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Azithromycin for genital mycoplasma intraamniotic infection in pregnant women with preterm premature rupture of membranes

A multi-centric, randomized, controlled, open label, clinical trial

BACHELOR THESIS

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1. List of abbreviations

AEMPS	Agencia Española del Medicamento y Productos Sanitarios
AF	Amniotic fluid
BMI	Body mass index
CEIC	Clinical Research Ethics Committee
CTG	Cardiotocography
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration
GA	Gestational age
IL-6	Interleukin 6
Mg	*Mycoplasma genitalium*
Mh	*Mycoplasma hominis*
MMP-8	Matrix metalloproteinase-8
PCR	Polymerase chain reaction
PROM	Premature/Prelabour rupture of the membranes
REDCap	Research Electronic Data Capture
Up	*Ureaplasma parvum*
Uu	*Ureaplasma uralyticum*

2. Abstract

BACKGROUND. Preterm premature rupture of membranes defined as rupture of membranes before the onset of labour is a complication of pregnancy whose main consequences are prematurity of the infant and infection of the amniotic fluid. The management in most of the cases is aimed to prolong the gestation to reduce the mortality and morbidity related to prematurity, as well as to reduce the incidence of chorioamnionitis which increases as the gestation is prolonged. This is why antibiotics are an important tool to prevent chorioamnionitis and to treat subclinical intraamniotic infections. Nevertheless, the antibiotic therapy of choice is not well stablished. Moreover, even if genital mycoplasma is the main microorganism identified as an agent implicated in the etiology of this pathology, most of the management protocols do not include specific tests to determine its presence, therefore, the possibility of a specific targeted therapy is not possible.

OBJECTIVES. The aim of this study is to compare the differences in latency period with targeted antibiotic with azithromycin and empiric antibiotic treatment in the management of singleton pregnancies with preterm premature rupture of membrane between 23 and 34 weeks of gestation with positive PCR in amniotic fluid for genital mycoplasma.

STUDY DESING AND POPULATION. A randomized, open label and controlled clinical trial will be done. It will be a multi-centric study involving the four provincial hospitals of Catalonia between June 2020 and May 2022. The sample size will be of 192 pregnant women who meet inclusion but not the exclusion criteria.

METHODS. Patients included in this study will be randomly assign to one group of intervention. 96 patients will receive empiric antibiotic (therapy A) and 96 patients will receive intravenous azithromycin (therapy B). The main outcome will be latency period (defined as time between the rupture of membranes and labour). The following covariates will be considered: age, body mass index, ethnicity, parity, time between onset of the symptoms and antibiotic initiation, socioeconomic status and sexual risk behaviour.

KEY WORDS. *Genital mycoplasma, amniocentesis, azithromycin, preterm premature rupture of membranes, real-time PCR.*

3. Introduction

3.1 Preterm Premature Rupture of Membranes

Premature rupture of membranes (PROM) is defined as the rupture of membranes before the onset of labour (1), in other words, in the absence of uterine dynamics. It is also referred in literature as prelabour rupture of membrane (2).

PROM is frequently classified by gestational age (GA) of pregnancy due to the implications it has in its management (2). We differentiate between “at term” PROM when gestational age is over 37 weeks and preterm PROM when gestational age under 37 weeks. There is a third group known as previable PROM when GA is under 23 weeks (3).

This text will be focused in preterm PROM, in particular, in those PROM under 34^{+6} weeks of GA. As PROM of more 34^{+6} weeks of GA are handled with the indication of concluding pregnancy, similarly as the “at term” PROM, they will be not taken into account (3).

3.1.1 Epidemiology of Preterm PROM

PROM is a complication in approximately 8% of pregnancies (2). The risk of PROM increases proportionally to GA, fortunately, most PROM occur at term and labour takes place spontaneously in the following 24 hours in around 60-95% of the cases (4).

On the other hand, preterm PROM occurs in up to 2-3% of singleton pregnancies (3), approximately 0.5% of pregnancies <27 weeks, 1% of pregnancies 27 to 34 weeks and 1% of pregnancies 34 to 37 weeks (5). It is also responsible of 30-40% of the spontaneous premature labour (2,3), which leads to an increase of mortality and morbidity such as cerebral palsy, sensory deficits, learning disabilities and respiratory illnesses resulting in enormous physical, psychological and economic costs (6). In women with preterm PROM, 50% will go into labour spontaneously within 24 to 48 hour and 70–90% within 7 days (7).

Incidence of PROM is higher in multiple than in singleton pregnancies (8), elevating the incidence up to 7-20% (4). Multiple pregnancies are handled according to different criteria because they suppose more risk for the mother and for the foetuses.

3.1.2 Risk factors of Preterm PROM

There are many factors that may be involved in the predisposition of preterm PROM. They include maternal physiologic, genetic and environmental factors (8). This risk factors are similar to those in preterm labour but most patients have no identifiable risk factors (2). The main risk factors are shown in **Table 1**.

Table 1: Main risk factors of preterm PROM

Risk Factor	Description
History of preterm PROM or preterm delivery	Many studies have shown that previous preterm PROM or premature labour is a strong risk factor for recurrences. The risk of recurrence during subsequent pregnancies ranges from 6% to 17% (5,8).
Antepartum bleeding	Bleeding during the first trimester and specially bleeding in more than one trimester increases the risk of preterm PROM. This relation is probably related to the high decidual concentration of tissue factor which thanks to its homeostatic properties, enable thrombin binds to decidual protease-activated receptors that up-regulate the expression of proteases such as matrix metalloproteases, which at the end degrade membranes (5).
Cigarette smoking	Smoking during pregnancy is associated with higher risk of preterm PROM as well as higher risk of *abruptio placentae* (9). The mechanism of the association in unclear (5)
Genital tract infection	Genital tract infection is the single most common identifiable risk factor for preterm PROM. Many of the microorganism that colonize the lower tract have the capacity to stimulate the production of prostaglandins which lead to the onset of uterine contractions. Moreover, the host's immune response to bacterial invasion of foetal membranes leads to the production of inflammatory mediators that can cause localized weakening of the foetal membranes and result in preterm PROM (5).

It has to be considered that patients with preterm PROM are more likely to have hypertension, diabetes mellitus, and anaemia, all of which are risk factors associated with *abruptio placentae* and may account both for the vaginal bleeding and for increased uterine irritability and intrauterine pressure (9).

Furthermore, there have been identified other risk factors such as polyhydramnios, acute trauma and several genetic polymorphisms of genes related to infection, inflammation, and collagen degradation (5).

3.1.3 Etiology of preterm PROM

The causes of PROM are not well understood but are likely to be multifactorial (10). Infection appears to have an important role, either as a cause or as a consequence of PROM (11). Intraamniotic infection has been shown to be commonly associated with preterm PROM, especially at earlier gestational ages (2). Some studies have shown a prevalence of microbial invasion of the amniotic cavity of 50% when tested with polymerase chain reaction (PCR). Moreover, viral invasion is exceptional (12).

In “Figure 1: Mechanisms of PROM” in can be shown the multifactorial character of preterm PROM.

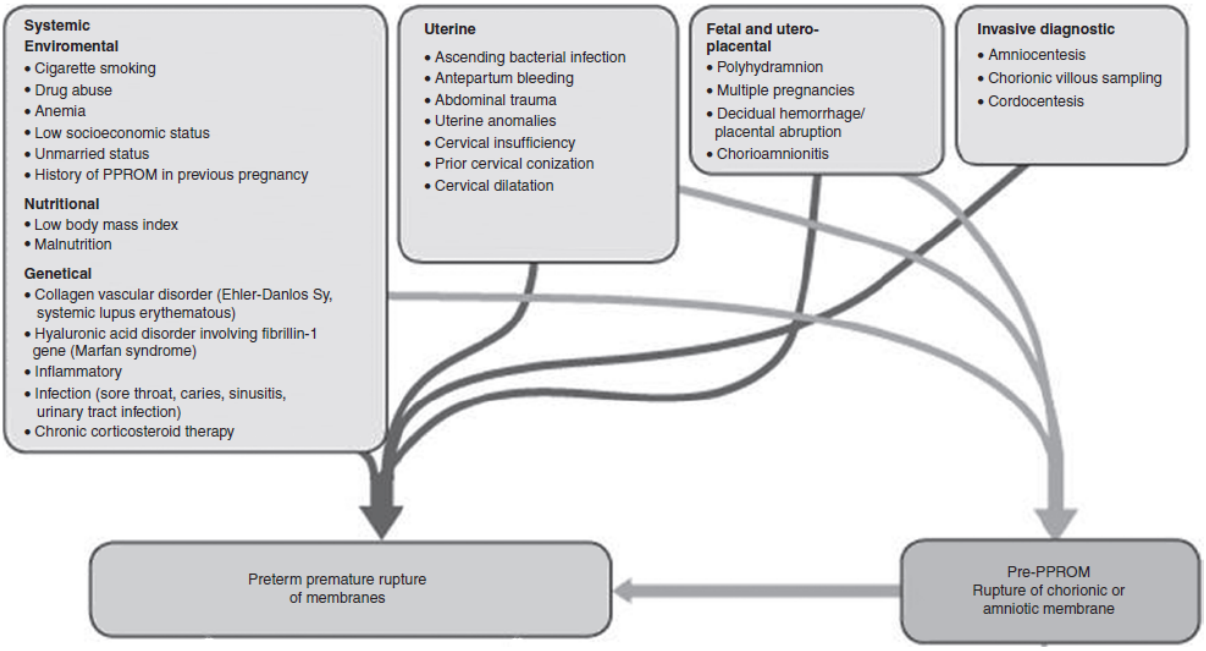

Figure 1: Mechanisms of PROM adapted from Tchirikov et al (12).

3.1.4 Complications

The establishment of preterm PROM aggravates pregnancy outcomes. Although all deliveries prior to 37 weeks of gestation are defined as preterm, most mortality and morbidity occur in infants born prior to 34^{+6} weeks of gestation (7). In this section, the main complications of preterm PROM are discussed.

- **Preterm delivery**. The most significant risk for the foetus after preterm PROM are complications of prematurity (2). Respiratory distress is the most common complication of preterm birth, nevertheless, sepsis, intraventricular haemorrhage, and necrotizing enterocolitis also are associated with prematurity.
- **Clinical chorioamnionitis**. It is frequently defined as acute intraamniotic infection and inflammation of the membranes and chorion of the placenta (13,14), typically due to ascending polymicrobial bacterial infection in the setting of membrane rupture (13). It

occurs in approximately 15-25% of preterm PROM and the incidence is higher at earlier gestational ages (2). The diagnosis is usually based on criteria adapted from Gibbs which are enumerated in “ANNEXE 1: Diagnosis criteria for clinical chorioamnionitis adapted from Gibbs”. In general, chorioamnionitis in the context of preterm PROM is commonly considered as the cause rather than the consequence of the rupture of membranes. The inflammation cytokines that are liberated as a result of the infection and consequent inflammation leads to an increase of prostaglandins in placental structures which results in uterine contractions, cervical maturation and reduction of membrane resistance (15).
In addition, chorioamnionitis is closely associated to infectious maternal complications for example caesarean delivery, endometritis, wound infection, pelvic abscess, bacteraemia and postpartum haemorrhage (13).

- **Neonatal morbidities** are significantly higher among pregnancies with preterm PROM complicated with chorioamnionitis when compared with pregnancies that are not (16), specially there is an increased risk of neurodevelopment impairment, neonatal white matter damage (2). As well as preterm PROM, clinical manifested intraamniotic infection causes premature labour and preterm birth together with other implications for the neonate such as preterm birth, neonatal sepsis, brain disease, respiratory distress syndrome and bronchopulmonary dysplasia (14).
- **Related to variation of amniotic fluid**. Preterm PROM, followed by prolonged oligo/anhydramnios, can lead to the development of bronchopulmonary dysplasia, with a perinatal mortality up to 80% (12). Other pathologies related to severe reduction of AL are pulmonary dysplasia (failure of maturation) and cord injury. This occurs in 1 to 2% of the cases of preterm PROM (2).
- **Cord prolapse.** Umbilical cord prolapse is related to the reduction of amniotic fluid following the rupture of membranes. The main risk is compression of the cord by the descending foetus during delivery which leads to hypoxia and bradycardia and posterior death or permanent disability. Outcomes have drastically improved due early recognition and intervention in the recent years, associated to extended caesarean availability (17).
- **Abruptio placentae**. Placental abruption is defined as the early separation of the placenta from the lining of the uterus before completion of the second stage of labour (18). Its principal sign is haemorrhage in different degrees and it is identified in 2-5% of pregnancies complicated by PPROM (2), being more common in pregnancies complicated by PPROM prior to 28 weeks of gestation (12). It is a rare but complicated condition which places the well-being of both mother and foetus at risk (18).
- **Postpartum infection**. Maternal complications after delivery of preterm PROM are related with infection. The most relevant is postpartum endometritis, which occurs in 2% to 13% of women with preterm PROM (19).

3.1.5 Diagnosis of preterm PROM

The diagnosis of preterm PROM is based on the confirmation of watery leakage from the vagina, together with medical history (20). In 80-90% of the cases, the use of an sterile speculum is enough to objective either amniotic fluid (AF) accumulation in the posterior vaginal fornix or the direct leakage from the cervical canal (12). There are additional test that can be performed when the diagnosis is uncertain:

- Niatrazine test: it detects changes in pH of vaginal fluid produced by AF. It is positive when pH is higher than 6.5, normally vaginal pH varies between 4.4 and 6.0. This test has 17% of false positives related to substances that changes pH such as blood, semen or the presence of bacterial vaginosis.
- Ultrasound: it does not give the diagnosis of PROM but the presence of oligoamnios supports the orientation. It is important to determine the maximum AF column.
- Biochemical parameters: the concentration of both of the following proteins in AF is 100 to 1000 times higher than in vaginal fluid of women without membrane rupture. They are commercialized in prepared kits which allows a quick diagnosis.
    - Insulin-like growth factor binding protein-1 (IGFBP-1).
    - Placental alpha macroglobulin-1 (PAMG-1).
- Amnio-fusion of indigo carmine: it consists on infusion of dye inside the amniotic cavity trough amniocentesis and the visualization of dyed fluid in the vaginal after 30 to 60 minutes. This test is only performed when the diagnosis of PROM is still uncertain after using the tests previously described.
- Manual vaginal examination is contraindicated due to the increment in risk of infection, especially of chorioamnionitis (21).

3.1.6 Management and treatment goals of preterm PROM

The management of PROM in general and especially in preterm PROM is not clear. There are some aspects of the management that are still controversial and there is the need of further investigation. Current evidence indicates that this patients with preterm PROM will benefit more from an expectant management, meaning extending as much as possible pregnancy after excluding the diagnosis of clinical chorioamnionitis, *abruptio placentae* and foetal compromise.

Initial assessment

There is the need of a general evaluation of the woman which consists in a physical exploration, the monitoring of her vital signs and a blood analysis. Vital signs refers to arterial tension, heart rate and temperature and blood analysis refers to complete blood count, coagulation, C-reactive protein, glucose, creatinine, urea and electrolyte panel. It is important

to explore the cervical characteristics (dilatation and length) and the presence the prolapse of the umbilical cord or foetal parts.

- Preterm PROM diagnosis: the diagnosis can be established with any of the technics explained above in “Diagnosis of preterm PROM”.
- Confirmation of gestational age with ultrasound. The most accurate GA assessment is first term ultrasound dating measuring crown-rump length rather than menstrual dating (22). If first term ultrasound was not performed or it is not available, routine ultrasound should be performed and dating by biometric parameters. This evaluation is crucial because it determines the management.
- The ultrasound exploration is also valid to determine the AF index and the highest column of AF which is principal to recognize oligohydramnios. If the AF index is <5cm or the highest column of AF is <2cm, there is a higher possibility of shorter latency period and higher risk of neonatal morbility.
- Use of cardiotocography (CTG) to determine foetus status, especially tachycardia and uterine contractions.
- Assess the risk of infection and rule out chorioamnionitis. This evaluation is essential in the management of preterm PROM.
    - Clinical diagnosis of chorioamnionitis is most frequently based on criteria adapted from Gibbs because individual clinical criteria have variable sensitivity and low specificity (13). They are enumerated in “ANNEX 1: Diagnosis criteria for clinical chorioamnionitis adapted from Gibbs”.
    - Vaginal and endocervical sampling and posterior culture and antibiotic sensitivity are crucial for the following medical handling. In case of a bacterial isolation, antibiotic treatment should be targeted towards this microorganisms.
    - Streptococcus B group test from vaginal and rectal secretions sampling is performed in addition to cultures.
    - Urine sediment analysis and urine culture

  Further information of the antibiotic use will be detailed in the section “Antibiotics”.

- Diagnostic amniocentesis: transabdominal amniocentesis is a feasible and safe (23) procedure which can be performed for the recollection of amniotic fluid (24) and subsequent assessment of intraamniotic infection defined as positive culture of the AF. The amniocentesis has the capacity to detect subclinical infection before clinical chorioamnionitis or foetal sepsis are established. The clinical value of vaginally obtained amniotic fluid is limited because of high sample-to-sample variability and contamination (25). The principal intention of this recollection is to process cultures, unfortunately, it requires at least 48-72 hours for a result and detection rates are low. Intraamniotic

infection is found in 30-60% of women with preterm PROM (20), being the most frequently found *Ureaplasma* spp. (12), a member of the group genital mycoplasma.

Cultures can be complemented with the analysis of some inflammation markers such as lactate dehydrogenase, glucose, interleukins 6 (IL-6), matrix metalloproteinase-8 (MMP-8), calgranulins and neutrophil-defensins (26,27).

There are more advanced molecular techniques to determine the presence of microorganism that can be performed such as probe hybridization assays and sequencing of ribosomal RNA (28). This technics increase rates of detection of higher than cultures, especially for those pathogens whose growth is challenging (29).

Antibiotics

- Prophylactic antibiotics. The use of prophylactic antibiotic in a rupture of membranes reduces the incidence of chorioamnionitis and neonatal infection, supposes a prolongation of latency period and brings improvements of short term neonatal outcomes, but not a significant reduction of perinatal mortality (2). Despite the absence of proven long term benefits, the short term advantages shown ensure the recommendation of systematic administration of antibiotic. However, there are no convincing results about the antibiotic of choice (11).

The recommended duration of the therapies varies from 7 to 10 days because shorter lines have not beneficial results. Most of the authors suggest starting with intravenous administration and after 48 hours changing to oral administration (20).

The antibiotics studied are ampicillin and erithromycin alone and in combine therapy, and clindamycin as an alternative. Nevertheless, the recommendation tends to be a broad spectrum antibiotic therapy even though the most frequent pathogens implicated in the intraamniotic infection are *Ureaplasma* spp.

Table 2. Antibiotic options and coverage (adapted from SEGO protocol (20)).

Antibiotic	Coverture
Ampicillin	Streptococcus group B, listeria, enterococcus spp., *Gardnerella vaginallis* and anaerobic bacteria
Gentamicin	*Escherichia coli* and enterobacteria
Cefotaxime	*Escherichia coli*, enterobacteria and anaerobic bacteria
Clindamycin	Anaerobic bacteria and mycoplasma. 15% streptococcus group B resistances
Macrolides (erithromycin,..)	*Ureaplasma* spp. 20-25% streptococcus group B resistances

The commonly used therapies as empiric prophylactic combination consist in combinations of the mentioned antibiotic to cover all the common pathogens, even though it may be reasonable to administer macrolides alone.

- Prophylaxis for streptococcus group B perinatal infection. Women with preterm PROM and a viable foetus who are candidates for intrapartum GBS prophylaxis (positive Streptococcus B group test from vaginal and rectal secretions sampling) should receive intrapartum prophylaxis to prevent vertical transmission (30).

- Treatment of stablished infection. If a clinical infection is confirmed, it is treated based on the pathogen detected and antibiotic sensitivity tests (3,20).

Corticosteroids

The use of corticosteroids for pulmonary maturation is recommended in patients with PROM between 24^{+0} and 32^{+6} because respiratory distress secondary to prematurity is the main morbidity and mortality complication. There is no clear evidence of its use if GA is 33^{+0} and 34^{+6}, anyhow, experts recommend the use in this gap (20,31) of a single course of antenatal betamethasone (2) should be considered routine for all preterm deliveries. It is controversial if there is the need of another course of medications because there is not enough data (31).

There is a proven reduction of risk of perinatal mortality [RR 0.72 (CI 95% 0.59-0.89)], respiratory distress syndrome [RR 0.66 (CI 95% 0.56-0.77)], intraventricular haemorrhage [RR 0.55 (IC 95% 0.40-0.76)], necrotizing enterocolitis [RR 0.50 (CI 95% 0.32-0.78)], need of mechanic respiratory assistance [RR 0.68 (CI 95% 0.56-0.84)], systemic infection during the first 48h of life [RR 0.60 (CI 95% 0.41-0.88)] and also there is no increment of incidence of chorioamnionitis nor endometritis (31).

Tocolysis

Tocolytic therapy is based on medications used to suppress premature labour. There are many options of tocolytic agents that are used to prevent delivery (32,33) in order to prolong pregnancy in women with preterm PROM, thereby reducing the consequences of prematurity. Every country has its own preferred therapy for tocolysis (12). However, the use of tocolytics in this patients is still controversial. Based on last Cochrane systematic review in 2014 and the American College of Obstetricians and Gynecologists most recent recommendation (2), there is no evidence of clear benefit in the prolongation of latency greater than the potential harm of maternal and perinatal infection which increases with longer latency. For this reason, the indication is to not use systematically tocolytics in preterm PROM.

The only reasonable situations where tocolysis really brings an advantage is when the pregnant woman must be moved to another centre, or if labour is imminent and time is crucial for the effect of corticosteroids (20).

Nevertheless, the studies in which are based this recommendations did not use systematically either corticosteroids or antibiotics, both of which have become part of standard management for reducing infection and improving neonatal outcomes (33,34).

Magnesium sulphate

Preterm neonates have higher risk of neurologic disease such as cerebral paralysis and physical and sensory disabilities related to prematurity (35,36). This is the reason why magnesium sulphate should be administered for neuroprotection of those who survive. It is indicated in women with preterm PROM before 32 weeks of gestation who are thought to be at risk of imminent delivery (2,20,36), which is considered an active phase (>4 centimetres of cervical dilatation) or in elective indication of termination (20).

The studies that have proved the effect of magnesium sulphate used different dosages, therefore, the exact recommendation is unknown (36) and there is the need of further homogenized investigations.

Pregnancy termination

Pregnancy termination is recommended in: chorioamnionitis and loss of foetal wellbeing. In general at 34^{+6} weeks because there is a reduction of preterm morbidity and the risk of infection is greater. The only reason to prolong pregnancy is in those cases where there is the need of corticosteroids administration (24 or 48 hours) (20).

3.2 Genital mycoplasma

Genital mycoplasma is a term used to identify microorganisms which are frequently found in the genitourinary tract of men and women. It consist of some species from the family Mycoplasmataceae (37), to be more specific, from the genus Mycoplasma and all the species identify of the genus Ureaplasma.

The most characteristic species and those associated with human pathology are *Mycoplasma hominis* (*Mh*)*, Ureaplasma ureatyticum* (*Uu*) and *Ureaplsma parvum* (*Up*)*.* It is often seen in bibliography *Mycoplasma genitalium* (*Mg*) included in this group (38), even thought, it has been lately identified as the cause of another group of pathologies such as sexual transmitted diseases (39). It was not until the development of genetic technics when it was possible to differentiate between species of the genital mycoplasma (group).

3.2.1 Microbiological characteristics and virulence factors

Genital mycoplasma have some qualities which makes them unique from any other group of species and worth being extensively studied just for the fact that they have the smallest known genome of any free-living organism (40).

First of all, the lack of cell walls is used to separate the family Mycoplasmataceae from other bacteria in a class named Mollicutes (mollis, soft; cutis, skin, in Latin) (37,41). The consequences of this property are basically two. In one hand, the glycopeptides and membrane proteins are the principal antigens, which produces cross reactions with human tissues and other bacteria (37). This produces an exaggerated inflammatory response and is related to the transition from colonization to infection (42).

On the other hand, the absence of wall makes them completely resistant to β-lactam antibiotic class (37,43), which is widely used in the clinical practice as empiric treatments.

Furthermore, this group of microorganisms have trouble growing in typical cultures (44) which makes them difficult to find through basic detection technics and gram stain. They require enriched growth medium supplemented with nucleic acid precursors, fatty acids, amino acids and sterols (45). Thus, it is needed to use specific detection technics which will be further explained in the section “Detection of genital mycoplasma”.

Another defining characteristic are the metabolism pathways. *Ureaplasma* spp. hydrolizes urea into ammonia and *Mycoplasma hominis* produces ammonia from arginine. This reactions incite to rise the pH of the genital tract which facilitate mixed infection with other bacteriam such as in bacterial vaginosis (46).

Finally, it cannot be forgot to mention that mycoplasmal colonization is essentially limited to mucosal surfaces as a result of the action of several adherence proteins (47) in the external layer. This location outside the cell facilitate the bacterial evasion of immune system.

3.2.2 Epidemiology of genital mycoplasma

Even considering the characteristics explained above, it is true that genital mycoplasma is a common colonizer of the genitourinary tract of men and woman with rate of prevalence around 50% for *Mycoplasma* and 80% for *Ureaplasma*. Different studies with different populations around the word shown similar rates of colonization (42).

Colonization increases according to the number of different sexual partners, and it is also influenced by age, race, socio-economic status, contraception, menstruation, menopausal changes and pregnancy (48).

Many studies have shown that rates of colonization between pregnant and non-pregnant women are similar but vary from 7.7% to 80% for *Ureaplasma* and 3% to 51% for *Mh*. However, others show that pregnant women are frequent vaginal carriers of all species of mycoplasmata, *Up* and *Uu* being the most frequent, *Mh* less frequent (39). This increase may be explained by the immunological and hormonal changes that are likely to affect colonisation. Therefore, the data available is not reliable (48).

3.2.3 Clinical manifestations related to genital mycoplasma

- **Genitourinary infection**. *Ureaplasma* spp., especially *Uu,* and *Mg* have been link to genitourinary infections such as urethritis, cervicitis, urine infections, calculus and also pelvic inflammatory disease and bacterial vaginosis (15).
- **Materno-foetal infection**: genital mycoplasma has been identify as pathogens responsible of a series of afflictions during pregnancy such as chorioamnionitis, PROM, abortion and preterm delivery. This microorganisms arrive trough ascending mechanisms from the vagina, as they are common colonizers, and produce an inflammatory response which at the same time generates uterine contractions, cervical changes and lesion in the membranes (15). Recent studies identify *Up* as the main agent (39). In addition, postpartum endometritis, postpartum fever and septicemia are other disorders which have been related to *Mh* and *Uu,* either associated to intraamniotic infection or direct ascending infection.
- **Neonatal infections**: some neonatal infections are associated with vertical transmission of genital mycoplasma from maternal vaginal microbiota to the infant. The risk is higher with the presence of chorioamnionitis. Some manifestations are pulmonary disease and systemic infection.
- Other infections: *Mh* and *Ureaplsma* spp. can cause various opportunistic infections in inmunodepressed patients such as wound infections and abscesses after surgical interventions, septic arthritis, nervous system and respiratory infections (15).

3.2.4 Detection of genital mycoplasma

The options currently available in the clinical practice for the detection of genital mycoplasma are mentioned below. The samples can be collected from different locations but are more frequently gathered from the genitourinary tract (vagina, endocervix and urethra), other organic fluids (urine, cerebrospinal fluid, amniotic fluid, and blood), wounds and solid tissues, everything depending on the case (28).

Culture

Genital mycoplasmas are difficult to grow in standard cultures due to their slow time of growth and the small colonies in which they develop (37), requiring two to five days for its

interpretation. In consequence, there is a high failure rate of microorganism identification which make the use of general cultures impractical. Publications have demonstrate that bacterial culture had a lower rate of detection (27,1%) compared to PCR (up to 72,9%) (12). There are specific growth mediums for *Ureaplasma* and *Mycoplasma* but each species best conditions of reproduction are different. This is the reason why molecular-based assays are gaining place in the identification of genital mycoplasma.

Cell cultures have the advantage of offering the possibility to perform antimicrobial susceptibility testing (15). Nevertheless, new generation sequencing technics are expanding their field of utility identifying antibiotic resistance genes (28).

Serological Analysis

Serological test methods for genital mycoplasma include micro-immunofluorescence, metabolism inhibition and enzyme immunoassay, but the elevated prevalence of *Ureaplasma* spp. and *Mh* in healthy people makes interpretation of antibody titres difficult. Additionally, it is challenging to distinguish between current/recent and past infection. Therefore, there is no standardize serological assay for genital mycoplasma (45).

Polymerase chain reaction

The introduction of polymerase chain reaction (PCR) methods for the detection of genital mycoplasma meant a change in the diagnosis of this microorganisms. Real-time PCR has the ability to detect and qualify the products simultaneously with amplification. This methods were shown to be superior to culture based methods for detection of the presence of organisms (42). Real time PCR detected ureaplasmal DNA in 40% of the cases versus 24% detected by culture (45).

Other advantages with PCR detection are the speed in which results are available (they can be ready in less than 2 hours) and, its high analytical sensitivity which allows the detection of low number of pathogens. Multiple pathogens can be tested at the same time in a sample, either with probe hybridization assays (specific targets for each specie) or DNA sequencing (for instance, sequencing of 16S rRNA and comparing the 9 variable locus with databases) (28).

As organism viability does not have to be maintained for Nucleic Acid Amplification Tests based detection, specimen collection, handling, and transport are somewhat simpler than for cultures, in that a specialized nutritive transport medium is not required. However, laboratories endorse basic instruction such as sterile material use, cold storage and rapid transportation in order to be processed (45) .

3.2.5 Antibiotic treatment of genital mycoplasma

The antibiotics of choice for the treatment are macrolides, either erythromycin, azithromycin, or clarithromycin. There are other macrolides such as josamicyn which is highly effective againsts ureaplasma and solithromycin which is a new macrolide but has not been tested in pregnancy yet (39).

The action mechanism is that they bind reversely to the V dominium of 23S ribosomal RNA trough hydrogen bridges, therefore they are effective against *Mycoplasma* and *Ureaplasma* unlike other antibiotics such as β-lactams because this last family attacks the cellular wall.

In the treatment of pregnancy infections related to this microorganisms, there are more things to take into account besides the potential biochemical effectiveness (action mechanism). For instance some agents are teratogens able to cause malformations or functional damage to de foetus or may have toxic effects on the neonate (49). Some examples are tetracyclines (doxycycline is highly effective against genital mycoplasma) and fluoroquinolones which are contraindicated in pregnancy (39).

In “Figure 2: Highlight properties of the main macrolides” are shown some characteristics of the antibiotics which should take into account when choosing the antibiotic in pregnancy to fight infections cause by genital mycoplasma. The information is summarized from (49–51).

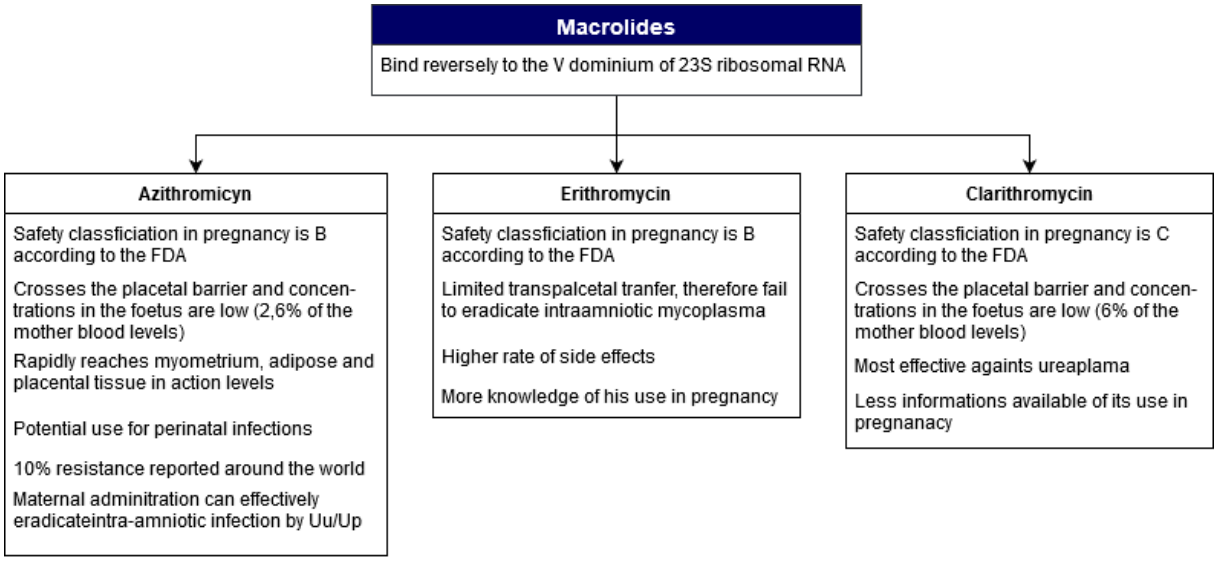

Figure 2: Highlight properties of the main macrolides

4. Justification

Preterm premature rupture of membranes (PROM), defined as rupture of membranes before the onset of labour before 37 weeks of gestation (1) is a complication that occurs in approximately 2-3% of pregnancies (3). Its etiology is not well stablished but is likely to be multifactorial (10) being subclinical intraamniotic infection an important agent, either as a cause or a consequence (11). The role of genital mycoplasma, in particular *Ureaplasma* spp., which produces inflammation through the release of cytokines, causes a weakening in the resistance of the amniotic membrane and subsequent rupture in certain zones (12).

The main problem with preterm PROM is the complications related to prematurity of the new-born (2). Preterm PROM is responsible of 30-40% of the spontaneous premature labour (2,3), which leads to an increase of mortality and morbidity such as cerebral paralysis, sensory deficits, learning disabilities and respiratory illnesses resulting in enormous physical, psychological and economic costs (6).

Nowadays, most of the reference guides (2,8,20) recommend a management of this pathology which includes measures to lengthen the time between rupture and delivery (known as latency period). This is done so that at least it is possible to administer a complete corticosteroid regimen for pulmonary maturation (31). Unfortunately, the risk of contamination of the amniotic fluid and subsequent manifested infection, which is related to the rise of bacteria from the vaginal flora, increases as the latency period is prolonged (2). In order to prevent this clinical infection of the membranes and the amniotic fluid, also known as chorioamnionitis, the use of antibiotics has been extended when the preterm PROM is settled. Studies have shown that antibiotics diminish the incidence of chorioamnionitis and neonatal infection, suppose a prolongation of latency period and bring improvements of short term neonatal outcomes (2,11). However, it is not clear which is the best antibiotic regimen to administer according to the different situations in which the patient can be found (11). The studies published of this subject are not conclusive.

Nowadays, in the Hospital Universitari Doctor Josep Trueta of Girona, the antibiotic treatment is an empiric therapy, which consist of intravenous ampicillin and gentamicin and one dose of oral azithromycin (52). This therapy is administered even when cell cultures of the genital and urine samples collected are negative, because it cannot be ruled out the presence of genital mycoplasma, a group of microorganism associated to subclinical intraamniotic infection that are difficult to grow in standard cultures and cannot be identify by gram stain (15,45,53) .

The negative consequences of using broad spectrum antibiotics when there is no clear infection stablished is that their administration may contribute to the emergence and spread of

microbial resistance Unfortunately, methodologically it is difficult to design a controlled clinical trial to measure the ecological impact of antibiotic empiric therapies when not necessary (54).

In order to diagnose intraamniotic infection, an amniocentesis can be performed to analyse the microbial colonization of the amniotic fluid (23,24). This practice is not extended as standard in many protocols (52), therefore, a targeted antibiotic regimen effective towards the probable cause of the preterm PROM is not possible. Moreover, the mere use of cell cultures to detect microorganisms is not enough as the main pathogen is *Ureaplasma* spp. (15,45,53). Publications have demonstrated that bacterial culture have a lower rate of detection (27,1%) compared to PCR (up to 72,9%) (12). Therefore, PCR should be the method of choice when it comes to the detection of genital mycoplasma, in order to administer a targeted antibiotic.

Seeing this situation, a randomized controlled clinical trial should be done to increase the amount of data supporting the implementation of the amniocentesis in the standard management of preterm PROM as well as the use of azithromycin (49–51) for genital mycoplasma infection in preterm PROM instead of a broad spectrum coverage when vaginal, endo-cervical and urinary cultures are negative.

5. Hypothesis and Objectives

5.1 Hypothesis

5.1.1 Main hypothesis

- The use of azithromycin when detecting genital mycoplasma by PCR in amniotic fluid increases the latency period (defined as time between the rupture of membranes and labour) in singleton pregnancies with preterm premature rupture of membrane between 23^{+0} and 34^{+6} weeks of gestation.

5.1.2 Secondary hypothesis

- The use of azithromycin when detecting genital mycoplasma by PCR in amniotic fluid decreases the incidence of clinical chorioamnionitis caused by genital mycoplasma in singleton pregnancies with preterm premature rupture of membrane between 23^{+0} and 34^{+6} weeks of gestation.
- Girona's prevalence of genital mycoplasma subclinical intraamniotic infection in pregnant women with preterm premature rupture of membrane is the same as shown in previous studies.

5.2 Objectives

5.2.1 Main objective

- Compare the differences in latency period (defined as time between the rupture of membranes and labour) with targeted antibiotic with azithromycin and empiric antibiotic treatment in the management of singleton pregnancies with preterm premature rupture of membrane between 23^{+0} and 34^{+6} weeks of gestation with positive PCR in amniotic fluid for genital mycoplasma.

5.2.2 Secondary objectives

- Compare the differences in incidence of clinical chorioamnionitis caused by genital mycoplasma with targeted antibiotic with azithromycin versus empiric antibiotic treatment in the management of singleton pregnancies with preterm premature rupture of membrane between 23^{+0} and 34^{+6} weeks of gestation with positive PCR in amniotic fluid for genital mycoplasma.
- Estimate the prevalence in Girona of genital mycoplasma subclinical intraamniotic infection in preterm premature rupture of membrane between 23^{+0} and 34^{+6} weeks of gestation.

6. Material and Methods

6.1 Study design

This study is a **multi-centric**, **randomized, controlled, open label** and **parallel** clinical trial to check if the latency period varies with empiric antibiotic treatment versus targeted antibiotic with azithromycin in the management of preterm premature rupture of membrane (between vweeks) with positive PCR for genital mycoplasma in amniotic fluid.

6.2. Study population

6.2.1 Subjects to study

The population of this trial will be the pregnant women who are diagnosed with preterm PROM (between 23^{+0} and 34^{+6} weeks) with positive PCR for genital mycoplasma in amniotic fluid who are admitted to the emergency service of the Catalan reference hospitals and meet the inclusion and not the exclusion criteria. These criteria are expressed in **Table 3**.

Table 3: Inclusion and exclusion criteria.

Inclusion criteria
• Age older than 18-year-old • Singleton pregnancy • Gestational age between 23^{+0} and 34^{+6} weeks • Diagnosed of preterm premature rupture of membranes • Positive PCR for genital mycoplasma (*Mycoplasma hominis, Ureaplasma uralyticum, Ureaplasma parvum*) in amniotic fluid • Patients who have read, understood and sign the informed consent
Exclusion criteria
• Criteria for clinical chorioamnionitis • Complications of preterm PROM such as abruptio placentae or cord injury or prolapse • Cervix dilation greater than 4cm • Positive PCR in AF for any other microorganisms that are not genital mycoplasma • Positive vaginal culture for pathogenic microorganisms • Congenital prolongation of QT interval • Allergy to penicillin, macrolides or gentamicin • Pregnancy complications such as preeclampsia and diabetes • Maternal chronic disease • Foetal genetic conditions and abnormalities

Withdrawal criteria

Patients may discontinue their participation in the study at any time. The research doctor, in his or her opinion or judgement, may also withdraw a patient from the study if required by the patient's clinical situation, for instance apparition of adverse effects of the treatment or complications of the amniocentesis. The cause and justification of the withdrawal will be reflected in the study development.

6.2.2 Sample size

In a bilateral test, accepting an **alpha risk of 5%**, a **statistical power of 80%,** assuming a moderate **size effect** (equivalent to a Cohen's d equal to 0.3, i.e. the difference in the effectivity between the two treatments will not be very large), the sample size will be 87 patients in each group.

It has been anticipated a **drop-out rate of 10%** and therefore the final **sample size will be 96 patients** in each group. The low drop-out rate is estimated at 10%, because preterm PROM is an acute medical condition with a treatment that is administered when the patient is admitted in the hospital and in most of the cases, the delivery will take place during the same admission.

Computations were carried out with the Prof. Dr. Marc Saez' software based on the 'pwr' package of the free statistical environment R (version 3.6.2).

6.2.3 Sample selection

The sample selection will be a **cluster sampling** which by definition is divided into two stages. First of all, there will be a selection of Catalan hospitals which are determined based on convenience. The hospitals will be:

- Hospital Universitari Arnau de Vilanova de Lleida
- Hospital Universitari Joan XXIII de Tarragona
- Hospital Universitari Vall d'Hebron
- Hospital Universitari Doctor Josep Trueta de Girona

This will not pose a problem considering that third level public hospitals in Catalonia are supposed to offer the same level of competence in human and material resources. In the second place, a non-probabilistic consecutive sample method will be followed in the gynaecology and obstetrics departments of the chosen hospitals. The patients who meet criteria will be offered to participate in the study.

6.2.4 Randomization

Once the patients accept the participation in the study by signing the informed consent, they will be randomly assigned to one of the two groups of treatment:

- Group A: control group (therapy A) // Group B: intervention group (therapy B)

This task will be done by a statistician who will randomly separate the patients into the two groups by means of a software in order to reduce biases of selection.

6.2.5 Masking techniques

This study is an open-label study, which means that the research doctor will be aware of the antibiotic treatment each patient is receiving and the patient will be aware as well of the therapy they are receiving. Masking the intervention is an added technical difficulty in this trial because the administration route and the duration of the treatment differs from therapy A to therapy B. The first one is one dose oral and the rest intravenous during 7 days and the second one is administered intravenous first and intravenous later during 7 days. The statistician who will analyse the results will not be aware of the group origin of the data.

6.2.6 Estimated time of recruitment

Based on professional experience, the media each month they are accepted 5 women with preterm rupture of membranes in a gynaecologic and obstetric department of the reference hospitals involved in the trial. Considering that 80% of the women will enrol in the trial accepting the performance of amniocentesis and that genital mycoplasma will be found in the amniotic fluid of 50% of the amniocentesis performed, we expect 97 cases a year to attend the services and meet the inclusion but not the exclusion criteria. This translates to an estimated time of recruitment of 2 years.

6.3 Study interventions

The independent variable of this study is the antibiotic treatment strategy. After the diagnosis of preterm PROM and inclusion in the trial, patients will be divided into two groups and each of them will receive different antibiotic treatment:

Group A: they will be given empiric antibiotic (therapy A) following the dosage:

- **Ampicillin.** 1 gram / 6 hours intravenous administration during 7 days.

Intravenous ampicillin is commercialized by Laboratorios Normon, S.A. as GOBEMICINA. It consist of a vial with 1 gram of sodic ampicillin and a blister with water for the solution. In each vial it can be found 2,9 mEq / each gram of ampicillin.

Before the administration of one vial it has to be reconstituted mixing the vial with the blister until all the solute is dissolved. It should be administered in continuous drip every 6 hours.

Expected adverse effects are allergic reaction with skin manifestations, blood cell alterations, transaminases elevation, nausea, vomiting and diarrhoea.

All this information is extracted from its technical sheet authorized in September 1978 (number of authorization 54341). More detailed information about the product can be found in AEMPS web page.

- **Gentamicin.** 240 milligrams / 24 hours intravenous administration during 7 days.

Intravenous gentamicin is commercialized by B. Braun Medical, S.A. as GENTAMICINA BRAUN. It consist of a container with a capacity of 100 millilitres with 80 millilitres of gentamicin in the form of gentamicin sulphate. Each container contains 283 milligrams of sodium as well as water.

The therapeutic rage of gentamicin is between 5 and 10 micrograms / litre which is achievable with an intravenous perfusion of 1 milligram / kilogram during 1 hour every 8 hours. The concentrations required in this therapy are elevated so milligrams are calculated for 80 kilograms.

Expected frequent adverse effects are neurotoxicity, ototoxicity, neuromuscular blockage and nephrotoxicity. It has been seen cases of foetus ototoxicity. Other less frequent are headache, tremors, nausea, vomiting, skin eruption and muscular debilitation.

All this information is extracted from its technical sheet updated in February 2015 (number of authorization 59658). More detailed information about the product can be found in AEMPS web page.

- **Azithromycin.** 1 gram oral administration in one isolated dose the first day of treatment.

Oral azithromycin is commercialized by PFIZER, S.L. as ZITROMAX. It consist of a tablet covered with a film that contains 500 milligrams of azithromycin dihydrated.

Each tablet contains 14,40 milligrams of lactose monohydrate. It also contains pregelatinized corn starch, anhydrous calcium hydrogen phosphate, croscarmellose sodium, magnesium stearate and sodium Lauryl Sulfate. The coating film is composed by lactose monohydrate, hypromellose, titanium dioxide (E171) and triacetin.

The administration will consist in the oral intake of two tablets at the same time the first day of antibiotic treatment. In total 1 gram of azithromycin that can be administered with water and with or without food.

Expected frequent adverse effects are headache, vomiting, nausea and abdominal pain. Other less frequent are mild infections, blood cell series alterations, insomnia, dizziness, palpitations, dyspnoea, mild gastrointestinal alterations, skin eruption, arthralgia and myalgia. It has been registered prolongations on QT interval.

All this information is extracted from its technical sheet updated in August 2019 (number of authorization 61272). More detailed information about the product can be found in AEMPS web page.

Group B: they will be given targeted antibiotic for genital mycoplasma (therapy B) following the dosage:

- **Azithromycin.** 500 milligram / 24 hours administered intravenously during the 2 first days and 250 milligrams in one oral take every 24h for the next 5 days.

Intravenous azithromycin is commercialized by PFIZER, S.L. as ZITROMAX. It consist of a vial with 500 milligrams of azithromycin in its dehydrated form (white powder).

Each vial contains 114 milligrams of sodium which is equivalent to 55 of the maximum sodium intake recommended by the World Health Organization). It also contains citric acid and sodium hydroxide.

Before the administration of one vial it has to be reconstituted adding 4,8 millilitres of sterile water or any prepared solution specified in the technical sheet and agitating the mix until all the solute is dissolved. Each millilitre of reconstituted solution contains 100 milligrams of azithromycin.

It is administered in a concentration of 1 milligram of azithromycin for 1 millilitre of solution during 3 hours. It is important to not administer the solution as a bolus or intramuscular injection.

Expected adverse effects are the same as the ones expected with the oral admiration but the exposure time is longer and it can appear inflammation of the injection area.

All this information is extracted from its technical sheet updated in August 2019 (number of authorization 64834). More detailed information about the product can be found in AEMPS web page.

Oral azithromycin is commercialized by PFIZER, S.L. as ZITROMAX. It consist in a tablet that contains 250 milligrams of azithromycin dihydrated.

Each tablet contains 151,55 milligrams of lactose monohydrate. It also contains anhydrous lactose, magnesium stearate, sodium Lauryl sulfate, cornstarch, gelatin, titanium Dioxide (E171), black Ink 10 A1 and black ink 10 A2.

The administration will consist in the oral intake of one tablets every day for 5 days after 2 days of intravenous administration. It can be administered with water and it must be spaced with food intakes (1 hour before and 2 hours after eating) because de bioavailability decreases 50% if taken with food.

Expected adverse effects are the same as the ones explained before.

All this information is extracted from its technical sheet updated in August 2019 (number of authorization 59616). More detailed information about the product can be found in AEMPS web page.

In both groups the treatment will be stopped 24 hours post-delivery if there is no clinical infection confirmed.

In table 4 “Summary of study’s intervention” it can be seen the antibiotics administered in each group each day of treatment as well as the quantity of drug.

The adverse effects registered during the trial because of the antibiotic intake will be registered in the patient’s digital medical record and reported to AEMPS. The intervention will only be stopped for this reason if the clinician in charge considers it is the best option under his or her clinical experience and under consensus of the rest of the department’s coworkers.

Table 4: Summary of study’s intervention.

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Tharapy A	Ampicilin	1 gram / 6 hours IV	1 gram / 6 hours IV	1 gram / 6 hours IV	1 gram / 6 hours IV	1 gram / 6 hours IV	1 gram / 6 hours IV	1 gram / 6 hours IV
	Gentamicin	240 milligrams IV	240 milligrams IV	240 milligrams IV	240 milligrams IV	240 milligrams IV	240 milligrams IV	240 milligrams IV
	Azithromycin	1 gram O	-	-	-	-	-	-
Therapy B	Azithromycin	500 milligrams IV	500 milligrams IV	250 milligrams O	250 milligrams O	250 milligrams O	250 milligrams O	250 milligrams O

IV: intravenous adminitration; **O**: oral adminitration

6.4 Study variables

6.4.1 Dependent variable

The dependent variable of the study is the latency period defined as the time between the rupture of membranes and the delivery of the infant. This continuous quantitative variable will be expressed in days, being day 0 when premature rupture of membranes is diagnosed.

6.4.2 Secondary variables

- **Incidence of chorioamnionitis**. It will be defined as the presence of fever >38ºC and two other signs based on criteria adapted from Gibbs which are enumerated in "ANNEXE 1: Diagnosis criteria for clinical chorioamnionitis adapted from Gibbs".
- **Prevalence of genital mycoplasma subclinical intraamniotic infection** after the event of preterm PROM which is defined as a positive PCR in amniotic fluid.

6.4.3 Independent variable

The independent variable of the study is dichotomic qualitative variable: the administration of a specific antibiotic strategy (intervention of the study).

- Therapy A: empiric antibiotic based on intravenous ampicillin and gentamicin and oral azithromycin.
- Therapy B: specific antibiotic for genital mycoplasma based on intravenous and oral azithromycin.

6.4.4 Covariates

- **Maternal age** at preterm PROM diagnosis (years). It will be treated as a categorical qualitative variable and divided into three groups.
- Anthropometric measurements: maternal weight (kilograms) and maternal height (metres) before pregnancy. This two variables will be analysed by means of the **Body Mass Index** (BMI) before pregnancy (kilograms/metre^2). They will be collected from the digital medical record of the patient.
- **Ethnicity**. It will be considered as a discrete variable divided into 5 categories: Caucasian, African, Latin-American, Asian and Other. It will be asked to the patient.
- **Parity**. It will be treated as a dichotomic qualitative variable separated in nulliparous and multiparous. This information will be gathered from the medical record.
- Time between **onset of symptoms of rupture of membranes** and antibiotic initiation. It will be collected from the digital medical record.
- **Socioeconomic status** (social class I to V) approached by the education level and occupation according to Domingo et al (55,56). It will be asked to the patient.

- **Sexual risk behaviours** defined as unprotected sexual relations and frequent changes in couples. This information will be asked to the patient during her stay in the department sheet. It will be treated as a dichotomic variable (yes/no).

In the Table 5: "Variables of the study" it can be found a summary of all the variables included in the study. All the information gathered will be recorded in the collection data sheet.

Table 5: Variables of the study

	Variable	Measure instrument	Categories or values
Independent variable	Intervention therapy	-	Therapy A
			Therapy B
Dependent variable	Latency period	Medical record	Time expressed in days
Secondary variables	Incidence of chorioamnionitis	Medical record	Yes / No
	Genital mycoplasma subclinical intraamniotic infection	PCR	Yes / No
Covariates	Maternal age at preterm PROM diagnosis	Patient's ID card or other documentation	• <25 years • 25-40 years • >40 years
	Body mass index before pregnancy	Medical record	• <18,5 → low weight • 18,5-24,→ normal weight • 25-29,9 → overweight • >30 → obesity
	Ethnicity	Asked to the patient	• Caucasian • African • Latin-American • Asian • Other
	Parity	Medical record	Nulliparous or multiparous
	Time between onset of the symptoms of rupture and antibiotic initiation	Medical record	Hours
	Socioeconomic status	Asked to the patient	Social class I to V
	Sexual risk behaviour	Asked to the patient	Yes / No

6.5 Study circuit and collection of data

6.5.1 Enrolment procedures

All patients that arrive to the hospital (or the services involved in the trial) under suspicion of premature rupture of membranes will be considered for the trial. In order to ensure the inclusion and exclusion criteria to the study, some test must be performed.

1. Determination of maternal clinical condition. Main vital signs (tension, heart rate and temperature) must be recorded form all pregnant women with the possible diagnosis of preterm PROM. This is essential for a correct evaluation and deciding the conduct to follow.
2. Diagnosis of premature rupture of membranes, defined as rupture of membranes before the onset of labour will be establish with the visualization of amniotic fluid in the vaginal cavity by means of a speculum. If there is uncertainty of the diagnosis, there is the possibility to perform complementary test such as the niatrazine test, or the Actim PROM test. This are the ones available in all obstetrics departments of the hospitals involved in the study.
3. Performance of cardiotocography (CTG) register to determine foetal status, especially tachycardia and uterine contraction.
4. Confirmation of gestational age with ultrasound. The most accurate GA assessment is first term ultrasound dating measuring crown-rump length. This information will be collected from the pregnancy booklet that the women will provide to the medical personal. If this information is not available, a trained obstetrician will assess gestational age based in ultrasound biometrical parameters. We will also evaluate cervical length and cervical dilatation.
5. Extraction of blood for basic tests that include complete blood count, coagulation, C-reactive protein, glucose, creatinine, urea and electrolyte panel. This parameters are essential to evaluate maternal conditions to undoubtedly adopt an expectant conduct.
6. An electrocardiogram will be performed in order to detect congenital QT elongation.
7. Assess the risk of infection and rule out chorioamnionitis. This evaluation is essential in the management of preterm PROM.
    - Clinical diagnosis of chorioamnionitis is most frequently based on criteria adapted from Gibbs because individual clinical criteria have variable sensitivity and low specificity. They are enumerated in “ANNEXE 1: Diagnosis criteria for clinical chorioamnionitis adapted from Gibbs”.
    - Vaginal and endocervical sampling and posterior culture and antibiotic sensitivity are crucial for the following medical handling. In case of a bacterial isolation, antibiotic treatment should be targeted towards this microorganisms.

- Streptococcus B group test from vaginal and rectal secretions sampling is performed in addition to cultures.
- Urine sediment analysis and urine culture are performed as well.

After the realization of this tests which are done systematically to all patients, we will inform the candidates to enrol to the clinical trial of the option of participating. The study will be explained to them and the information document will be given. See “ANNEXE 2: Information document”. In order to enter, we need additional information which will be provided from the amniocentesis and posterior PCR.

8. Diagnostic amniocentesis: if not contraindicated following standard recommendations (57). A real-time PCR will be performed. It will be applied a probe hybridization assay to identify *Ureaplasma urealyticum, Ureaplasma parvum* and *Mycoplasma hominis*, as well as *Neisseria gonorrhea*, *Chlamydia trachomatis* and *Trichomonas* because the available material is a multiplex kit for sexually transmitted infections. The results are expected to be available for the clinicians in 24 hours due to laboratory logistics.
   Only those patients with negative cultures (urine, endocervical, vaginal and amniotic fluid) for any microorganisms but genital mycoplasma or PCR positive in amniotic fluid for genital mycoplasma will be included in the study.

In order to officially enter to the study, patients will have to read, understand and sign the informed consent. See “ANNEXE 3: Informed consent”.

6.5.3 Intervention and study circuit

9. Randomization. The patients will be randomly assigned to one of the two groups of treatment by a software:
   - Group A: control group (therapy A).
   - Group B: intervention group (therapy B).
10. All patients will receive the same standard treatment that is given to patients with preterm PROM:
    - Corticosteroids 12 milligram of intramuscular betamethasone as soon as it is possible and repeat in 24 hours. If the pulmonary maturation was done before the week 28 of pregnancy, there is a destabilization of the clinical status which make us think that the delivery is imminent and the first cycle of corticosteroids was administered more than 15 days before, a new dose as a reminder can be given.
    - Tocolytics. There are two situations in which it is justified giving tocolytics: the pregnant woman must be moved to another centre, or if labour is imminent and time is crucial for the effect of corticosteroids. Only in this cases it will be considered.

- General recommendations during the admission:
  - Relative rest during the first 72 hours (only for personal hygiene and seating eating). After the first 72 hours, it is possible to walk around the department
  - Diet rich in fibre. If there is the need, a laxative can be administered.
  - Low molecular weight heparin if there are no contraindications (20).
  - Control of vital signs (arterial tension, heart rate and temperature) every 8 hours.
  - Repeat initial blood analysis every 24 hours the first 3 days and every 48h from then on.
  - Repeat CTG and ultrasound every 24 hours to assess the foetal wellbeing.
- Empiric antibiotic treatment: as soon as the patients are diagnose with preterm PROM and right after all samples for cultures and other analysis are gathered, an antibiotic therapy will be administered with ampicillin 1 gram / 6 hours intravenous administration, gentamicin 240 milligrams / 24 hours intravenous administration and azithromycin 1 gram oral administration (single dose).

11. **Intervention treatment**: this treatment must be started as soon as all the results from all the samples are gathered. As it has been already mention, all patients will start with empiric antibiotic treatment as soon as they are diagnosed but as soon as the results for cultures and PCR are generated, the intervention will start: half of the patients will continue with the same therapy (Therapy A) and the other half will receive the targeted antibiotic (Therapy B). See “Intervention of the study”.

If the latency period is longer than 7 days, there is the possibility (under clinical expertise criteria) to discharge the patient and continue the observation in external appointments. Nonetheless, for a better control of variables, we will maintain admitted this patients in the hospital until the delivery. As they represent less than the 10% of the preterm PROM, the additional cost of he stay will not raise the price of the study exaggeratedly.

Following the general recommendations of SEGO (Sociedad Española de Ginecología y Obstetricia), the gestation will be finish if there is chorioamnionitis, loss of foetal wellbeing or the pregnancy exceeds $34+^{6}$ weeks of gestation.

6.5.2 Collection of data

All the information will be recorded in the data collection sheet by the professional who is responsible for the patient during the patient stay in the hospital. There will be an investigator in charge in each hospital, who will check the information collected during the admission and if it is not possible, as soon as he is able. They will validate the data collection sheet assuring that all the information in correctly recorded.

All the doctors in the department susceptible of being in charge of a woman with preterm PROM will be instructed on how to complete the collection of data sheet. One informational meeting will be arrange in each of the hospitals to explain the procedures of this study.

The information regarding the patient's hospital admission following information will be collected in the data collection sheet. See "ANNEXE 4: Data collection sheet" for the parameters requested from each patient.

There will be an additional register with the results of the positive PCR for mycoplasma and ureaplasma, the number of amniocentesis performed and the number of patients admitted with preterm PROM to the obstetrician departments of the hospitals enrolled in the trial. It will be the investigator in charge in each hospital the responsible to enter this information to the data base.

All information collected in this trial will be introduce to **REDCap** (Research Electronic Data Capture available in https://www.project-redcap.org/) a secure web application for building and managing online surveys and databases, available at no cost for not-for-profit institutions. This tool can be used by researchers from multiple sites and institution so it will greatly facilitate this multi-centric clinical trial.

7. Statistical plan and statistical analysis

7.1 Statistical plan

The following section describes the items that will be necessary to evaluate and relate in order to analyse the data collected from each patient throughout the study.

7.2 Statistical analysis

7.2.1 Descriptive analysis

The latency period in each one of the groups will be summarized by the median and the interquartile range.

We will estimate and draw the survival curves of the latency period, stratified by the intervention groups, using the **Kaplan-Meier** estimator.

The incidence of chorioamnionitis and the prevalence of genital mycoplasma, stratifying by the intervention groups, could be summarized by proportions in a **contingency table**.

All these analyses will be stratified by the covariates. The quantitative variables, except BMI, will be categorized in quartiles. BMI will be categorized in four categories: low weight, normal weight, overweight and obese.

The rest of covariates will be summarized based in their characteristics. Ethnicity, parity, socioeconomic status, sexual risk behaviour will be expressed in proportions (qualitative variables) and BMI, maternal age and time between onset of the symptoms of rupture and antibiotic initiation will be expressed in medians and interquartile ranges (discrete quantitative variables). In all cases it will be stratified by the intervention groups.

7.2.2 Bivariate analysis

The difference in the survival curves will be tested using the **log-rank test**.

The difference of the proportions of the incidence of chorioamnionitis and the prevalence of genital mycoplasma between the intervention groups, will be tested using the **Chi-square** and the **exact Fisher's test** when the expected number of counts in any of the cells of the contingency table will be lower than 5.

All these analyses will be stratified by the covariates. The quantitative variables, except BMI, will be categorized in quartiles. BMI will be categorized in low weight, normal weight, overweight and obese.

The difference between the proportions, means and medians of the covariates between the intervention groups will be tested by the Chi-square, Student's t, and the Mann-Whitney's U, respectively.

7.2.3 Multivariate analysis

Main objective

The effectivity of the intervention groups on the latency period will be assessed in a **Cox regression**, where the dependent variable is the latency period (defined as time between the rupture of membranes and labour) and the independent variable will be the intervention (Therapy A and Therapy B) controlling for all the covariates.

Secondary objectives

The prevalence of genital mycoplasma and the incidence of chorioamnionitis will be assessed in **logistic regressions** with dependent variables will be the positive PCR test in amniotic fluid and the meet the criteria adapted from Gibbs, respectively. The independent variable will be the intervention group (Therapy A and Therapy B). We will control for all the covariates.

8. Work plan and chronogram

STAGE 0 – STUDY DESIGN AND PREPARATION OF STUDY PROTOCOL

(3 months, November 2019 to January 2020)

Activity 1: Bibliographic research in major databases.

Activity 2: Problem identification (definition of study objective and variables), proposal change and protocol development and evaluation of final protocol.

STAGE 1 – PROTOCOL VALIDATION

(2 months, February 2020 to March 2020)

Activity 3: Application for a registry number to the EudraCT (European Union Drug Regulating Authorities Clinical Trials).

Activity 4: Ethical assessment from the CEIC (Clinical Research Ethics Committee) of the Hospital Universitari Doctor Josep Trueta in Girona.

Activity 5: Participation acceptance of hospitals directive committees to who the participation in the trial is proposed.

Activity 6: Presentation of the protocol to AEMPS (Agencia Española del Medicamento y Productos Sanitarios) and posterior authorizations receive.

This two first stages will be carried out principally by the research team. This stages are indispensable for the realization of the trial.

STAGE 2 – COORDINATION

(2 months, April 2020 to May 2020)

Activity 7: Organization meetings. The research team and other collaborators will have several meeting in the Hospital Universitari Dr. Josep Trueta in Girona and will be in close contact to the directors of the hospitals involved in the trial. As a result, they will be a detailed chronogram.

Activity 8: Programme formation sessions in the four hospitals participating in the study. In each Obstetric Department of the hospitals participating in the study the research team will have a meeting to talk about the trial, read and understand the protocol and assign someone to be the investigator in charge. This figure will be in charge of entering the data to the REDCap database.

STAGE 3 – PATIENTS RECRUITMENT AND DATA COLLECTION

(2 years, June 2020 to May 2022)

Activity 9: Patients recruitment and data collection. Patients will be enrolled in the study by consecutive sampling once ensure they accomplish the inclusion and exclusion criteria and if they accept the informed consent. The data collection referred to the variables and covariates will take place during the admission of each patient. This activity will be carried out by the physicians in each of the hospitals involved in the trial.

Activity 10: Protocol follow-up. For the correct development and guarantee of quality of the procedures, the investigator in charge of each hospital will maintain regular contact with the research team and three meeting will be organized annually.

STAGE 4 – DATA ANALYSIS AND FINAL EVALUATION

(2 months, June 2022 to July 2022)

Activity 11: Statistical analysis. After receiving the last results from the 192 patients of this trial (estimated July 2022), the statistician will proceed to recollect all the data from the database and do the statistical analysis.

Activity 12: Elaboration of the final document, results distribution and redaction of scientific articles. This articles will be presented in national and international congress.

Figure 3: Chronogram of the study

Stage		Actitivy		2019		2020							2022			
				NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	APR	MAR	JUN	JUL
0	STUDY DESIGN AND PROTOCOL ELABORATION	1	Bibliographic research													
		2	Proposal elaboration													
1	VALIDATION	3	EudraCT application													
		4	CEIC approval													
		5	Hospital direction approval													
		6	AEMPS													
2	COORDINATION	7	Organization meetings													
		8	Programme formation													
3	PATIENTS RECRUITMENT AND DATA COLLECTION	9	Patients recruitment and data collection													
		10	Protocol follow-up													
4	DATA ANALYSIS AND FINAL EVALUATION	11	Statistical analysis													
		12	Final document and dissemination													

9. Ethical and legal considerations

This clinical trial has been proposed according the **Principles of Biomedical Ethics of Beauchamp and Childress** (incorporated in the Spanish law "Ley 41/2002") and following the principles of the **World Medical Association Declaration of Helsinki** of 1964 (last revised in 64th General Assembly, Fortaleza, Brazil, in October 2013).

The principle of justice will be taking into consideration in the fact that every woman that meets the inclusion and not the exclusion criteria will be asked to enrol the trial, without any discrimination. At the same time this trial respect the patient's autonomy because after receiving the information document (see "ANNEXE 2: Information document") and solving their doubts with the physician in charge, they will decided if they willing to join the trial which decision will be reflect in signing the informed consent (see "ANNEXE 3: Informed consent").

The confidentiality of the database used will be respected during the procedure according to the Spanish law "**Ley Orgánica 3/2018**, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales". All women's data will be managed anonymously in order the patient will be guaranteed as well as the right of modifying or erasing their personal data.

This study is written in concordance to the Spanish "**Real Decreto 1090/2015**, de 4 de diciembre, por el que se regulan los ensayos clínicos con medicamentos, los Comités de Ética de la Investigación con medicamentos y el Registro Español de Estudios Clínicos" as a non-commercial clinical research and the "**Ley 14/2007**, de 3 de julio, de Investigación biomédica".

This trial doesn't have a third arm of patients treated with placebo for ethical issues. Both treatments showed effectiveness compared with expectant management in the most recent publications, and it will not be ethical to leave a group of pregnant women without an effective treatment.

This protocol will be review by the **Clinical Research Ethics Committee** (CEIC) of the Hospital Universitari Dr. Josep Trueta de Girona, whose dictum will be valid for the rest of the hospitals involved in this multi-centric clinical trial.

In order to be carried out, this trial must be registered in EudraCT database and AEMPS must give its authorization as this is a trial with drug interventions.

All the research team commits that, whatever the results of the trial are, all the data will be published with transparency, and they will not exclude unfavourable events or data.

10. Strengths and limitations of the study

In clinical trials, the most important bias to avoid is the selection bias. Sample selection will be carried by a non-probabilistic sampling method because the population to study need to fulfil some specific characteristics that are not highly prevalent in the population. The participants will be randomly assigned to the intervention groups in order to distribute equally the possible confusing factors. Moreover, as this factors may influence association between dependent and independent variables, a multivariate analysis will be made.

There is also the possibility that the acceptance rate of participation of the women that meet the inclusion but not the exclusion criteria is low, increasing the risk that the selected sample does not represent the targeted population. In order to minimize this bias, even if it cannot be complete avoided, the informed document will be detailed and clinicians in each hospital and the research team will be available to answer any question the potential participants have. See “ANNEXE 2: Information document”.

In addition, we will consider the drop-out of the sample. The pregnant woman who refuse continuing in the project will not provide results to the study and this can affect the validity of the statistical results. To deal with this, we have anticipated these losses and we have calculated the sample size with a drop-out rate of 10%.

Due to the size of the sample, we will not be able to take conclusions because we would need a larger sample size. However, a larger sample will mean even longer estimated period of recruitment which can be a limitation.

This study is multi-centric, so it could create variability because in each hospital has a specific method. In order to reduce this variability, there will be meetings in each hospital between the obstetric clinicians from the hospitals which participate in the train and the research team before the beginning of the trial and the investigator in charge of each hospital will be in constant contact with the research team during the recruitment and data collection. Being a multi-centric study in different hospitals of Catalonia, the results could be more generalizable

One of the main limitation of our study is the lack of blinding of the intervention therapy for clinicians and participants (this is an open label clinical trial). In order to minimize this bias, the statistician will not know the group of precedence of the data analyzed.

11. Budget

Staff. Research team and medical assistance personnel are employed by the hospitals included in this study. Therefore, there is no need of contracting additional services. It will only be included the sub-contraction of the professional services of a statistician to analyse the data results. We estimate that there will be needed 40h of work which are paid 35€ /hour.

Material. For the budget, we will only take into consideration the additional materials that are not included systematically in the care of a patient diagnosed with preterm premature rupture of membranes in the hospitals of study. This materials are:

- **Amniocentesis.** The material has a cost of 269€. This procedure will be performed to more patients that the ones included in the study as it is a test necessary to know if the patient can participate in the study.
- **Real-time PCR-multiplex (for 7 pathogens)**. It needs sterile material, reactants to do DNA extraction and PCR process per see and a thermal cycler. It costs 142€ assuming that the cost of personnel and transportation from the hospital to the laboratories will not add any additional cost. This test will be performed to more patients that the ones included in the study as it is a test necessary to know if the patient can participate in the study.
- **Azithromycin.** The amount needed is 500 milligrams for intravenous administration every 24 hours for 2 days and 250 milligrams for oral administration for 5 days. The price will be 23 € / day the first 2 days and 6€ / day for the following 5 days. This therapy will be administered to 96 patients. We will not take into account the price of therapy A because it is the standard treatment that patients receive and the National Health System will assume the costs.

Fees. There is no fee in this clinical trial.

Insurance. For the possible realization of this clinical trial, there is the need contracting an additional lability insurance to protect the research team from the risks of liabilities imposed by lawsuits and similar claims. The estimation of this cost is 40.000€.

Coordination. An external supervisor company will be hired to ensure the correct development of the different stages of this clinical trial. This figure is indispensable due to the multi-centric character of the study. A Contract research organization also known as CRO will be contracted.

Considering that this study is a multi-centric clinical trial, we have to take into account extra transportation expenses, accommodation and meetings facilities in which the explanations

Publication and result's dissemination. Once the study is finished, its results will be disseminated to the scientific community. For the publication of the protocol and other scientific papers, there will be budgeted part of the monetary resources. We also expect some expenses for the assistance of two people to the reference congress, national and international. The total expenses are estimated to be 5.000 €.

In the "Table 6: Summary of budget" it can be found a summary of the budget.

Table 6: Summary of budget

	Description	Quantity	Cost	Subtotal
STAFF	Statistician	60 hours	35 € / hour	2.100 €
MATERIAL	Amniocentesis	385	269 €	103.565 €
	Real-time PCR Multiplex	385	142 €	54.670 €
	Azithromycin	7 days	76 € / patient	7.300 €
INSURANCE	Lability insurance	1	40.000 €	40.000 €
COORDINATION	CRO	1	15.000 €	15.000 €
PUBLICATION and DISSEMINATION	Protocol and results	1	2.500 €	2.500 €
	International congress	1	1.500 €	1.500 €
	National congress	1	1.000 €	1.000 €
			Total:	**228.100 €**

12. Feasibility

The hospitals that will be involved in this trial have been chosen because they are third level hospitals in Catalonia, therefore they are equipped with competent medical staff and adequate technological resources sufficient to accomplish the objectives of the trial, especially those related to the performance of amniocentesis and amniotic fluid analysis.

In addition, they are provincial reference for complicated pathologies such as preterm PROM, therefore we expect to have enough patients to recruit (we need a total of 192) in a period of 2 years taking into consideration the number of patients seen each year.

A Clinical Research Organization will be hired to coordinate and control data quality, due to the fact that this multi-centric study comprises many hospitals and there must be an extra effort or reinforcement on these aspects to avoid rectifiable errors than can reduce easily the value of the study. We will hire a statistician, as well, to process the statistical analysis implicated.

The chronogram of the study will be elaborated in consensus with all the implicated parts from the four hospitals in this trial and they will take into consideration any punctual matter that can affect the development of the different phases of the study.

Finally, we expect that the budget will not suppose a barrier to the development of this clinical trial as the research team is putting a lot of effort in funding this project by public money, research centres or voluntary groups such as patients associations so it stays independent and there is no conflict of interests with the sponsors.

13. Clinical and health care impact

The prevalence of genital mycoplasma colonization of genital tract is widely extended in many communities around the world and studies show that it is higher in pregnant women. It is a known pathogen involved in premature rupture of membranes, thanks to its characteristic properties such as a trigger of inflammation and consequent weakness of the membrane. In this context, the establishment of a more targeted antimicrobial therapy against this microorganisms, when it is identified as the only pathogen in amniotic fluid, will decrease the amount and duration of broad spectrum antibiotic therapies.

In consequence, this management will not contribute to the increase of bacterial resistances as a result of the use of too many antimicrobial agents especially when they are not indispensable because the clinical infection is not stablished. Moreover, the iatrogenic harm to the mother caused by the adverse effects will decrease as well as the cost of unnecessary antimicrobial therapy which will be beneficial to the National health system.

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14. Annexes

14.1 ANNEXE 1: Diagnosis criteria for clinical chorioamnionitis adapted from Gibbs

1. Fever Temperature >38ºC
2. Maternal tachycardia > 100/min
3. Foetal tachycardia >160/min
4. Fundal tenderness tenderness on palpation
5. Vaginal discharge foul-smelling discharge
6. Elevated maternal white blood cell count (>15,000 cells/m^3)

Typically, the presence of fever >38ºC is required in addition to two other signs. In the absence of other etiologies the combination of 3 clinical criteria provides a highly accurate diagnosis of chorioamnionitis (13).

14.2 ANNEXE 2: Information document

Título del estudio: *Azithromycin for genital mycoplasma intraamniotic infection in pregnant women with preterm premature rupture of membranes*

Investigador/a pricipal:_____

Centro:_____

Nos dirigimos a usted para informarle sobre un estudio de investigación en el que se le invita a participar. El estudio ha sido aprobado por un Comité de Ética de la Investigación con medicamentos y por la Agencia Española de Medicamentos y Productos Sanitarios, de acuerdo a la legislación vigente, el Real Decreto 1090/2015 de 4 de diciembre y el Reglamento Europeo 536/2014 de 16 de abril, por los que se regulan los ensayos clínicos con medicamentos.

Nuestra intención es que usted reciba la información correcta y suficiente para que pueda **decidir si acepta o no participar en este estudio**. Para ello lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir. Además, puede consultar con las personas que considere oportuno.

Participación voluntaria

Le invitamos a participar en el estudio porque ha sido diagnosticada de rutura prematura de membranas pretérmino. Con relación a esta condición médica, usted necesitará de la administración de antibiótico para reducir su riesgo de presentar una infección de las membranas y el líquido amniótico.

Debe saber que su **participación en este estudio es voluntaria** y que puede decidir NO participar. Si decide participar, puede cambiar su decisión y retirar el consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni se produzca perjuicio alguno en su atención sanitaria.

Objetivo del estudio

El objetivo de este estudio es comprobar que una pauta más simple de antibiótico es igual de efectiva que una pauta formada por 3 antibióticos, en pacientes con rotura prematura de membranas con infección pero sin clínica del líquido amniótico, únicamente por mycoplasmas genitales y ninguna otra infección activa ni cultivo positivo.

Descripción del estudio

En primer lugar, para saber si tiene usted una infección intraamniótica por mycoplasma genital, necesitaremos realizar una amniocentesis diagnóstica. Esta prueba consiste en la obtención de líquido amniótico a través de una punción con una aguja fina que se introduce a través de la pared abdominal y del útero. A la vez que la punción se realiza una ecografía para guiar correctamente la dirección de la aguja y extraer el líquido del sitio adecuado. Esta prueba se realizará al ingreso.

Para esta autorización, deberá firmar usted el consentimiento informado para la realización de esta prueba que su médico le proporcionará.

Este estudio inluirá un total de 192 mujeres embarazadas con rotura prematura de membranas antes de las 34 semanas y 6 días de gestación que acudan a alguno de los 4 hospitales provinciales de la Comunidad Autónoma de Cataluña. Esta investigación separará a las mujeres al azar en dos grupos de 96 pacientes. El primer grupo recibirá 1 gramo de ampicilina intravenosa cada 6 horas, 240 miligramos de gentamicina intravenosa cada 24 horas y 1 gramo de azitromicina oral. Este tratamiento durará 7 días o 24 horas tras el parto. El segundo grupo recibirá 500 miligramos de azitromicina intravenosa cada 24 horas durante 7 días.

Actividades del estudio

Este estudio tiene una duración variable ya que una vez se ha entrado en el estudio, el seguimiento continuará hasta el parto. La fase de tratamiento tendrá lugar en el hospital ya que el ingreso a causa de la ruptura de membranas será hasta el parto. Una vez este ocurra, su participación en el estudio habrá concluido y el seguimiento se desarrollará según el protocolo de cada hospital, el cual será explicado por el facultativo a su cargo.

Además de la amniocentesis que se realizará antes del inicio del estudioen sí, la única intervención adicional que difiere del cuidado convencional que reciben todos los pacientes con su condición será que se le realizará será una entrevista orientada a cumplimentar cuestionario el cual recoge datos importantes para el estudio.

En la siguiente tabla encontrará las interveciones rutinarias que reciben tdas las pacientes con rotura prematura de membranas que desean ser tratadas:

Intervención	**Frecuencia**	**Observaciones**
Exploración física	Al ingreso y diariamente	
Cardiotocografía	Al ingreso y diariamente	
Analítica de sangre	Al ingreso, diariamente los 3 primeros días y posteriomente cada 2 días	
Obtención de muestras para cutivos	Al ingreso	Perianales, vaginales y endocervicales
Análsis de orina	Al ingreso	
Heparina de bajo peso molecular	Diariamente durante todo el ingreso	
Corticoides intramusculares		

Los fármacos incluidos en este estudio son ampliamente usados en la práctica clínica y están autorizados pors la Agencia Española del Medicamento y Productos Sanitarios,a demás de comercializados en toda España. Sin embargo, dentro de sus usos previstos en las fichas técnicas,

no se encuentra el tratamiento en embarazadas con rotura prematura de membrana. Es por eso que este estudio puede contribuir a su inclusión.

Es posible que derivado de los antibióticos sienta dolor de cabeza, vómitos, náuseas, debilidad muscular, molestias gastrointestinales, alteraciones leves de la piel, entre otras. Al ser fármacos aprobados por las autoridades sanitarias competentes, existe información al acceso de todo el mundo sobre los efectos secundarios. Por favor, hable con el médico de su estudio para obtener una lista completa de los efectos secundarios comunicados con este fármaco y en cualquier caso se le entregará el prospecto del fármaco.

Además, puede haber posibles riesgos o acontecimientos desconocidos en este momento y que no se puede descartar que ocurran.

Las responsabilidades de las participantes con respecto al estudio son las siguientes:

- Cumplimiento con actividades del estudio

- Notificar cualquier evento adverso que le suceda o cambios en medicación, de manera que no debe modificarse la medicación que está tomando ni tomar otros medicamentos o “plantas medicinales” sin consultar antes con el médico del estudio.

Tratamientos alternativos

En el caso de no participar en el estudio, los mejores cuidados le serán proporcionados durante su estancia en el hospital. Le será administrada la pauta convencional de antibioterapia que se usa en el hospital al cual usted ha acudido, pudiendo ser en alguno de los casos el mismo que podría haber recibido en su participación en el estudio ya que se trata de un ensato clínico en fase IV. El médico investigador responsable del hospital en el cual usted se encuentra le dará más información si así lo desea.

Seguro

El grupo de investigación del estudio dispone de una póliza de seguros que se ajusta a la legislación vigente (Real decreto 1090/2015) y que le proporcionará la compensación e indemnización en caso de menoscabo de su salud o de lesiones que pudieran producirse en relación con su participación en el estudio, siempre que no sean consecuencia de la propia enfermedad que se estudia o de la evolución propia de su enfermedad como consecuencia de la ineficacia del tratamiento. Si desea más información relativa a este apartado, consulte con el investigador principal del estudio en su centro.

Le informamos que es posible que su participación en este ensayo clínico pueda modificar las condiciones generales y particulares (cobertura) de sus pólizas de seguros (vida, salud, accidente...). Por ello, le recomendamos que se ponga en contacto con su aseguradora para determinar si la participación en este estudio afectará a su actual póliza de seguros.

Protección de datos personales

El promotor se compromete al cumplimiento de la Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales. Los datos recogidos para el estudio estarán identificados mediante un código, de manera que no incluya información que pueda identificarle, y sólo su médico del estudio/colaboradores podrá relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a persona alguna salvo excepciones en caso de urgencia médica o requerimiento legal. El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los participantes se ajustarán a lo dispuesto en esta ley. Los datos se recogerán en un fichero de investigación responsabilidad de la institución y se tratarán en el marco de su participación en este estudio.

El promotor adoptará las medidas pertinentes para garantizar la protección de su privacidad y no permitirá que sus datos se crucen con otras bases de datos que pudieran permitir su identificación.

Si usted decide retirar el consentimiento para participar en este estudio, ningún dato nuevo será añadido a la base de datos, pero sí se utilizarán los que ya se hayan recogido.

Los datos codificados pueden ser transmitidos a terceros y a otros países pero en ningún caso contendrán información que le pueda identificar directamente, como nombre y apellidos, iniciales, dirección, nº de la seguridad social, etc. En el caso de que se produzca esta cesión, será para los mismos fines del estudio descrito o para su uso en publicaciones científicas pero siempre manteniendo la confidencialidad de los mismos de acuerdo a la legislación vigente.

Gastos y compensación económica

El promotor del estudio es el responsable de gestionar la financiación del mismo. Para la realización del estudio el promotor del mismo ha firmado un contrato con el médico del estudio y centro donde se va a realizar.

Usted no tendrá que pagar por los medicamentos ni por pruebas específicas del estudio. Su participación en el estudio no le supondrá ningún gasto adicional a la práctica clínica habitual y le serán reintegrados los gastos extraordinarios (p. ejem. comidas y traslados) que la participación en el mismo le generen.

Contacto en caso de dudas

Si durante su participación tiene alguna duda o necesita obtener más información, póngase en contacto con (espacio para poner los datos del médico del estudio, incluyendo nombre, servicio, forma de localizarle, teléfono de contacto del hospital correspondiente).

Muchas gracias por su atención,

*(This **Information Document** will be also be available in Catalan, the other official language of Catalonia)*

14.3 ANNEXE 3: Informed consent

Yo, _____, con DNI/NIE _____

☐ He leído la hoja de información que se me ha entregado sobre el estudio.

☐ He podido hacer preguntas sobre el estudio.

☐ He recibido suficiente información sobre el estudio..

☐ He hablado con Dr/Dra _____

☐ Comprendo que mi participación es voluntaria.

☐ Comprendo que puedo retirarme del estudio:

- Cuando quiera.
- Sin tener que dar explicaciones.
- Sin que esto repercuta en mis cuidados médicos.

Recibiré una copia firmada y fechada de este documento de consentimiento informado.

Presto libremente mi conformidad para participar en el estudio.

Firma de la paciente Firma del investigador/a

Lugar y fecha: _____, ___ de _____ del 20___

- -

Revocación del consentimiento informado

Yo, _____, con DNI/NIE _____ revoco el consentimiento previamente firmado para la participación en el ensayo clínico especificado arriba.

Firma de la paciente Firma del investigador/a

Lugar y fecha: _____, ___ de _____ del 20___

*(This **Informed Consent** will be also be available in Catalan, the other official language of Catalonia)*

14.4 ANNEXE 4: Data collection sheet

Date of admission: _____

Hospital of admission: _____

Physician in charge of the patient: _____

Gestational age (weeks + days): _____

- Maternal information:

Maternal age	(years)
Maternal weight before pregnancy	(kilograms)
Maternal height before pregnancy	(metres)
Parity	(nulliparous / multiparous)
Ethnicity [1]	
Social Class [2]	
Sexual risk behaviour [3]	(yes / no)

- Preterm PROM information:

Intervention therapy	(A / B)
Time between diagnosis of preterm PROM and antibiotic initiation	(hours)
Latency period	(days)
Perinatal mortality	(yes / no)
Chorioamnionitis	(yes / no)

Validation of the data:

Date of validation:

[1] Maternal ethnicity: Caucasian, African, Latin-American, Asian, Other.

[2] Social Class: Questions - CSO-SEE12 (Valid in Spanish)

a). ¿Cuál es la ocupación que desempe˜na en la actualidad o la última que ha desempe˜nado?

b) ¿Cuál es su situación laboral actual, o en la última ocupación que ha desempe˜nado?

Trabajador/a por cuenta ajena	Trabajador/a por cuenta propia, autónomo/a
Empresario/a / empleador/a de 10 o más asalariados/as	Empresario/a / empleador/a de <10 asalariados/as
Gerente de empresa de 10 o más asalariados/as	Gerente de empresa de menos de 10 asalariados/as

[3] Sexual risk behaviour: defined as unprotected sexual relations and frequent changes in couples