

Biological markers for the diagnosis and the prognosis of preterm premature rupture of membranes

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PPROM

Preterm Premature Rupture Of Membranes

- **PPROM : implication for perinatal health**
- **Management**
 - **Positive impact on perinatal health**
 - **But Invasive treatment**
 - **Consequences of false positive and false negative results**
- **Effective diagnosis**
 - **Clinical**
 - **Biological**
- **Prognosis in cases of PPRM**
 - **Preterm delivery or infection ?**
 - **Many biomarkers studied**
 - **Biomarkers used currently**

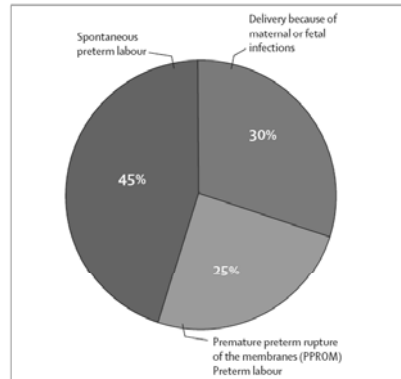
PPROM : frequency and impact on prematurity

•PPROM: 3 % of pregnancies

–20 to 40 % of the PROM

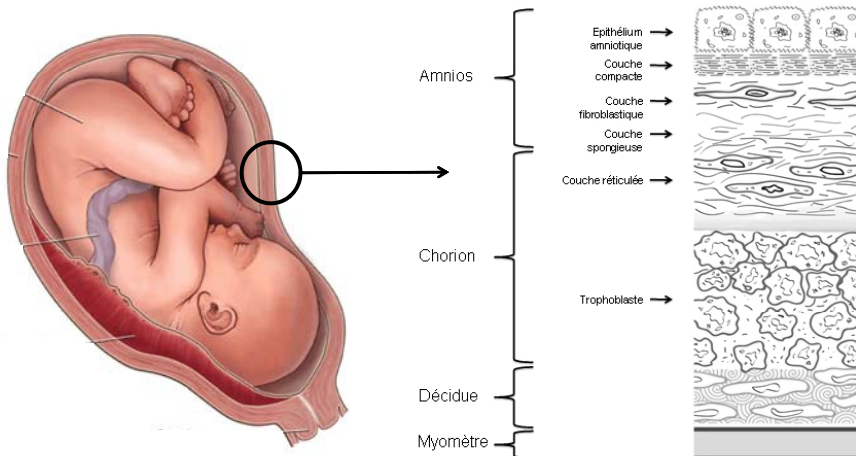
–30 to 40% of preterm deliveries

*Mercer BM et al.
Am J Obstet Gynecol 2000*

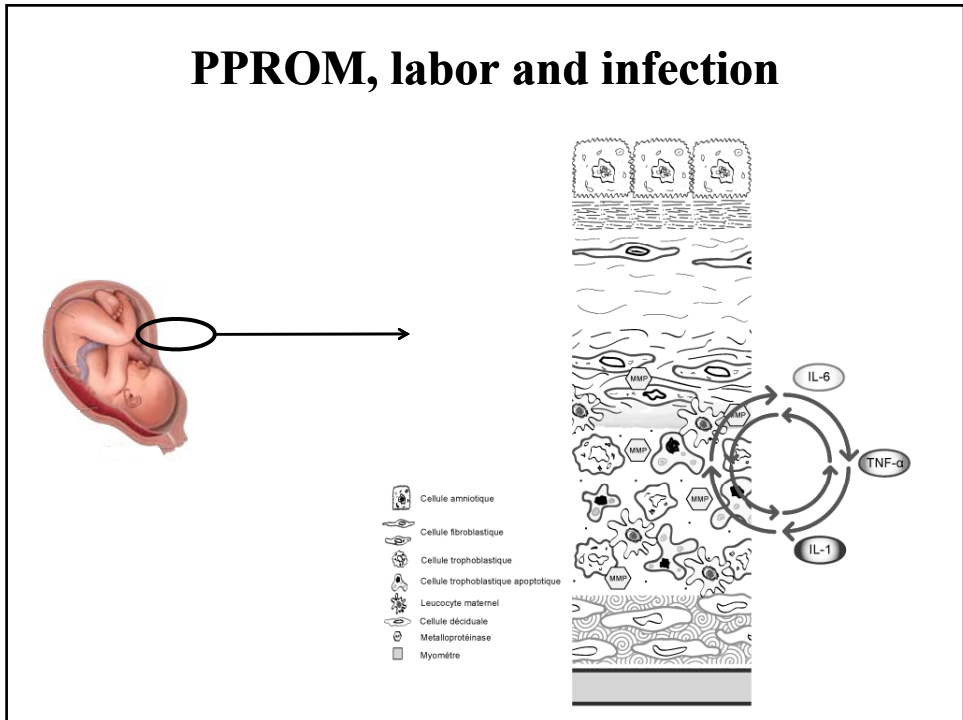


Goldenberg RL et al. Lancet 2008

PPROM, labor and infection

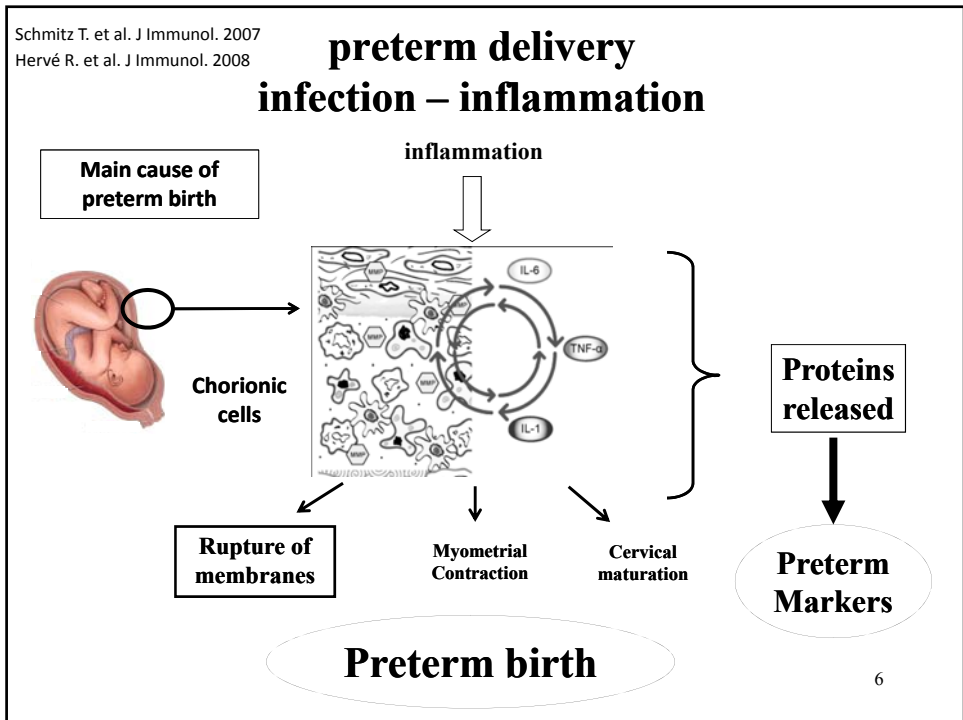


PPROM, labor and infection



Schmitz T. et al. J Immunol. 2007
 Hervé R. et al. J Immunol. 2008

preterm delivery infection – inflammation



PPROM consequences

- **Maternal complications:**
chorioamnionitis (2-8%), endometritis
- **Perinatal complications = preterm birth**
 - Perinatal mortality
 - **Short term complications: Enterocolitis, bronchodysplasia, periventricular leukomalacia,**
 - **Long term outcome : cerebral palsy, sensorial deficit, mental retardation**
- **Infection = additional pejorative factor**

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Management in cases of PPRM (1) no signs of infection

before 32-34 Weeks



Hospitalisation
Antibiotics
Maternal transfer
Tocolysis 24-48 hours
Corticosteroids

Search for infection

after 32-34 weeks



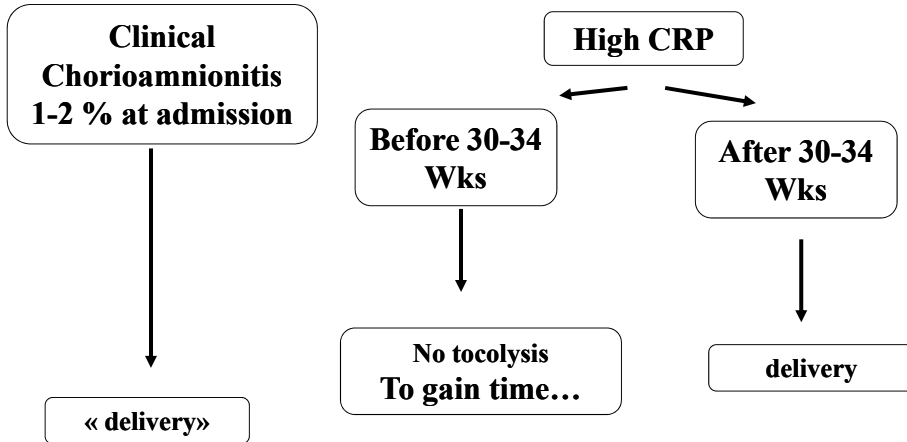
Hospitalisation
Antibiotics
Maternal transfer

Search for infection

Induction of labor
at 34-36 weeks

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Management in cases of PPRM (2) use of a high CRP in decisions



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Diagnosis of PPRM : clinical

Verspyck E et Al. J Gynecol Obstet Biol Reprod. 1999
Diagnostic methods and prognostic criteria in the case
of premature rupture of the membranes

**History taking and clinical
examination are often sufficient**

80%



**Sterile speculum examination:
leakage of amniotic fluid
from cervical os continued
and expanded by the
mobilization**

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Biomarkers: Quality required

NO « GOLD STANDARD »
To confirm the diagnosis of
rupture of membranes

No perfect
biomarkers

Strictly specific molecule of the AF
Present at all gestational ages
Slow degradation
Easily detectable at low concentration
The test should be rapid and available
24 h a day

- prevent false positive results
 - Contamination (blood, seminal liquid)
- prevent false negative results
 - Rupture of membranes very early in the pregnancy
 - Disappearance of the marker
 - Concentration too low

Published results methodological drawbacks

Verspyck E, Landman T, Marpeau L J Gynecol Obstet Biol Reprod 1999

No Gold standard for diagnosis of PPRM

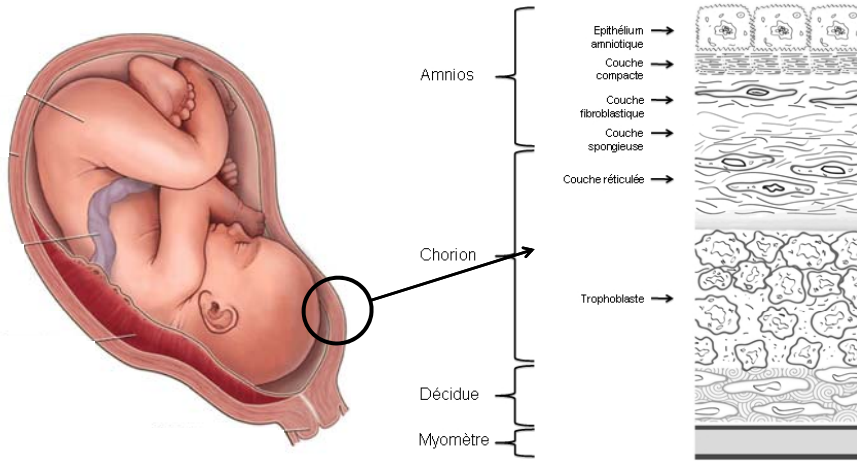
Very few studies compared diagnostic value of different tests

Cut-offs to define an abnormal test could be different in the studies

Comparison groups : No rupture of membranes versus cases with clear liquid flow

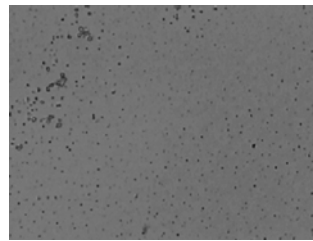
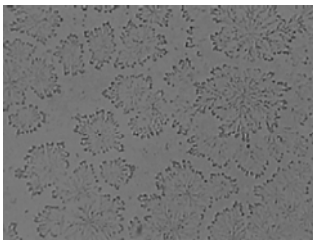
Target population : suspicion of PPRM (intermittent or no leakage of fluid) - cases in which clinical diagnosis is not evident

Localization of the biomarkers used



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amniotic fluid crystallization testing – fern test



	Sens	Spec	PPV	NPV
Fern test				
-Gibbs, 1982	42%	76%	-	-

Contamination with cervical mucus (false positive result)
Learning required (microscope)
subjective

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A lot of biomarkers studied

	<i>Nb</i>	Sens	Spec	VPP	VPN
DAO (DiAmine Oxydase)					
- <i>Gaucherand, 1997</i>	100	83,7	100	100	89
- <i>De Meeus, 1997</i>	71	90,9	100	100	98,3
AFP (Apha Feto-Protein)					
- <i>Kishida, 1995</i>	103	100	97,4	92,5	100
- <i>Gaucherand, 1995</i>	131	88	84	86	87

Others : hCG, Prolactin, urea, creatinin, lactates ...

amniotic fluid/serum ratio low

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FIBRONECTINE

	<i>Nb</i>	Sens	Spec	VPP	VPN
Fibronectine					
- <i>Gaucherand, 1995</i>	131	94	97	97	94
- <i>Rutanen, 1993</i>	54	92	80	79	-

Extracellular matrix protein of membranes > AF
Released in the case of preterm labor or labor with intact membranes
Protein present in seminal fluid
amniotic fluid/serum ratio low

NITRAZINE paper testing simple, cheap

cervical pH= 5-6
Amniotic fluid : alkaline
Dipstick colorimetric

Interpretation of Amnicator® colour change			
Intact membranes		Ruptured membranes	
pH 5.0 yellow		pH 6.5 blue-green	
pH 5.5 yellow		pH 7.0 blue-green	
pH 6.0 yellow		pH 7.5 blue-black	

	<i>Nb</i>	Sens	Spec	VPP	VPN
Nitrazine					
- Gaucherand, 1997	100	90,7	77,2	75	91,7
- De Meeus, 1997	71	81,1	83,3	52,6	96,1

bacterial vaginosis
cervicitis
semen, alkaline urine,
blood, soap and
antiseptic solutions

**false
positive**

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IGFBP-1 (ActimProm®)

insulin-like growth factor binding protein-I

Produced by decidual cells (placental protein)

Immunoenzymometric assay for quantitation

Concentrations = 100 to 1000 fold higher than those in serum

Very low concentration in urine, cervical mucus and seminal fluid

Rapid strip test (5 min) => 95th percentile of serum levels

ActimProm – IGFBP-1	<i>Nb</i>	Sens	Spec	VPP	VPN
<i>Lockwood et al. 1994</i>	105	74,4	92,6	96,7	55,6
<i>Rutanen et al. 1996</i>	130	100	94,7	93,2	100
<i>Gaucherand et al. 1997</i>	100	95,3	98,2	97,6	96,5
<i>Marcellin et al. 2011</i>	90	95,3	95,6	97,7	93,6



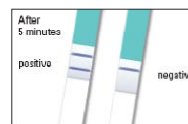
A. Extraction
Brief instructions for use



B. Dipping

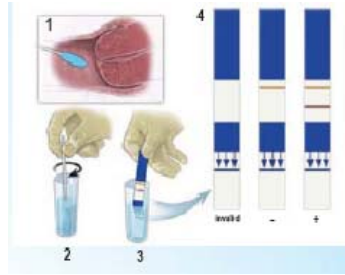


C. Note liquid front



D. Result

PAMG-1 (AmniSure®)



placental alpha-microglobulin-1

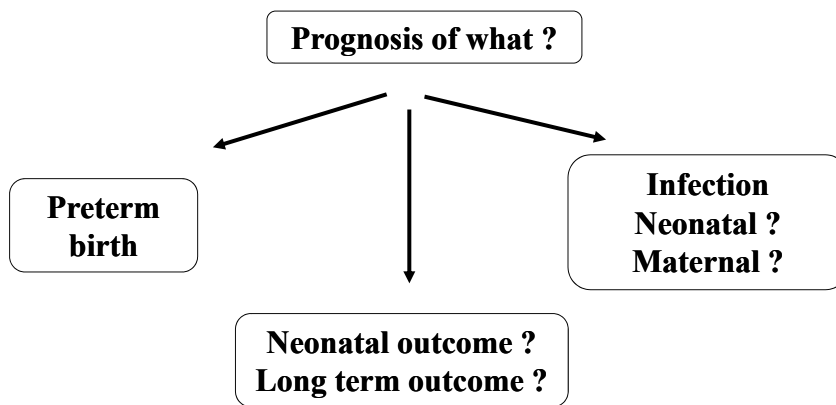
Produced by decidual cells (placental protein)

Concentrations = 1 000 to 10 000 fold higher than those in cervical mucus

Amnisure – PAMG-1	Nb	Sens	Spec	VPP	VPN
<i>Cousin et al. 2005</i>	203	98,9	100	100	99,1
<i>Marcellin et al. 2011</i>	90	92,8	91,6	90,7	93,6

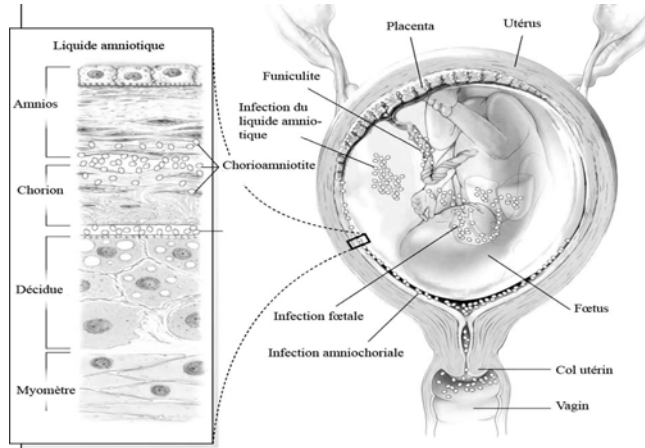
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Prognosis in case of PPRM



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Intra uterine inflammation or infection



Clinical chorioamnionitis:

Admission : 1-2 %

Subsequently: 3-8 %

Positive culture by
amniocentesis: 25-40 %

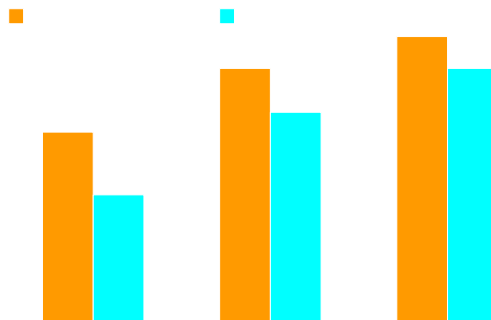
Neonatal infection

5-10%

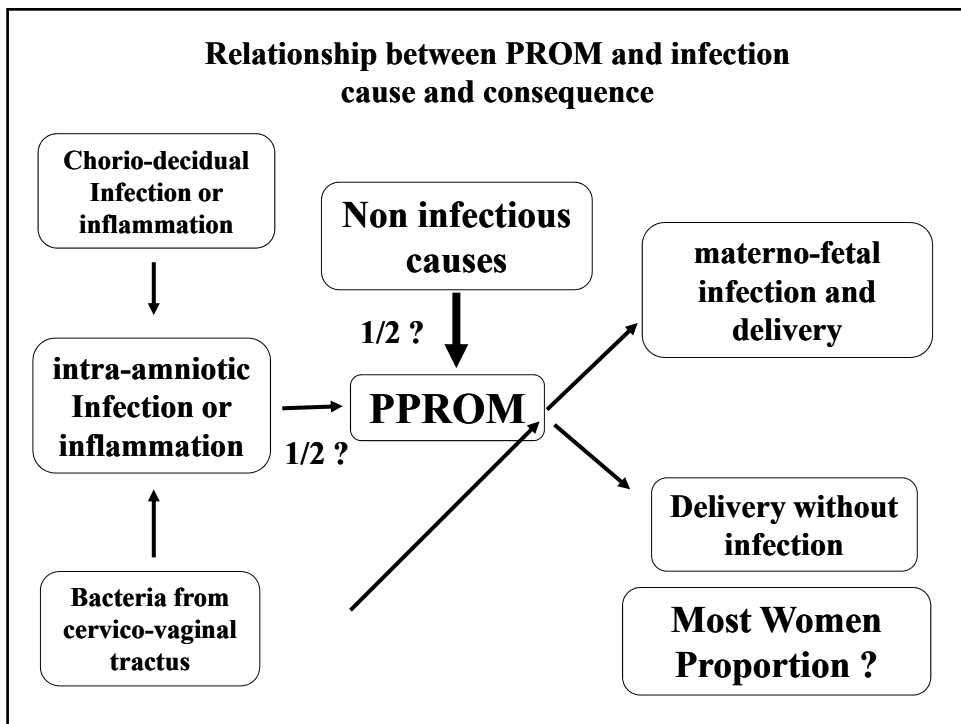
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Natural history of PPRM

(Savitz et al, *Am J Obstet Gynecol* 1997 et Johnson, *Obstet Gynecol* 1981)



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Best way : diagnosis of inflammation or infection in amniotic fluid (amniocentesis)

- **Culture could be the gold standard for the diagnosis of infection**
 - **Direct diagnosis of the inflammation or the infection**
 - **Identification of the germs**
- **Many short term tests studied**
 - **Gram stain, white blood cell count, leucocyte esterase, glucose concentration**
 - **Interleukines, metalloproteinase**
 - **Etc etc**

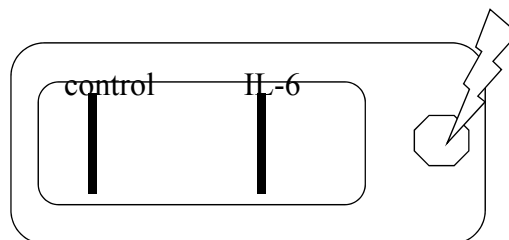
Disadvantages of amniocentesis

- **Disadvantages of the procedure**
 - Results of the culture (48 hours) after the delivery frequently
 - Short term test : low specificity and meaning ?
 - Success of amniocentesis is associated with amount of amniotic fluid remaining (oligohydramnios in PPRM)
 - Complications associated with amniocentesis ?
- **No evaluation of the use of test**
 - Decision ? : antibiotics, Cesarean section, corticosteroid before extraction
- **Few team published (many, many) papers on the diagnostic value of these tests, many methodological weaknesses**

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Cytokines in serum or vaginal secretions ?

- **Parturition: IL-6, TNF α , IL-1 β , IL-8**
- **Glycoproteins involved in inflammation**
 - released mainly after activation of macrophages
 - result in the production of prostaglandins and proteases (MMP)
 - measurement difficult and costly (ELISA in most studies)
- **Prospective study (73 women with PPRM) (Kayem et al 2005)**
 - diagnostic value of IL-6 in vaginal secretions for neonatal infection in cases of PPRM
 - Immunochromatographic bed side test (20 minutes)



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Odds of neonatal infection as function of maternal markers, based on logistic regression

	Congenital proven or probable sepsis n =16	Without congenital sepsis n = 68	OR	Adjusted OR
Vaginal IL6 positive	12 (75.0)	29 (42.6)	4.0 (1.3-13)	5.5 (1.2-17.7)
Maternal serum C-Reactive Protein				
> 5 mg/dl	9 (52.9)	31 (41.3)	1.2 (0.5-4.6)	
> 20 mg /dl	4 (23.5)	5 (7.4)	3.4 (1.0-16.5)	5.6 (0.95-32.6)
Maternal WBC (x 1000 :)				
> 15	3 (18.9)	21 (30.9)	0.6 (0.13-1.97)	
> 20	1 (6.2)	4 (5.9)	1.0 (0.1-10.2)	

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Conclusion

- **Diagnosis**
 - **Clinical**
 - **Biomarkers necessary in 10-20 %**
 - Nitrazine +/- PROM test or Amnisure test
 - Perspective : Biomarker strictly specific of the AF and easily detectable at low concentration
- **Prognosis**
 - **To predict subclinical infection before onset of labor, but**
 - Which kind of management ?
 - No simple biomarkers available

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