)	25.5[22.0-28.0]~~25.6±4.9~~	23.0[20.5-26.5]~~23.1±3.8~~	0.13	-	-
Nulliparous	11(42.3)	7(63.6)	0.23	-	-
Caucasian	17(65.3)	9(81.8)	0.32	-	-
Gestational Age at admission (Weeks)	28.6[25.8-31.5]~~28.8±4.1~~	34.7[29.6-35.5]~~32.3±4.2~~	0.03	1.24 (1.0-1.5)	1.16 (0.9-1.4)
WBC admission (n x10⁹/L)	11.2[8.8-13.3]~~11.3±2.9~~	11.2[10.1-12.6]~~12.0±3.3~~	0.57	-	-
CPR at admission (mg/L)	6.0[3.3-11.0]~~10.0±14.3~~	5.2[3.6-14.7]~~17.2±31.9~~	0.39	-	-
WBC maximum value (n x10⁹/L)	13.9[10.7-15.3]~~13.7±3.9~~	11.5[10.1-17.3]~~14.4±5.8~~	0.68	-	-
CPR maximum value (mg/L)	12.6[6.5-27.4]~~21.7±24.1~~	5.2[6.5-16.6]~~18.3±31.6~~	0.73	-	-
Genito-urinary infections	18(69.2)	7(63.6)	0.74	-	-
Cervical lenght at admission (mm)	26.0[15.8-34.0]~~26.6±11.92~~	22.0[18.0-27.0]~~21.6±6.7~~	0.20	-	-
Hyper-echoic membranes	3(11.5)	6(54.5)	0.005	9.2 (1.7-49.8)	6.1 (1.~~00~~-36.9)

					p=0.04

*for variables significantly different at univariate analysis (e.i. Gestational age at admission, Hyperechoic membranes)
WBC: White Blood Cells; CPR: C-Reactive Protein

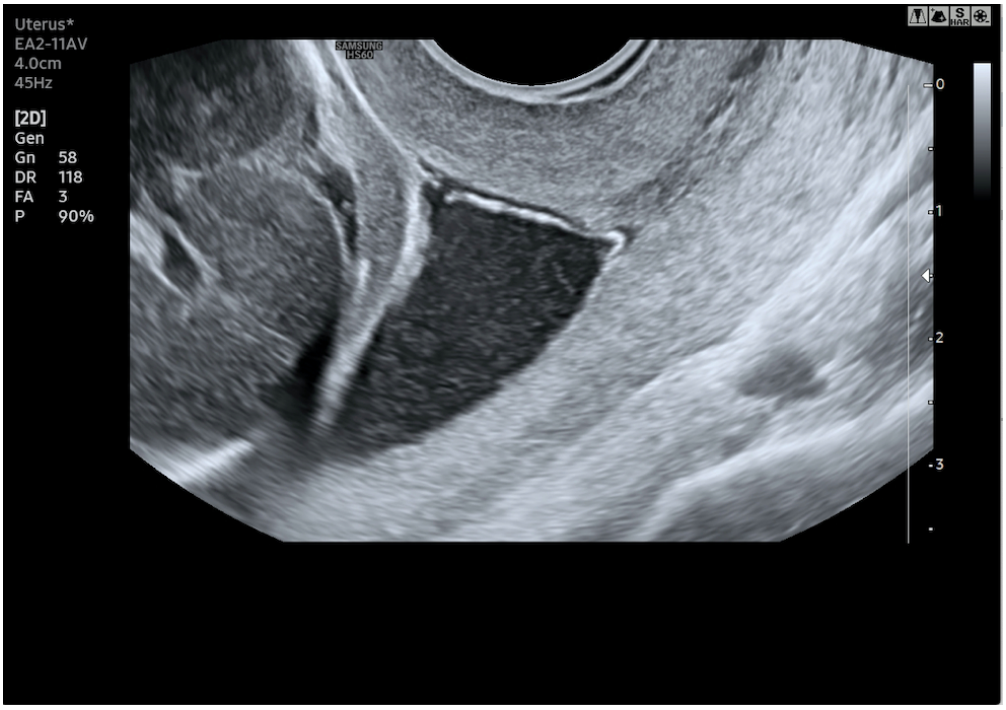

Figure 1. Transvaginal ultrasound assessment of hyperechoic membranes with the fetus in cephalic presentation

347x243mm (72 x 72 DPI)

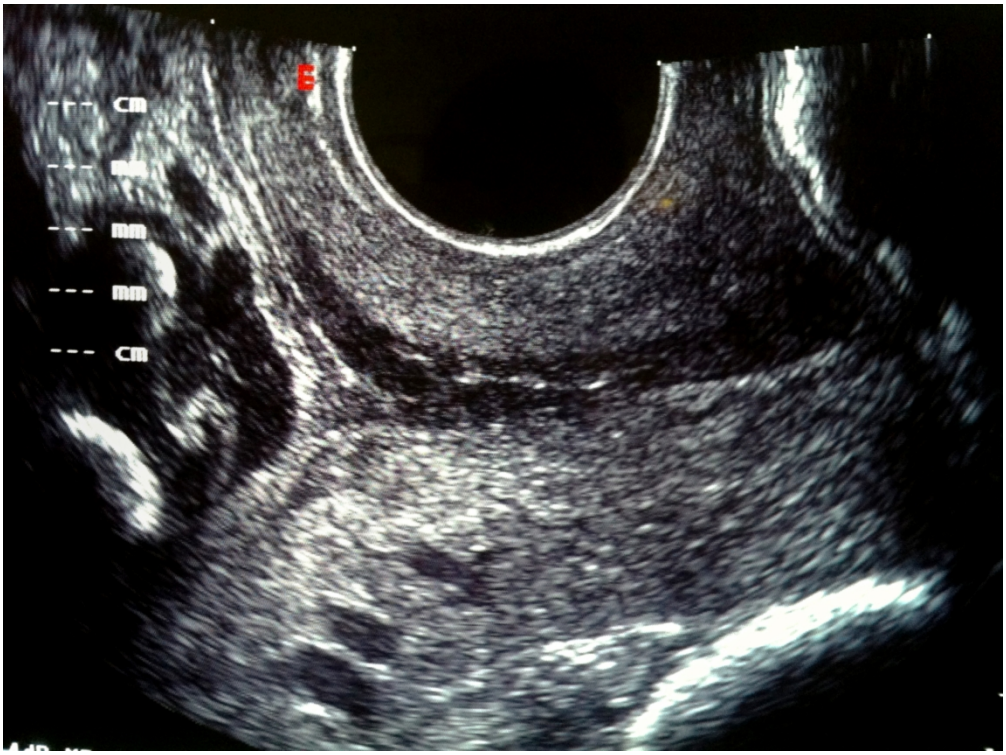

Figure 2. Transvaginal ultrasound assessment of hyperechoic membranes with the fetus in breech presentation

914x682mm (72 x 72 DPI)

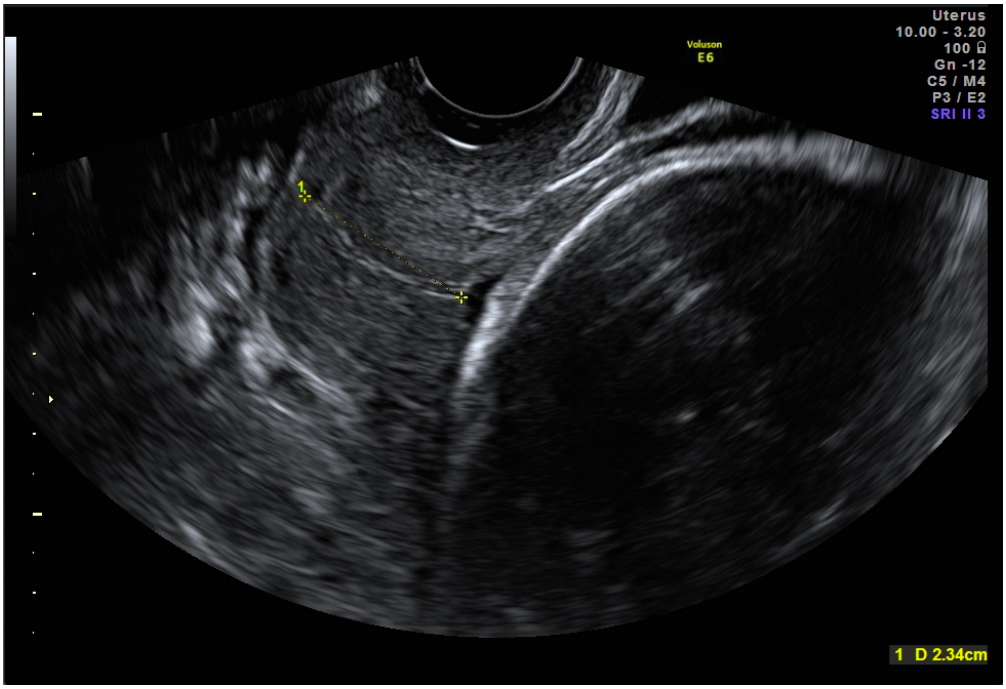

Figure 3. Transvaginal ultrasound assessment of normoechoic membranes with the fetus in cephalic presentation

343x234mm (72 x 72 DPI)

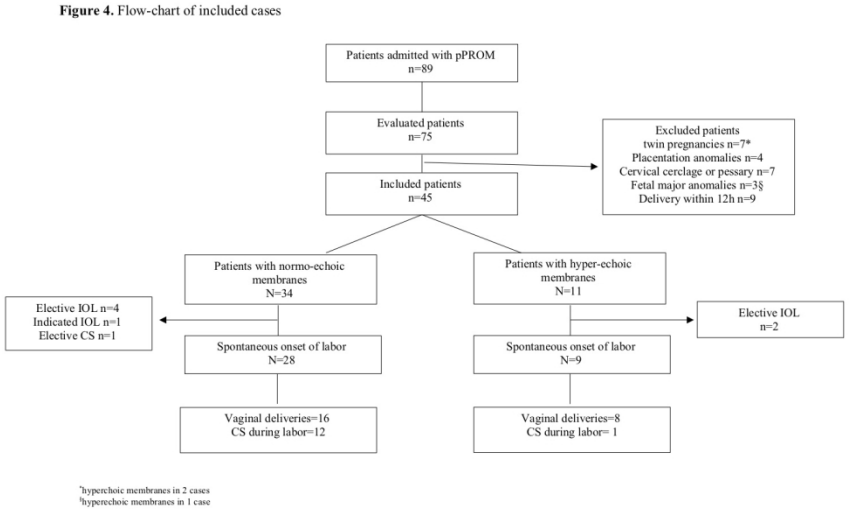

Figure 4: Flow-chart of included cases

296x209mm (150 x 150 DPI)

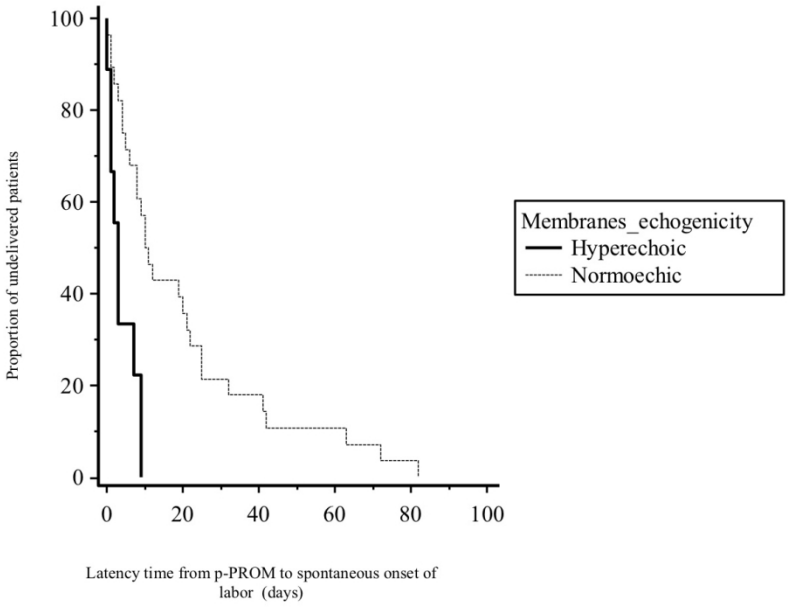

Figure 5. Kaplan-Meier estimates of latency time from pPROM to spontaneous onset of labor according to the presence/absence of hyperechoic membranes

254x190mm (150 x 150 DPI)