TORCH INFECTIONS IN PREGNANCY

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INTRODUCTION:

TORCH stands for a group of infections that can cause serious congenital defects or severe fetal conditions when acquired during pregnancy, especially before 20 weeks. TORCH includes: (T) toxoplasmosis, (O) other agents, the most common being chickenpox, syphilis, parvovirus B19 and, recently zika virus (R) rubella, (C) cytomegalovirus, (H) herpes simplex virus. Although many of these infections produce similar fetal abnormalities and can present with similar ultrasound abnormalities (see Appendix 1: "Maternal Serological Study in Case of Ultrasound Markers Suggestive of Fetal Infection"), each one results in a specific fetal pathology.

1. TOXOPLASMOSIS:

1.1. Pathophysiology, maternal clinical presentation, and epidemiology:

Toxoplasma gondii is a protozoan parasite. Infection is primarily acquired by ingestion of raw or undercooked meat or meat products containing cysts or tachyzoites. Water, soil, or contaminated vegetables are the second source of infection. Contact with domestic cats is considered a low-risk factor. The incubation period is variable, between 5 and 23 days.

Toxoplasmosis is typically an asymptomatic infection in the immunocompetent adult. The seroprevalence of women of childbearing age is highly variable, ranging from 15% to 77%. In recent years, seroprevalence has decreased in our setting and is currently 20%. The incidence of congenital infection is unknown in our setting, but in countries such as France it currently represents 0.3/1000 births.

There are different strains of *Toxoplasma gondii*, and genotype II is the most common in Europe. Genotype I and other atypical genotypes are the most common in South America, and they produce more severe forms of the disease, as well as more severe eye sequelae in congenital infections. Infection with *Toxoplasma gondii* confers long-lasting immunity in immunocompetent patients, but isolated cases of reinfection with different genotypes have been described in the context of travelling to other continents.

1.2. Congenital toxoplasmosis:

Vertical transmission of the parasite occurs during acute infection. The risk of transmission increases significantly as pregnancy progresses: 5% < 12 weeks, 15% 12 to 16 weeks, 25% 17 to 23 weeks, and 60% after 24 weeks, but fetal involvement has an inverse evolution: < 16 weeks: 60%; 17-23 weeks: 25% and >24 weeks: 15%. Ocular disease is the most common, but *Toxoplasma gondii* can occasionally cause serious neurological impairment after maternal infection in the first trimester.

Gestational age	Vertical Transmission	Fetal infection	Clinical Manifestations
< 14 weeks	< 10%	60%	Ocular and intracranial lesions Can be severe
14-28 weeks	15-55%	25%	Mainly ocular In general, they are not serious
> 28 weeks	55-80%	15%	Ocular lesions Exceptional intracranial involvement

There is no risk when infection occurs during the preconception period.

1.2.1. Symptomatic newborns: 15% of infected foetuses are symptomatic at birth, mainly from infections acquired before 24 weeks, except for ocular disease, which can also occur in infections acquired during the third trimester**.**

The Sabin's triad (the classic presentation of congenital toxoplasmosis) includes: hydrocephalus, intracranial calcifications, chorioretinitis, and seizures, but it is very rare. Only 4% of symptomatic newborns will have permanent neurological sequelae, death, or bilateral blindness.

Less specific signs may include: rash, jaundice, hepatosplenomegaly, anaemia, thrombocytopenia, cardiomegaly, and microphthalmia. Clinical signs at birth do not necessarily imply functional deterioration (e.g. intracranial calcifications).

1.2.2. Asymptomatic newborns: 85% of newborns with congenital infection are asymptomatic at birth, but a significant proportion of these (20-30%) may have long-term ocular involvement, especially chorioretinitis.

1.3. Maternal infection diagnosis: serology

After infection, the immune response starts with IgM, which appears during the first week, and from 8 weeks onwards, it starts to decrease until it disappears after a variable amount of time (which can even last months or years). IgG appears during the second week of infection, and its levels rise for 6-8 weeks before decreasing and persisting for life (long-lasting immunity).

Routine universal screening is not recommended for pregnant women at low risk in the majority of Western European countries. Nevertheless, if any ultrasound findings suggest fetal infection with Toxoplasma (see point 1.5), or if a patient undergoes a serological determination, the interpretation will be as follows:

IgG positive with IgM negative: it suggests that the patient has immunity and probable pregestational infection. However, if the reason for testing for these antibodies is due to the presence of abnormalities in the ultrasound, the absence of IgM does not rule out infection at the beginning of pregnancy.

IgG positive with IgM positive: can indicate a recent infection but a positive IgM is not always accurate, and the timing of the infection cannot be determined with certainty, as it can persist for more than a year. In addition, the diagnosis of IgM can be a false positive and should be confirmed with a second sample, using a different kit if possible, and adding the determination of IgG avidity. It is advisable to wait for the result of the IgG avidity assay and to start maternal treatment only if infection during pregnancy is suspected.

1.3.1. IgG avidity is the parameter that is most closely correlated with the start of maternal infection. It requires precise testing methods, and the interpretation of the results can vary depending on the laboratory that is conducting the test. Interpretation with the bioMerieux® kit:

- Low IgG avidity (< 20%): cannot exclude a recent maternal infection (< 12 weeks of evolution)
- Intermediate avidity (20-30%): probable infection > 12 weeks
- High avidity (> 30%): confirms an infection > 20 weeks
- Very high avidity (> 45%) probable infection > 40 weeks.

1.3.2. The determination of IgA does not add any advantage over IgM and is no longer used.

1.3.3. **Seroconversion during pregnancy**: if seroconversion is diagnosed in the context of serial gestational screening, it confirms maternal infection and treatment should be initiated immediately (see treatment guidelines at the end of the Guide).

1.4. Diagnosis of fetal infection:

If maternal infection is suspected at any stage of pregnancy, amniocentesis will be indicated for the amplification of *Toxoplasma gondii* DNA in amniotic fluid (AF). This is the procedure of choice. It is important to remember that the study of toxoplasma in AF has a higher sensitivity for the diagnosis of congenital infection than the postnatal study (serology or polymerase chain reaction (PCR) in the different neonatal fluids) and therefore will provide very relevant information for neonatologists. With real-time PCR of the Re-sequence gene (which we use in our centre), a sensitivity of 90-92% and a specificity of nearly 100% is obtained, but each centre must know the PCR it uses, because older conventional techniques have lower sensitivity. To obtain the maximum sensitivity of the technique, It is also essential to adhere to two conditions at all times:

- Perform the amniocentesis **at least 4 weeks after** the start of maternal infection
- **Do not perform** the amniocentesis **before 18 weeks** of pregnancy.

Analysis of the result:

- **Toxoplasma DNA not detectable:** With current diagnostic techniques, the negative predictive value of AF is very high, especially after a first-trimester maternal infection due to the low risk of transmission (the negative predictive value is considered 99% after an infection in the first trimester, but slightly lower in third trimester infections (84%). This result can help to reassure the patient and the usual follow-up care for the pregnancy will be indicated, with the addition of a supplementary ultrasound in the third trimester. Paediatric follow-up is also recommended until congenital infection is ruled out.
- **Toxoplasma DNA positive:** indicates that the foetus is infected. The patient will be informed that an infected foetus is not synonymous with a symptomatic foetus or newborn, and that the risk of serious impairment (neurological sequelae or bilateral blindness) is low, even in first-trimester maternal infections. Ultrasound follow-up will be scheduled every 2-3 weeks and monthly neurosonography. Due to the type of brain lesions caused by toxoplasma, intracranial MRI provides little additional information, but it can be performed in the third trimester, especially in cases with visible intracranial abnormalities on ultrasound, to confirm or complete the study of lesions.

1.5. Ultrasound findings:

Ultrasound can detect most severe abnormalities (except retinal abnormalities), but certain markers of impairment may not be visible until later in the pregnancy. In cases where there is severe damage to the retina, there may also be associated brain abnormalities.
The most characteristic ultrasound findings are:

- **Ventriculomegaly**: generally poor prognosis, especially if it is severe (> 15 mm), hydrocephalus.
- **Hyper-refringent intraparenchymal foci or nodules (cerebral calcifications).** When they appear in isolation, the prognosis is uncertain. In general, they are not associated with neurological sequelae, but they increase the risk of chorioretinitis.

Cases of:

- Porencephaly (due to periventricular parenchymal destruction), microcephaly, ascites, hydrops, hepatomegaly, splenomegaly, intrahepatic calcifications, and thickened placenta have been described.

There is no evidence of association with fetal growth restriction.

1.6. Maternal treatment:

The therapeutic recommendations are as follows:

- Whenever there is a well-founded serological suspicion of maternal infection during pregnancy, antibiotic treatment should be started as soon as possible. Scientific evidence shows that the effectiveness of treatment depends on the timing of its initiation, with a significant decrease in fetal transmission especially when it is initiated within the first 3 weeks of maternal infection. Treatment should be administered until the amniocentesis result is available. If the AF PCR result is negative and at least 4 weeks of treatment have been completed, the treatment could be suspended, except in cases of documented seroconversion during pregnancy in which treatment is indicated until delivery to prevent late vertical transmission, as well as in patients who refuse amniocentesis. Therapeutic options are:
- **Spiramycin 1 g/8 h PO** (Rovamycine® 2 tablets/8 h, preferably on an empty stomach). It is the most frequently used treatment. It has a mild parasiticide effect and accumulates in the placenta, although it has little transplacental passage. It is well tolerated, with no adverse fetal effects in any trimester.
- **Contraindications**: allergy (rare) and long QT Syndrome. Better to avoid in patients with G6PD deficiency.
  It is the treatment of choice in the first trimester and in all cases of maternal infection suspected by serology, but without absolute certainty (unconfirmed gestational seroconversion).
- **Pyrimethamine 50 mg/24 h orally** (Daraprim® 2 tablets/24 h) **+ sulfadiazine 1.5 g/12 h orally** on an empty stomach (Sulfadiazine Reig Jofre® 500 mg, 3 tablets/12 h) **+ folinic acid 7.5 mg/day** (Folaxin® 7.5 mg/24 h). **Pyrimethamine is contraindicated if < 14 weeks of gestation**. They are synergistic antibiotics, inhibitors of folic acid synthesis, with greater toxicity but good parasiticide activity. They cross the placental barrier. A randomised study *(Mandelbrot et al., Am J Obstet Gynecol 2018)* that compared it with Spiramycin suggested (could not be confirmed due to the sample size) that it decreased

vertical transmission and showed that it significantly reduced the sequelae of newborns who became infected.

Side effects: **Pyrimethamine**: bone marrow suppression (5%). **Sulfadiazine:** skin reactions (Lyell's syndrome and Stevens-Johnson syndrome), crystalluria. Avoid in G6PD deficiency. Treatment with sulfadiazine + pyrimethamine requires folinic acid supplementation, blood cell count at the beginning and every 1-2 weeks, good hydration (2 L/24 h) and urinary alkalinisation (citrus fruits intake). Once the treatment is finished, folinic acid should be continued for one more week. If leukopenia < 1500/mm^3, stop treatment, keep folinic acid and repeat the blood cell count after 15 days to evaluate restarting or switching to spiramycin.

This antibiotic regimen can be offered in case of confirmed maternal infection (documented seroconversion) from 14 weeks and especially in third trimester infections after 32 weeks due to a high risk of fetal infection (60-70%) with no risk of neurological impairment but with risk of ocular disease.

Maintain until the result of the amniocentesis and in case of negative amniocentesis, switch to spiramycin except in late maternal infections (> 32 weeks) in which, in the absence of intolerance, pyrimethamine + sulfadiazine + folinic acid can be maintained until delivery.

- **In case of allergy** or shortage of spiramycin**, azithromycin* 500 mg/24-48 h PO** (taking into account the drug's half-life, a dosage every 48 h probably has the same efficacy and lower toxicity) or **co-trimoxazole 160/800 mg/12 h orally** + folinic acid 7.5 mg/24 h** could be used.

**A baseline ECG will be recommended in those cases of prolonged treatment with azithromycin, repeating it after 7-10 days, since cases of QTc prolongation, especially in patients with multiple comorbidities, have been described.*

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1.6.1. If fetal infection is confirmed (positive PCR-toxoplasma in amniotic fluid), the treatment of choice is:

- **Pyrimethamine 50 mg/24 h PO** (Daraprim® 2 tablets/24 h, contraindicated if < 14 weeks) **+ sulfadiazine 1.5 g/12 h orally** on an empty stomach (Sulfadiazine Reig Jofre® 500 mg, 3 tablets/12 h) **+ folinic acid 7.5 mg/day (Folaxin® 7.5 mg/24 h) until delivery.** Contraindicated if allergy to sulphonamides or G6PDH deficiency**.** During treatment, keep good hydration (2 L/24 h), urinary alkalinisation (citrus fruits intake) and request a full blood cell count every 1-2 weeks due to the risk of bone marrow aplasia.

Once the treatment is finished, folinic acid should be continued for one more week. In case of leukopenia < 1500/mm^3, stop treatment, maintain folinic acid and repeat the full blood cells count after 15 days to evaluate restarting treatment or switching to an alternative regimen after expert consultation.

Alternative regimen in case of infected foetus and drug allergy:

- If allergy to sulphonamides: pyrimethamine 50 mg/24 h orally + folinic acid 7.5 mg/24 h + azithromycin* 500 mg/24-48 h. Perform a blood cell count at the start and every 2 weeks.
- If allergic to pyrimethamine: co-trimoxazole 160/800 mg/12 h orally + folinic acid 7.5 mg/24 h. This regimen has a lower parasiticidal effect.

** A baseline ECG will be recommended in those cases of prolonged treatment with azithromycin, repeating it after 7-10 days, since cases of QTc prolongation, especially in patients with multiple comorbidities, have been described.*

1.7. Primary prevention:

Seronegative pregnant women should be informed of the hygiene measures that help reduce the risk of exposure and infection during pregnancy. This includes:

- Cook food to safe temperatures (> 70-80ºC). Freezing at low temperatures (< -18º) for 48 hours destroys the cysts. Deli meats and cured meats may also contain the parasite.
- Peel or wash fruits and vegetables thoroughly before eating.
- Wash cooking utensils and surfaces where food has been prepared.
- Wash hands with hot water and soap before and after handling food.
- Use gloves for gardening and soil handling tasks.
- If you have a cat at home: avoid changing the cat litter if possible, do not feed it raw meat, and keep it away from the street. Keep cats indoors to prevent them from hunting and to thus reduce the chances they will become infected with Toxoplasma.

1.8. Newborn follow-up:

In the case of demonstrated intrauterine transmission (positive toxoplasma DNA in amniotic fluid), neonatologists will admit the newborn for study and treatment. In the case of unconfirmed transmission (negative toxoplasma DNA in amniotic fluid), it should also be recorded in the paediatric history, and neonatologists will refer the child to the specific Paediatric Infections Dispensary of the Reference Centre for clinical and serological follow-up until 12 months of age.

Breastfeeding is not contraindicated.

2. RUBELLA:

2.1. Description of the pathogen, maternal clinical presentation, and epidemiology:

Rubella is a respiratory tract infection caused by the rubella virus, an RNA virus in the *Togaviridae* family. It has an incubation period of about 14 days (12-23 days). It causes a mild macular rash that initially appears on the face and then spreads to the trunk and limbs; characteristic lymphadenopathy (suboccipital, postauricular, and cervical), which usually persists after the rash and, occasionally, arthritis, especially in adult women. In 50% of cases, rubella is an asymptomatic infection. Viremia and the infectivity period begin 7 days before the rash appears and persist for 7-10 days after its onset. Viremia is transient and is detectable during the week before the rash.
With the current vaccination programme in our area (2 doses of triple viral vaccine), immunity is lifelong in more than 95% of cases. This is why the transmission of infection has been virtually eradicated, with cases of congenital infection described exceptionally and are generally imported from countries without systematic vaccination.
Reinfection with the rubella virus is extremely rare but has been described after primary infection or vaccination with confirmed immunity. It tends to be asymptomatic or clinically very mild. The risk of congenital defects, if it occurs during pregnancy, is very low (<5%).

2.2. Congenital rubella:

Congenital rubella syndrome (CRS) is a chronic infection that causes significant fetal impairment and serious sequelae that may appear later. Transmission and fetal involvement depend on the stage of pregnancy.

2.2.1. **Risk of transmission**: < 12 weeks: 90%; 12-17 weeks: 55%; 18-24 weeks: 25% with a new increase in the risk of transmission (> 60%) after 36 weeks

There is no risk of vertical transmission during the preconception period. There are no reported cases of intrauterine infection in mothers with rash onset before the first day of the last menstrual period (LMP) or during the following 11 days. It is important to remember that the onset of the rash coincides with the end of viremia and the end of fetal transmission.

2.2.2. Risk of CRS in infected foetuses based on gestational age:

- <12 weeks: there is a high risk (80-90%) of cardiovascular defects, ocular disease, central nervous system abnormalities, deafness, and psychomotor retardation (delays in physical and mental development).

Risk of fetal impairment in maternal infections during the 1st trimester without knowing fetal transmission: 85%,

- 12-16 weeks : 30-35% unilateral or bilateral deafness and occasionally retinopathy and microcephaly

Risk of fetal impairment in maternal infections of 12-16 weeks without knowing fetal transmission: 15%

- 16-20 weeks: minimum risk of deafness
- > 20 weeks: no fetal risk has been described

Gestational Age	Vertical Transmission	CRS	Risk with Unknown transmission	Tipo de disorder
< 12 weeks	90%	80-90%	85%	Cardiovascular defects (mainly < 8 weeks) Ocular defects, deafness, psychomotor retardation Miscarriage 20%.
12-16 weeks	55%	30-35%	15%	Unilateral or bilateral deafness Retinopathy/microcephaly (occasionally)
16-20 weeks	25%	0%		Deafness (minimum risk)

2.3. Rubella screening during pregnancy:

Systematic screening should be performed: Rubella IgG for all pregnant women in the 1st trimester.
If the titres are protective (≥ 10 IU/ml), determination of IgM is not indicated.
A positive IgM in an asymptomatic pregnant woman has a very low positive predictive value in countries like ours where the incidence of the disease is almost non-existent and significant interpretation problems are involved. It is necessary to rule out a cross-immune reaction with other viral infections (Epstein-Barr, CMV, measles, Parvovirus B19) or with a positive rheumatoid factor. Serial determination of IgM with stable titres and high IgG avidity can help rule out acute infection. In seronegative or non-protective titres (IgG titres < 10 IU/ml) pregnant women and in the absence of compatible clinical presentation, it is not necessary to repeat serology during pregnancy, but vaccination must be indicated in the postpartum period before being discharged home. In fully vaccinated (≥ 2 doses) pregnant women who do not generate IgG titres, there also seems to be protection against primary infection.

2.4 Maternal infection diagnosis:

It will be based on maternasymptomatology but especially on serology and pharyngeal exudate samples for viral RNA detection (PCR).

In the case of non-vesicular maternal rash, rubella serology (IgG and IgM) must be requested.

- **Specific IgM:** it is detected 3-6 days after the onset of the rash and lasts up to 8 weeks. Occasionally, it can persist for longer (e.g. post-vaccination)
- **Specific IgG:** it appears 7-9 days after the onset of the rash and lasts a lifetime. The avidity of IgG after primary infection goes from a low index to a high index in 2-3 months. After vaccination, the maturation of IgG is slower (IgG avidity assay is only carried out in a few reference centres)
- **Pharyngeal exudate sample:** use a nylon swab without transport medium (sterile urine culture jar) for viral RNA detection (PCR). The samples must be kept in the fridge for transport (do not freeze). The rubella virus can be detected in the pharynx from one week before to 2 weeks after rash onset, but maximum sensitivity is obtained during the first 4 days after the onset of the rash.

2.5 Fetal infection diagnosis:

Maternal rubella confirmed during the first 12 weeks of pregnancy: due to the very high risk of serious fetal impairment and sequelae, the patient will be informed, and termination of pregnancy will be considered without the need for vertical transmission confirmation.

Indications for amniocentesis for detection of viral RNA (PCR) in AF:

- Maternal primary infection between 12 and 20 weeks
- Uncertain maternal infection before 20 weeks (inconclusive maternal serological study)
- Documented maternal re-infection < 20 weeks (low risk of transmission and congenital impairment)
- Ultrasound markers of rubella infection

There is little experience with PCR techniques on amniotic fluid (AF) in congenital rubella infection, but it is estimated to have a sensitivity > 90% and a specificity of 100%.

To obtain good sensitivity in AF, the following is necessary:

• An interval between maternal infection and amniocentesis > 6 weeks (preferably > 8 weeks)

• Never perform amniocentesis before 18 weeks (preferably starting at 21 weeks)

• With negative result < 21 weeks and high suspicion of maternal infection, it is recommended to repeat the sample at 21-22 weeks.

2.5.2. Indications for cordocentesis:

In the case of clinical or serological suspicion of maternal infection and negative RNA-PCR in AF, or if this result is not available, a cordocentesis can be performed to check for fetal IgM. If positive, it allows the diagnosis of infection, but has variable sensitivity.
Cordocentesis should not be performed before 22 weeks, as the foetus rarely produces IgM below this gestational age.

2.6 Ultrasound features of CRS:

- Cardiac abnormalities: pulmonary artery stenosis and/or pulmonary valve stenosis, aortic valve stenosis, interventricular septal defects. Request an echocardiography

• Microcephaly (< -3 SD)
• Cataracts and/or microphthalmia. Ocular MRI can help with diagnosis
• Hepatomegaly (see normal values in Appendix 6)
• Splenomegaly
• IUGR (intrauterine growth restriction)
• There are cases described of hydrops secondary to cardiopathy.

In newborns of mothers with rubella during pregnancy or in cases of congenital rubella syndrome, breastfeeding is not contraindicated.

2.7 Rubella vaccination during pregnancy and puerperium:

Rubella vaccination (MMR) is a live-attenuated vaccine and therefore is contraindicated during pregnancy, and it is recommended to avoid pregnancy for 1 month after its administration. However, there are no described cases of congenital rubeola syndrome after accidental administration of the vaccine during the first trimester and hence it is not justified to terminate the pregnancy in these circumstances.

It is essential to remember to administer the vaccine to all susceptible individuals during the puerperium, preferably before discharge. Breastfeeding does not contraindicate vaccination. The patient should receive the 2nd dose in 1-2 months.

Since it is a vaccine with live-attenuated viruses, the administration of a previous transfusion (e.g. packed red blood cells, plasma,, postpartum transfusion) can decrease the effect of the vaccine and requires delaying its administration. In these cases, vaccination will be postponed until 5 months (packed red blood cells) or 7 months (plasma or platelet transfusion) after the transfusion. Previous administration of an immunoglobulin (except for anti-D) can also decrease the effects of the MMR or chickenpox vaccine.

Rubella immunity should be ensured in all women before pregnancy

3. CYTOMEGALOVIRUS:

3.1. Pathogenesis, maternal symptoms and epidemiology:

CMV is a DNA virus of the *Herpesviridae* family that establishes a state of cellular latency after primary infection and has the ability to reactivate. Reinfection with different strains is also possible. CMV is transmitted through close contact via saliva, urine, vaginal secretions, semen, through the placenta, and also through breast milk. In pregnant women, the main cause of infection is contact with children under 3 years of age, as, when they become infected, they shed the virus in saliva and urine for long periods of time. The incubation period is 3 to 4 weeks. The virus generally causes asymptomatic infection in immunocompetent adults, but occasionally can cause a flu-like illness with fever, asthenia, and arthralgia. The seroprevalence in pregnant women in our environment is approximately 65%, but it is higher in patients from developing countries. CMV is one of the most common congenital infections, with an incidence of primary maternal infection of 1-1.5% and an estimated prevalence of infected newborns between 0.6% and 0.7%. Of these, 17-20% will have immediate or long-term sequelae. CMV is one of the leading causes of hearing loss in childhood. Neonatal infections acquired at the time of the delivery (through birth canal) or through breast milk do not affect later neurological development, but there are cases described of sepsis in premature infants < 32 weeks.

3.2. Congenital CMV infection:

3.2.1 **Vertical transmission: primary maternal infection** results in global vertical transmission of 35-40%, with an increased risk at later gestational ages (see Table). Due to the persistence of viremia, fetal infection in perigestational infections is frequent, and there have been cases described of vertical transmission in maternal infections up to 12 weeks before conception (9-10 weeks before the LMP), but with lower vertical transmission (see Table).

Due to the presence of previous immunity, **non-primary maternal infections** (reactivation or reinfection) have a very low risk of fetal transmission (0.5-3%), although as reactivation-reinfection phenomena are very common in the immune population, recurrences are the main cause of congenital infection in populations with maternal seroprevalence > 50%.

3.2.2 Fetal impairment:

Globally, 10-15% of infected newborns are symptomatic at birth and 85-90% are asymptomatic. Most symptomatic newborns will have significant neurological sequelae, but, in addition, 10-15% of asymptomatic newborns may have late-onset sequelae, especially hearing loss. However, this proportion is very variable depending on the timing of infection. Due to the virus's tropism for the fetal central nervous system and its initial vulnerability, the most severe damage and neurological and hearing sequelae almost exclusively occur when the foetus becomes infected during the first

trimester of pregnancy. Currently, infection is primarily considered an embryopathy. A recent meta-analysis including nearly 3000 pregnancies with CMV infection calculated the risk of vertical transmission and fetal/neonatal injuries and long-term sequelae based on gestational age at the time of maternal infection with the results shown in the table below.

Timing of infection	Vertical transmission (VT)	Risk of fetal/neonatal symptoms if VT	Risk of long term severe sequelae (neurologic and hearing)	Risk of fetal/neonatal symptoms with unknow VT
Pregestational (up to 10-12 weeks pre LMP)	5-6%	No data	No data	No data
Perigestational (4 weeks pre-6 weeks post LMP)	21%	29%	No data but estimated > 1st trimester	6%
1st trimester	37%	19%	23%	7%
2nd trimester	40%	<1%	<1%	<1%
3rd trimester	66%	<1%	0%	<1%

Table: Risk of vertical transmission (VT) and central nervous system injury in the foetus and newborn and risk of severe neurological and hearing sequelae after primary maternal CMV infection. Source*: Chatzakis et al., AJOG 2020*

3.2.3. Clinical signs and symptoms of congenital infection:

They can occur in newborns from both primary and non-primary maternal infection.

1) **Symptomatic newborns at birth:**

- **Neurological or auditory symptoms:** Most of these cases are from infections in the first trimester or perigestational period, and present with neurological or auditory symptoms and neuroimaging abnormalities. They may also have intrauterine growth restriction (50-60%).

  Of these children, most will have sequelae:

  - Psychomotor retardation, epilepsy, hypotonia (45-90%)
  - Neurosensory hearing loss (30-65%) which may be of late onset
  - Chorioretinitis and visual deficit (15-30%)

- **Congenital cytomegalic inclusion disease,** which includes involvement of the reticuloendothelial system (thrombocytopenia, anaemia, jaundice, hepatosplenomegaly) and often prematurity, is also part of the clinical spectrum of symptomatic congenital CMV infection. It generally appears in cases of later and closer-to-delivery fetal infection and does not necessarily imply a poor long-term neurological prognosis**.**

2) **Asymptomatic newborns at birth with negative examination and normal neuroimaging:** most will be from maternal infections after 14 weeks, but also around 70% of newborns infected in the first trimester or perigestational period will be asymptomatic at birth. Among those infected in the first trimester, although neuroimaging tests (including MRI) are normal, up to 17% may have mild, late-onset hearing sequelae. Among foetuses infected after 14 weeks, the vast majority will not have any long-term sequelae.

3.3. Maternal infection diagnosis:

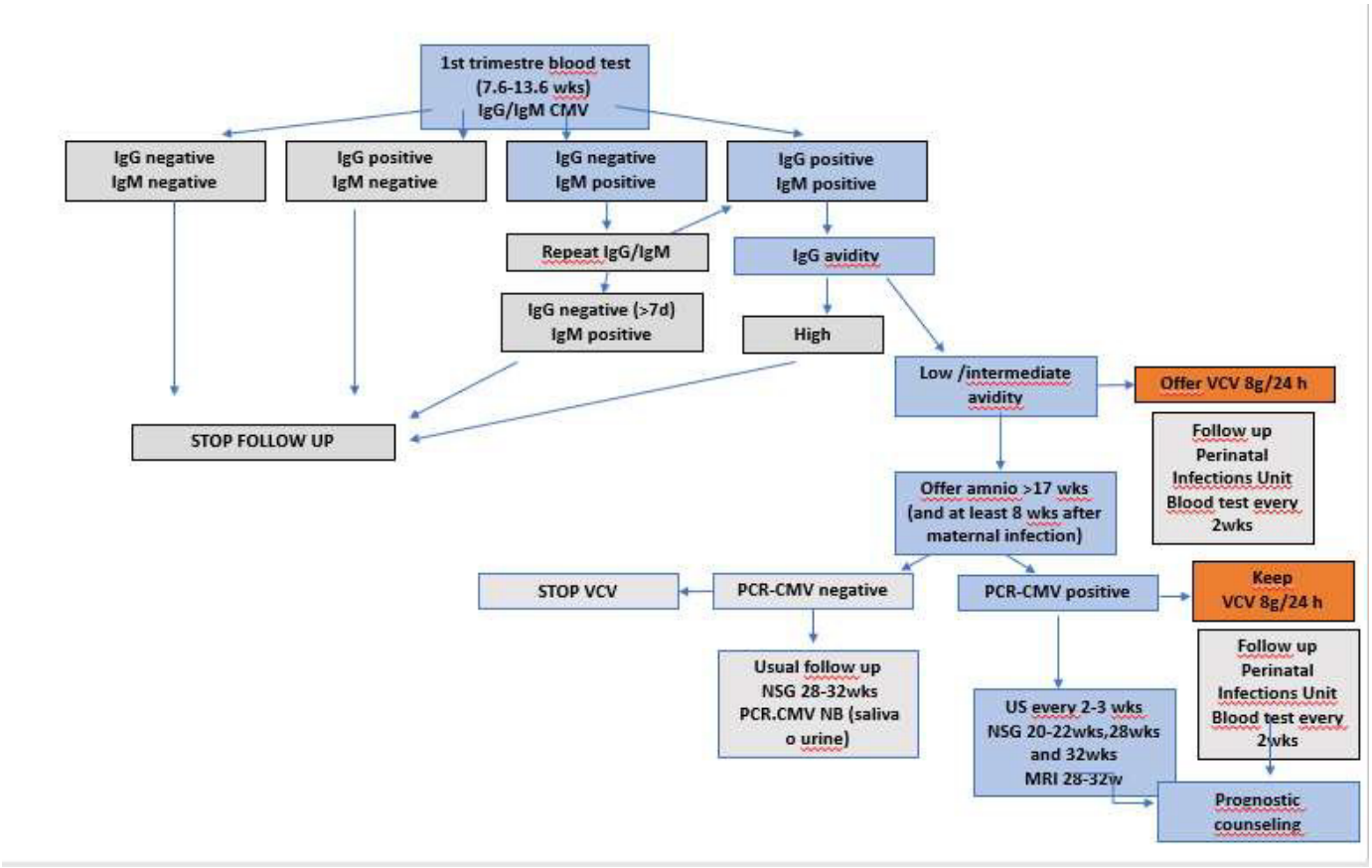
3.3.1. Routine CMV screening during pregnancy:

Most scientific societies do not currently recommend routine serological screening during pregnancy because the negative consequences of implementation (anxiety and iatrogenic terminations of pregnancy) would outweigh the possibility of avoiding significant sequelae. However, this recommendation should be revisited based on the results of a recent randomised controlled trial (*Shahar-Nissan et al., the lancet, 2020*) that demonstrated that maternal administration of high-dose valaciclovir after primary infection during the first trimester or perigestational period reduced vertical CMV transmission by 63% (11% vs 30%; RR 0.37). The benefit of treatment was greater when initiated early, but was still effective when started up to 7 weeks after maternal infection.

In our centre, we screen for CMV in all pregnant women (IgG and IgM-CMV) at the time of the first trimester blood test (between 7.6 and 13.6 weeks) (see algorithm). In non-immune pregnant women, there is no indication to repeat serology in the following trimesters due to the low fetal risk in infections starting after 14 weeks.

In the case of positive IgM, it is essential that the laboratory automatically generates a determination of IgG avidity and an alert to the clinician in order to inform the patient and start treatment early, if it is indicated.

It is important to consider that, with this strategy, we can prevent vertical transmission of a part of primary first trimester and perigestational maternal infections, but we will not detect or prevent non-primary infections (reactivations or reinfections).



Algorithm: CMV infection screening in the first trimester. Management and follow-up in cases of suspected or confirmed primary maternal infection.

3.3.2. Other indications to request CMV serology during pregnancy (IgG and IgM):

- Maternal signs/symptoms consistent with CMV infection
- Identified risk contact
- Ultrasound findings consistent with fetal infection (see specific section)
- Early IUGR (EFW < p3 and < 28 weeks)
- Persistent increased nuchal fold (> 99th percentile) (> 16 wks) with normal karyotype/array CGH

3.3.3. Serology interpretation:

Diagnosis of primary maternal infection:

 Seroconversion (if previous negative serology is available).

 Positive CMV IgG and IgM: does not confirm a primary infection during pregnancy or perigestational period. It may be a previous primary infection since IgM can remain positive for more than 12 months (it can have a slow decline pattern) and very rarely may correspond to a non-primary infection. IgG avidity will be requested to determine the time of infection.

- Interpretation of IgG avidity using the VIDAS® bioMerieux kit (the one used in our centre):

- **High avidity (≥ 0.65):** infection > 12 weeks. In a serology taken in the first trimester, it can reliably rule out a gestational or perigestational infection.
- **Low avidity (< 0.40):** suspected infection < 12 weeks.
- **Intermediate avidity (0.40-0.65):** indeterminate time of infection.

In general, a low avidity index is strongly suggestive of a recent primary infection (i.e. within the past 3 months), while a high avidity index is strongly suggestive of a past infection (i.e. more than 3 months previously)

- A positive IgG and negative IgM indicates a past primary infection ≥ 3 months ago. It should be noted that a positive IgG with negative IgM in the context of ultrasound markers suggestive of infection cannot exclude a primary infection at the start of pregnancy.

Carrying out serology in reference laboratories and expert interpretation of the results has a high sensitivity for identifying pregnancies at risk of fetal infection, while at the same time significantly reducing maternal anxiety and terminations of pregnancy in non-infected foetuses.

3.3.4. Diagnosis of non-primary maternal infection:

Serology is not very useful because IgM is only positive a few times and viremia or viruria, if they occur, are transient and not proven to be related to vertical transmission. Therefore, the diagnosis of reinfection or reactivation is very difficult. The possibility of this should also be considered in cases of a compatible ultrasound anomaly in pregnant women with positive IgG and negative IgM.

3.4. Diagnosis of fetal infection:

Amniocentesis with amplification of viral DNA in amniotic fluid (PCR) is the method of choice. Real-time PCR techniques have very high sensitivity (92%) and specificity (98-100%) if amniocentesis is performed at least 6-7 weeks after maternal infection and after 21 weeks. False negatives correspond to late transmission of the virus and have never been associated with neurological sequelae.

A study from *Enders et al., Prenat Diagn, 2017* showed that amniocentesis from 17 weeks would have the same sensitivity as from 21 weeks in cases of infection at the start of pregnancy, provided that at least 8 weeks had passed since maternal infection. Therefore, this is the recommendation since it shortens the duration of maternal anxiety and the duration of secondary prevention with high-dose valaciclovir. However, early amniocentesis would not be applicable in cases where the duration of maternal infection is unknown.

What's more, there is recent evidence that diagnosis of placental infection following maternal primary infection with cytomegalovirus (CMV) in early pregnancy, i.e. when the foetus is at risk of sequelae, can be achieved at the end of the first trimester (at 13-14 wks) by chorionic villus sampling (CVS) and PCR amplification of the viral genome in chorionic villi, with a high specificity and negative predictive value. Therefore, the French group leading the study *(Faure-Bardon et al., Ultrasound Obstet Gynecol 2021),* proposes that negative CMV-PCR in the trophoblast after 12 weeks could be used to exclude CMV-related embryopathy leading to sequelae. However, until further evidence is available, CVS needs to be performed under maternal valaciclovir treatment and negative CMV viremia and its result needs to be confirmed through a second invasive procedure: an amniocentesis after 17 weeks, which is the gold standard for diagnosis. These findings could help to establish CVS as the diagnostic test of choice in the future following maternal serology screening in early pregnancy.

Indications for amniocentesis:

- Serological suspicion of maternal infection during pregnancy or perigestational period
- Fetal ultrasound abnormalities and positive maternal serology (positive IgG with positive or negative IgM).

Interpretation of results:

- **DNA-CMV undetectable:** this likely rules out fetal infection and allows the patient to be reassured. It does not exclude the possibility of vertical transmission after amniocentesis (8-10%). Nevertheless, no cases of symptomatic newborns have been described that are associated with these late transmissions. Supplementary ultrasounds at 28 and 32 weeks are recommended, and in cases that have received prophylactic treatment with valaciclovir before amniocentesis, a neurosonography (NSG) at 32 weeks may be added due to the possibility of a transient false negative in amniotic fluid. In all cases, the absence of congenital infection in the newborn should be confirmed with a determination of CMV DNA in urine/saliva before 15 days of life.

- **Positive CMV DNA:** this shows that the foetus has been infected. The prognosis will depend on the time of maternal infection. Subsequent management will be directed towards detecting fetal abnormalities that can help determine neonatal prognosis. There is controversy as to whether quantification of the viral load in amniotic fluid is related to

fetal prognosis, as the viral load increases over time from infection, regardless of the degree of fetal impairment.

3.5. Diagnosis of fetal impairment:

3.5.1. Ultrasound findings/abnormalities:

They are usually progressive defects, especially in the CNS, and may not appear until the third trimester. A targeted neurosonography is highly sensitive for detecting CMV-induced lesions (80-85%). Intracranial MRI at 32 weeks provides additional information, especially of cortical, cerebellar, and posterior fossa lesions. Therefore, neurosonography and MRI are complementary techniques that, used together, can achieve a diagnostic sensitivity of close to 100% in terms of a newborn being at risk of sequelae, except for hearing loss.

- **Anomalies of the central nervous system:** they almost exclusively appear after infections in the first trimester. Their identification has generally been associated with sequelae in the newborn. However, due to the increased sensitivity of ultrasound and MRI in detecting subtle lesions, nowadays anomalies can be classified as "poor prognosis abnormalities" with a high risk of a symptomatic newborn and high risk of neurological sequelae, and "uncertain prognosis abnormalities."

Poor prognosis defects/severe CNS abnormalities:

- Severe ventriculomegaly (> 15 mm), hydrocephaly
- Microcephaly (< -3 SD)
- Increased subarachnoid space (microencephaly)
- Agenesis of the corpus callosum
- Hypoplastic cerebellum
- Destructive and haemorrhagic lesions. Porencephalic cysts
- Anomalies of sulcation and cerebral gyri

Lesions with an uncertain prognosis:

- Mild ventriculomegaly (10-14.9 mm)
- Periventricular hyperechogenicity. "Halo"
- Isolated calcifications (often in caudate nuclei)
- Intraventricular synechiae. Germinolytic cysts
- Hyperintense vessels in the thalami ('candle lights'). They are exclusively associated with hearing deficit
- Small isolated parenchymal cysts
- Increased signal in white matter (MRI)

- **Extra-CNS anomalies.** They can also appear after fetal infections in the second and third trimesters. With the exception of severe anomalies (fetal hydrops), ultrasound anomalies that do not affect the CNS generally do not imply poor prognosis or a high risk of the newborn having neurological sequelae. However, when they appear early in the second trimester, they often precede severe CNS anomalies, and the risk of moderate/severe long-term sequelae is 30%. Their presence requires thorough follow-up (ultrasound and MRI). The most frequent extra-CNS anomalies are:
- Small for gestational age (SGA)/IUGR
- Bowel hyperechogenicity
- Hepatomegaly (see normal values in Appendix 6)
- Splenomegaly
- Ascites, hydrops
- Cardiomegaly
- Oligoamnios/Polyhydramnios (less frequent)
- Placentomegaly (see cutoff points in Appendix 7)
- Signs of fetal anaemia (PVS-ACM Doppler > 1.5 MoM)

3.5.2. Fetal blood markers:

The only marker that has been correlated (weak evidence) with the birth of a symptomatic newborn at risk of neurological sequelae is thrombocytopenia (< 100,000/mm^3), but in the case of a first trimester fetal infection, a cord blood platelet count would not be useful beyond 28 weeks. In our experience, elevated gamma-glutamyl transferase (GGT) levels (> 183 IU/ml) could be useful at any gestational age for the prediction of severe brain damage, but evidence is still scarce. Therefore, routine cordocentesis does not seem to be justified as neurosonography/MRI are better prognostic indicators. Its indication should only be carried out on an individual basis, discussing the benefit/risk with the parents.

3.6. Advice, treatment and follow-up in case of suspected maternal infection and/or confirmed fetal infection:

3.6.1 **Suspected maternal infection:** a serological evaluation is required by the Perinatal Infections Unit team and in the case of first trimester serological screening with suspicion of gestational or perigestational infection, treatment with Valaciclovir and amniocentesis will be offered from 17 weeks of gestation and > 8 weeks after maternal infection (see Algorithm and section 3.4

"Diagnosis of fetal infection")

Treatment for the prevention of vertical transmission:

-Valaciclovir (see point 3.3.1 "Gestational Screening")

Drug profile: Valaciclovir is an antiviral with high oral bioavailability and an excellent safety profile during pregnancy. The dosage of 8 g/24 h is licensed for use and is used in the transplanted adult to prevent CMV infection. There is previous experience of its use at these doses during pregnancy for the treatment of the infected foetus The best dosage regimen to prevent rare but described renal complications (reversible acute renal failure secondary to drug accumulation and precipitation of crystals in the proximal tubule), is 2 g/6 h (4 doses) (see point 3.6.2).

Contraindications: liver disease, kidney disease, allergy to valaciclovir, inability to swallow capsules, hyperemesis gravidarum

Valaciclovir is the option we recommend for the prevention of vertical transmission in pregnant women with infection in the first trimester or perigestational period and with a serological diagnosis before 14 weeks (positive IgG/IgM with low or intermediate IgG avidity). Its administration should be started as soon as possible after diagnosis, and treatment should be maintained until the result of amniocentesis (see Algorithm). In pregnant women who accept treatment but reject amniocentesis, treatment will be maintained until the week in which amniocentesis would be indicated.

Prior to its prescription: provide information to the patient about the available evidence of its benefits and risk of side effects.

Due to its off-label use, in our setting the patient signs a consent form.

Dosage: Valaciclovir 2 g/6 h orally. It is recommended to keep well hydrated (2 L/24 h).

Post-administration follow-up: full blood count, liver profile (transaminases) and renal profile the first week after initiation, and subsequently every 2 weeks until the end of preventive treatment.

3.6.2 Confirmed fetal infection by PCR in AF: targeted ultrasound evaluation and follow-up, preferably in a specialised centre, is required in order to determine the fetal/neonatal prognosis. Likewise, at the time of diagnosis, and in the absence of evident ultrasound abnormalities, the prognosis and risk of sequelae will be related to the trimester in which the maternal infection occurred, the risk being almost exclusive for infections in the first trimester. The patient should be informed of the high predictive value of neurosonography (NSG), but also that ultrasound or MRI abnormalities may have a late onset. The recommended follow-up is:

- Ultrasound in the Perinatal Infection Unit every 2-3 weeks.
- Neurosonography every 4 weeks
- Intracranial MRI at 30-32 weeks. An early MRI at 26-28 wks, although less defining, is also advisable
- Evidence of severe brain abnormalities in any trimester of pregnancy diagnosed by ultrasound and/or MRI has a very high positive predictive value (> 90%) in terms of the birth of a newborn

with injuries and risk of serious sequelae. In these cases, and in those countries where it is legal, the patient should be informed of the possibility to request a termination of pregnancy. On the other hand, in the absence of CNS markers on the NSG and the MRI performed around 32 weeks, the negative predictive value in terms of neurological damage and sequelae, except mild hearing sequelae, is 100%. The long-term prognosis of isolated CNS anomalies of uncertain prognosis that could be related mainly to hearing sequelae remains to be defined.

Treatment of infected foetus:

Antiviral medications administered postnatally (ganciclovir, valganciclovir) are contraindicated during pregnancy due to their potential fetal toxicity.

Valaciclovir: a pilot study that evaluated the administration of high doses of valaciclovir (8 g/24 h), in a group of pregnant women with an infected foetus showed that therapeutic levels were achieved both in the pregnant woman and in fetal blood (*Jacquemard F et al. BJOG 2007*) and, later on, a phase II and single-arm study (*Leruez-Ville et al., AJOG 2016*), observed that the administration of valaciclovir at a dose of 8 g/24 h to pregnant women with an infected foetus and mild-moderate involvement was associated with a significant increase in asymptomatic newborns, compared to a historical cohort of foetuses with similar characteristics and without treatment (82% asymptomatic versus 43%).

Despite the lack of evidence on the matter, treatment with valaciclovir may be offered as an off-label indication for pregnant women with fetal infection in the first trimester, which has been confirmed in amniotic fluid (positive CMV PCR). It may be offered to both those with evidence of mild fetal ultrasound abnormalities (of uncertain prognosis) and those who do not present defects at the time of diagnosis, in order to try to prevent late onset abnormalities **(see the drug profile and contraindications of its administration in section 3.6.1.1)**. Treatment would no longer be useful in foetuses with severely poor prognostic ultrasound findings.

Before offering treatment, the prognosis of the infection should be explained to the parents, as well as the legal assumptions about termination of pregnancy, and the limited evidence about the effectiveness of treatment with valaciclovir. In case of acceptance, due to its off-label use, the patient signs an informed consent in our setting.

- **Dose:** Valaciclovir 2 g/6 h orally. Recommend abundant water intake (2 L/24 h).
- **Post administration laboratory follow-up:** full blood count, liver profile (transaminases) and kidney profile in the first week after the start of treatment, and later every 2 weeks until delivery.
- **Fetal follow-up:** targeted ultrasound and MRI as specified in point 3.6.2**.**

3.7. Diagnosis of Congenital CMV Infection in Newborns and Follow-up: the suspicion or diagnosis of fetal

CMV infection should be recorded in the paediatric history since it must be confirmed in the newborn during the first 2 weeks of life (also when the AF is negative) by determining CMV DNA in urine or saliva. CMV positive results 3 weeks after delivery may correspond to an intrapartum or postnatal infection. The identification of a newborn with congenital CMV infection requires an initial assessment of symptoms and a potential need for treatment. In asymptomatic newborns, follow-up in the first years of life is also needed for detection of possible hearing sequelae, and to make an early intervention possible. Neonatologists will refer the child to the specific Paediatric Infections Unit for follow-up.

3.8 Primary prevention of CMV infection:

All women, especially those known to be seronegative, but also those with unknown immunity, as well as immune patients, due to the risk of re-infection, should receive information during the perigestational period or as soon as possible in the first trimester of pregnancy on prophylactic hygienic measures to prevent CMV infection. Children under 3 years of age are the main source of contagion. Washing hands with hot water and soap after contact with saliva and urine (diaper changing, feeding, touching toys, etc.) and avoiding intimate contact with young children (kissing on the mouth, sharing cutlery and glasses, etc.) are the most effective measures.

3.9. Determination of maternal CMV IgG in preterm births (< 32 weeks) with neonatal weight < 1500 g: in seropositive women, the excretion of CMV through breast milk is very common, but it carries a risk of sepsis only in preterm newborns < 32 weeks and especially when the weight is < 1500 g. For this reason, CMV IgG should be requested either before delivery or in the immediate postpartum period for all mothers of extreme preterm (< 32 weeks) infants with weight <1500 g (unless previous seropositivity is already known) in order to proceed with freezing the milk before its administration.

3.10. Study of CMV infection in stillborn foetuses: in addition to determining maternal serology (IgG and IgM), the study of second trimester gestational loss and the study of a stillborn foetus includes amniocentesis for CMV PCR determination before delivery. If the amniotic fluid is not obtained, a placenta fragment (1 cm^3) will be obtained for CMV determination (DNA-PCR) from an area near the umbilical cord that includes fetal membranes. The sample must be sent to Microbiology in a urine culture bottle with physiological serum.

3.11. Preconception advice in cases of previous CMV infection: it is recommended to wait 6 months before attempting a new pregnancy. For those patients not willing to wait, or who become pregnant inadvertently, a possible option that can be used to detect possible maternal infectivity and risk of vertical transmission (very low) is the determination of serial viruria up to 14 weeks of pregnancy. In case of positivity, an amniocentesis should be offered at the appropriate time (≥ 17 weeks).

Following the IgM until it turns negative is not a good tool as it can have a very variable pattern of decline (2-3 months or > 1 year).

4. CHICKENPOX:

4.1. Pathogenesis, maternal clinical features, and epidemiology

Chickenpox is an exanthematic disease caused by a highly contagious DNA virus of the *Herpesviridae* family. It is transmitted from person to person by direct contact with the vesicular fluid of the skin lesions and/or by secretions from the respiratory tract. Varicella-zoster virus (VZV) can be transmitted through the placenta, and fetopathy due to chickenpox has been described. Perinatal transmission around the time of delivery can cause a very serious neonatal infection.

The disease is characterised by widespread outbreaks, especially at the end of winter and spring, and seroprevalence in women of childbearing age is very high (> 90%), but may be lower in pregnant women from tropical and subtropical countries (25-85%). It is estimated that the incidence of chickenpox during pregnancy is 2-3 per 1000.

4.2. MATERNAL MANIFESTATIONS

4.2.1. Maternal symptomatology:

It produces a characteristic maculopapular-vesicular skin rash that generally starts on the face and spreads to the trunk and abdomen, often accompanied by mild fever.

The incubation period is 13 to 17 days and produces 2 periods of viremia: the first at 4-6 days after infection and the second at 10-14 days, 2 days before the appearance of the exanthem. The most infectious period (also for vertical transmission) is 2 days before the onset of rash until the vesicles crust over, 5-7 days after onset of the rash.

Shingles: although the risk is low, contact with shingles lesions, a form of reactivation of latent VZV, can also cause chickenpox infection in non-immune people. In immunocompetent pregnant women, an episode of shingles during pregnancy does not pose any risk of vertical transmission.

There have been case reports of clinical reinfections, but the risk of severe maternal disease and fetal impairment is much lower.

4.2.2. Diagnosis of maternal infection:

Chickenpox in adults is symptomatic and the diagnosis is clinical, but during pregnancy it is always recommended to obtain a diagnostic confirmation:

-**In the presence of active lesions**: VZV PCR (extraction and amplification of viral DNA) of the lesions, preferably in the vesicular phase, with a nylon swab and transport of the swab in a virus transport medium. Keep the sample in the fridge (2-8ºC).

-**Serology**: both IgG and IgM (the first to appear) do not become positive until 3-5 days after the onset of the rash. IgG persists for life and IgM usually disappears within 2-3 months.

4.2.3 Maternal Complications:

Maternal varicella pneumonia

This is the main complication in adults and affects 10-15% of cases. Pneumonia during pregnancy can be more severe, particularly towards the end of the 2nd and beginning of the 3rd trimester (27-

32 weeks), with a maternal mortality of up to 3% despite treatment. Pneumonia usually occurs during the 1st week of the rash and initial symptoms are fever, dry cough, and difficulty breathing. Risk factors for pneumonia: smoking; chronic obstructive pulmonary disease; immunosuppression (e.g. patients on chronic corticosteroid therapy); > 100 skin lesions; 2nd and 3rd trimester of pregnancy.

Prevention of pneumonia:

- Oral aciclovir (800 mg 5 times a day for 5 days) or oral valaciclovir (1 g/8 hours). Indicated if early onset (first 24-72 hours after onset of rash) in:
  -Pregnant women > 20 weeks.
  -Pregnant women with risk factors for pneumonia (described above) at any gestational age.
- Hospitalisation (with isolation) and treatment with intravenous aciclovir in cases of:
  -Very extensive skin lesions of > 6 days' evolution
  -Haemorrhagic rash
  -Persistent high fever
  -Respiratory symptoms

Treatment of pneumonia: hospitalisation (with isolation from other pregnant women) and intravenous aciclovir 10-15 mg/kg every 8 hours for 5-10 days, and in severe cases (respiratory failure) admission to the intensive care unit.

Other less frequent complications of chickenpox in adults can be hepatitis and encephalitis.

4.2.4. Managing a suspected case of chickenpox in a pregnant woman:

- Isolation of the patient from the other pregnant women (individual emergency box minimising the time in the waiting room). Mask placement.
- Clinical examination.
- Blood extraction for serological confirmation.
- Assess indication for treatment with aciclovir/valaciclovir or admission.
- Symptomatic treatment and hygiene measures for the prevention of bacterial superinfections of skin lesions will be indicated for all pregnant women with chickenpox.
- If they do not meet admission criteria, discharge home as soon as possible with instructions for a new consultation (persistent high fever, respiratory symptoms, haemorrhagic rash)
- Schedule an appointment at the Infectious Diseases Clinic for advice and follow-up, never before 15 days to avoid contagion.

4.3. FETAL MANIFESTATIONS: Fetal varicella syndrome

Congenital chickenpox syndrome includes scarring skin lesions following dermatomes, musculoskeletal lesions (limb reduction), peripheral neurological involvement, hypotonia, eye disease (microphthalmia, chorioretinitis, cataracts) and oesophageal and urinary sphincter abnormalities.

It can also produce other serious, less specific anomalies, such as IUGR and CNS defects (cortical atrophy, psychomotor delay, and seizures) with a mortality rate up to 30%.

Children of mothers with chickenpox during pregnancy, especially after 20 weeks, have a risk of developing an episode of herpes-zoster in the first 2 years of life (3.8%).

4.3.1. Risk of vertical transmission and fetal impairment:

The risk of vertical transmission until 24 weeks is 10-15%, but it is higher in the third trimester. The risk of fetal impairment, that is, fetal chickenpox syndrome, is much lower, but it can occur in the case of maternal chickenpox in the first and second trimesters. Most of the cases described have been in maternal infections before 20 weeks, but there is a case described up to 28 weeks.

MATERNAL CHICKENPOX	RISK OF FETAL IMPAIRMENT
< 12 weeks	0.5-1%
12-20 weeks	1.5-2%
21-24 weeks	< 0.5%
24-28 weeks	exceptional

It has not been demonstrated that the administration of aciclovir in the mother reduces the risk of fetal chickenpox syndrome.

4.3.2. Diagnosis of fetal infection:

Recommended method: **amniocentesis** for detection of viral DNA in amniotic fluid (PCR). Real-time PCR techniques for VVZ have a very high sensitivity as long as they are performed at least 5-6 weeks after maternal infection and always after 18 weeks of gestation. Amniocentesis is contraindicated in the presence of maternal skin lesions, due to the risk of vertical transmission during the procedure.

Viral DNA can remain positive in maternal blood for several weeks and it is recommended to request a maternal DNAemia before amniocentesis to ensure negativity at the time of the procedure. This could avoid false positives in the amniotic fluid result.

Consider performing amniocentesis after discussion with the patient if:

- Maternal chickenpox ≤ 24 weeks.
- Maternal chickenpox between 24-28 weeks. In maternal chickenpox at this gestational age, the risk of the invasive procedure exceeds the risk of fetal impairment from the infection.

Evaluation of the result:

- **Undetectable VVZ DNA:** low risk of infection and fetal impairment. However, additional ultrasound controls are recommended (e.g. at 28 and 36 weeks).
- **Positive VVZ DNA*:** indicates fetal transmission and infection but not necessarily fetal impairment. In the absence of ultrasound markers at the time of obtaining the result, the risk of congenital chickenpox is unlikely, but it should be noted that these markers can appear late and occasionally not appear. There is little information on the fetal prognosis in the case of positive amniotic fluid, but the risk of some type of sequelae (including mild sequelae) could be up to 20%. The doctors in the Infection Unit will give the patient detailed advice on the risks and follow-up to be performed.

The follow-up includes: **specific/targeted ultrasound every 2-3 weeks, monthly NSG and intracranial and ocular MRI (30-32 weeks)**.

**Although it is very uncommon with reverse transcription-polymerase chain reaction (RT-PCR) techniques, false positives for VVZ PCR in amniotic fluid have been described. For this reason, whenever a positive result is obtained in the absence of specific ultrasound markers, it is recommended to confirm the result with a second sample of amniotic fluid before deciding on any action, especially in the absence of a negative maternal viremia at the time of amniocentesis.*

4.3.3. Fetal varicella syndrome: ultrasound findings/markers

- Limb defects, malpositions, partial amputations or limb shortening
- Calcification of soft tissues
- Microcephaly (< -3 SD)
- Hydrocephaly
- Porencephaly
- Anomalies of the cerebral cortex (polymicrogyria)
- Echogenic foci: CNS, bowel, lungs
- IUGR
- Polyhydramnios
- Placentomegaly

3D ultrasound can help in the diagnosis of limb defects and soft tissue defects.

Intracranial and ocular MRI, mainly at 32 weeks, supports the diagnosis of CNS defects, especially cortical abnormalities and ocular defects (microophthalmia, cataract)

4.4. Maternal chickenpox at the time of delivery and neonatal chickenpox:

When maternal chickenpox appears in the 5 days prior to delivery or in the 2 days following (onset of viremia), the risk of severe neonatal chickenpox is very high as vertical transmission is high (> 50%) and the newborn may not yet have protective antibodies from the mother, which progressively appear from 3-7 days after the onset of the rash.

For this reason, it is recommended to try to postpone/delay the delivery for 7 days after the onset of the rash:

- Admission with isolation measures
- Tocolytic treatment

- Oral aciclovir (800 mg 5 times/day) or valaciclovir (1 g/8 h orally) for prophylaxis of

pneumonia. The administration of aciclovir to prevent fetal transmission or the severity of neonatal infection has not been proven.

- Intravenous aciclovir (10-15 mg/kg every 8 hours) in the case of severe chickenpox or chickenpox pneumonia. In these cases, the delivery also poses a significant risk to the mother (thrombocytopenia, bleeding, sepsis, and hepatitis). This must be taken into account in order to apply the necessary supportive measures.

During the highly contagious phase (period of viremia: 2 days prior to rash until the first 5-7 days of the rash), local-regional anaesthesia is contraindicated due to the risk of viral transmission to the central nervous system (intrathecal anaesthesia carries a higher risk than epidural anaesthesia). In the regressive phase of the infection (> 5-7 days from the onset of the rash), local-regional anaesthesia in skin areas free of lesions is no longer contraindicated.

4.4.1. Isolation measures at the time of the delivery and in the postpartum period:

The mother will remain isolated from other pregnant women until all vesicles crust over. The newborn requires isolation from other newborns, but does not require isolation from the mother.

Breastfeeding is not contraindicated.

4.5. Neonatal treatment:

Early prophylactic administration of varicella-zoster immune globulin (foreign medicine) or, if not available, polyvalent immune globulin intravenously to newborns born within 7 days before or after the onset of maternal rash reduces mortality and the severity of the condition. The signs of infection in the newborn should be monitored until 28 days of life in order to start intravenous aciclovir treatment early.

4.6. Follow-up of children of mothers with chickenpox during pregnancy:

They will be referred to the specific Paediatric Infections Unit of their reference centre to confirm/rule out intrauterine transmission and detect any possible ocular disease.

The evidence of varicella-zoster IgM in the newborn's blood (low sensitivity, around 25%), the persistence of varicella-zoster IgG beyond 7 months of life, or the onset of herpes zoster in the first 2 years of life confirm the diagnosis of intrauterine transmission. The negativisation of maternal IgG at 7 months of life will allow the ruling out of congenital infection.

4.7. Maternal post-exposure prophylaxis:

In non-immune pregnant women, after significant exposure to chickenpox (household contact, "face-to-face" contact with index case or in same room > 15 min) at any trimester of pregnancy, it is recommended **to perform a post-exposure prophylaxis with an antiviral (Aciclovir or Valaciclovir) from day 7 to day 14 after exposure**. In non-immune patients who consult from 7 days after contact (< 14 days) it is also recommended to start the treatment and continue it until day 14. The first day of exposure is considered to be the first day of contact with the infected person and, in the case of a household contact, the first day on which the skin rash appeared shall be

considered.

Dosage: Aciclovir 800 mg/6 h or Valaciclovir 1 g/8 h. Both are well tolerated, can be used during pregnancy and have few contraindications [renal failure or intestinal malabsorption (severe inflammatory bowel disease…)].

Post-exposure prophylaxis with an antiviral is more effective than the classic preventive treatment, which was the use of polyvalent immunoglobulin. Antivirals are also considered the prophylactic treatment of choice given the shortage of immunoglobulins. VZV-specific IgG is scarcely available in our environment. In case of contraindication to antivirals, the alternative would be to **administer a dose of a polyvalent immunoglobulin (IG) for prophylaxis during the first 72-96 h** of contact (IG administration has some effect up to 10 days after contact). You can use **Hizentra® 3200 mg, 16 ml (1 ml = 200 mg)** (the vials are 5, 10 and 20 ml). The route of administration approved in the Summary of Product Characteristics (SmPC) is the subcutaneous one, but intramuscular administration is recommended because of its faster absorption. As the volume > 5 ml, it is advisable to distribute the dose in three different anatomical regions. Due to its off-label use, in the Clinic Hospital it must be requested through the Subcommittee on Medicines in Special Situations (https://intranet.clinic.cat/?q=ca/sots-comissio-de-medicaments-en-situacions-especials).

Since there are very few susceptible pregnant women (< 10%), before post-exposure prophylaxis, the **absence of immunity should be confirmed by requesting an urgent varicella-zoster IgG** (result within 24-48 hours prior to contact with the Microbiology Service) for all pregnant women who do not remember having had the infection or being vaccinated against it. **IgM determination is not recommended as it could delay the process and does not provide any benefit in the context of a chickenpox exposure**. The algorithm of action and the treatment guidelines are available in Appendix 3.

Post-exposure prophylaxis reduces, but does not eliminate the risk of infection, although if it appears, it is usually milder. There is no information on the usefulness of prophylactic treatment for the prevention of congenital chickenpox in case of prophylaxis failure.

In case of a new exposure, the antiviral prophylaxis should be repeated, but it is advisable to again confirm the absence of IgG as the chickenpox could have been subclinical. With regard to IG, its effect lasts for 3 weeks. In the event of a new risk exposure after this period of time, it is advisable to administer a new dose, after confirming the absence of antibodies first.

4.8. Chickenpox immunisation:

As it is a vaccine with attenuated virus, its administration is contraindicated during pregnancy. After its administration, it is recommended to avoid pregnancy for 1 month. However, there are no reported cases of congenital impairment and interruption of pregnancy would not be justified after accidental administration during the first trimester.

Vaccination in adults (2 doses separated by an interval of 6 to 8 weeks) provides 99% protection. Vaccination is not contraindicated during breastfeeding.

It would be advisable to ensure varicella immunity in all women before becoming pregnant.

5. HERPES SIMPLEX VIRUS:

5.1. Pathogenesis, maternal symptoms and epidemiology:

HSV-1 and HSV-2 are DNA viruses in the *Herpesviridae* family, with common antigens, and they cause the host to produce heterologous antibodies with some capacity to neutralise both viruses. Transmission of the virus requires direct and close person-to-person contact. HSV has a variable incubation period of between 2 and 12 days. The initial contact is usually with HSV-1 during early childhood, causing subclinical infection in 90% of cases, or gingivostomatitis and herpes labialis in the remaining 10%. From the onset of sexual activity, especially HSV-2 (but also HSV-1) causes genital infection, which is transmitted, in most cases, from an asymptomatic carrier.
Seroprevalence in adults is 60-75% for HSV-1 and 11-30% for HSV-2.
Vertical transmission of the virus at the time of delivery can cause severe neonatal infection.

Depending on the presence of previous antibodies, genital herpes can present in 3 different stages:

 Primary genital herpes: occurs in the absence of HSV-1 or HSV-2 antibodies. It can be an asymptomatic infection or be associated with severe symptoms with herpetic lesions, systemic symptoms, and inguinal lymphadenopathy. Viral excretion through the genital tract can persist for 3 months.

 First non-primary genital herpes episode: first episode of genital lesions in a patient with previous HSV-1 antibodies. The symptoms are usually less intense and shorter in duration, without systemic manifestations and with shorter genital viral excretion.

 Recurrent infection: HSV remains latent and has a high capacity for reactivation. Lesions tend to be limited and shorter in duration. Viral excretion also occurs during subclinical episodes and there may be possible transmission.

5.2. Vertical transmission:

Neonatal herpes is a serious systemic infection with high morbidity and mortality and variable incidence (1.6-20/100,000 births). Most infections are acquired from infected maternal secretions when passing through the birth canal, but neonatal herpes can also be acquired postnatally. Haematogenous transmission is also described. Therefore, the 3 forms of perinatal transmission are:

- **Congenital infection with HSV (5% of neonatal infections):** intrauterine transmission from maternal viremia (haematogenous transmission) or ascending with intact membranes. Intrauterine transmission of HSV is extremely rare and only occurs in < 5% of primary herpes infections (which can be asymptomatic). If transmission occurs, there is a higher risk of abortion and premature delivery. HSV has little teratogenic capacity, but there are isolated cases of defects after maternal infection in the first and second trimesters. It

produces a disseminated infection with a predominance of skin, ocular and CNS lesions. HSV-2 has a greater capacity to affect the CNS.

Ultrasound findings described: microcephaly, hydranencephaly, intracranial calcifications, microphthalmia, skin lesions (thickening of the skin, hyperechogenicity of the skin), IUGR, fetal hydrops**.**

- **Neonatal infection by ascending transmission during labour**: it is the most frequent form (85% of neonatal infections). It occurs from infected maternal secretions (most commonly HSV-2) and after the rupture of membranes. The risk of acquiring the infection during delivery is variable and depends on the type of maternal disease:
- Primary genital herpes: 50% vertical transmission
- First non-primary genital herpes: 33% vertical transmission (presence of heterologous Ab)
- Recurrent herpes: 1-3% vertical transmission

- **Postnatal infection: (10% of neonatal infections).** The newborn acquires the postpartum infection through horizontal transmission (more common with HSV-1)

It should be noted that 70% of infected newborns come from mothers with asymptomatic or unrecognised infection.

5.3. Diagnosis of HSV infection:

5.3.1. Cell culture/PCR of genital lesions:

In the presence of compatible lesions, material from the base of the lesions should be obtained for viral culture with a nylon swab, preferably from the liquid contained in the vesicles, and placed in a tube with a virus transport medium, keeping the sample at 4°C (DO NOT FREEZE).
The sensitivity of culture in active primary lesions is high (80-90%), but decreases in recurrent lesions, over crust lesions (70%), or in treated patients, and is lower in mucosal samples without lesions (30%). Negative culture does not exclude genital herpes infection because virus excretion is intermittent.
The sensitivity of PCR techniques (viral DNA extraction and amplification) is higher, especially in the absence of lesions or in recurrent lesions (> 90%). In this case, the nylon swab is collected in the same virus transport medium.
Both cell culture and PCR techniques are specific for the virus (HSV-1 and 2).

5.3.2. Serology:

The available tests in general are not specific for the virus type and do not distinguish between HSV-1 and 2. They are positive in 70-80% of pregnant women. Therefore, serological determination is not indicated in patients with compatible lesions, nor is it useful in pregnancies with IUGR foetuses, ultrasound findings compatible with congenital infection, or in cases of intrauterine fetal demise. Type-specific viral serology is available in some laboratories, but its indication is reserved for selected cases such as a pregnant woman with a first episode of genital herpes in the last weeks of pregnancy (diagnosed by PCR or culture) to confirm the absence of homologous antibodies before indicating an elective caesarean section.
The determination of HSV IgM is not validated and is not used.

5.3.3. HSV-DNA in AF:

The detection of viral DNA in AF (RT-PCR) has high sensitivity and specificity for the diagnosis of congenital infection by HSV-1 and 2. It is part of the study recommended in AF when there are ultrasound abnormalities compatible with fetal infections (see **Appendix 1** at the end of the protocol) and, in particular, if there are abnormalities of the CNS, or fetal hydrops.

In pregnant women with suspicion of primary herpes infection during the first and second trimesters, there is no indication for an amniocentesis to study HSV DNA in the AF. The procedure can be individualised only in case of maternal infection with systemic clinical repercussion (risk of viremia and absence of protective maternal antibodies) and always after 5-6 weeks of maternal infection and after 18 weeks of pregnancy .

5.4. **Treatment of genital herpes during pregnancy, intrapartum management, and mode of delivery (see Table):**

The main objective of the treatment of genital herpes during pregnancy is the prevention of vertical transmission at the time of delivery. The performance of a **caesarean section in early labour in the presence of herpes lesions** (especially primary lesions) and the reduction of asymptomatic viral excretion by administering **aciclovir/valaciclovir** to selected pregnant women, are the best prophylactic measures available. The recommended suppressive doses of aciclovir and valaciclovir during pregnancy are higher than those given to non-pregnant patients with recurrent herpes. They have been shown to reduce recurrences (RR 0.25) and the need for caesarean section (RR 0.27).

5.4.1 Maternal primary infection during pregnancy:

- **Aciclovir oral 400 mg/8 h** (aciclovir 200, 2 tablets/8 h) or **valaciclovir oral 1 g/12 h** for 7-10 days at the time of clinical diagnosis in any trimester of pregnancy. Treatment reduces the healing time of lesions and the duration of viral excretion. In severe maternal herpes or disseminated herpes, intravenous aciclovir (5-10 mg/kg every 8 h for 2 to 7 days) will be administered and treatment will continue with oral therapy until 10 days are completed.

- All pregnant women with primary infection during pregnancy are recommended to take a **suppressive regimen with aciclovir oral 400 mg/8 h** (aciclovir 200, 2 tablets/8 h) or **valaciclovir oral 500 mg/12 h** from 36 weeks, until the day of delivery, to prevent viral excretion and the appearance of lesions at the time of delivery.
- Whenever a first episode of genital herpes is diagnosed at the time of delivery, a caesarean section will be performed as soon as possible regardless of the time of rupture of membranes. An elective caesarean section will also be performed on all pregnant women who have had a **primary genital herpes infection in the previous 6 weeks before delivery in the absence of homologous antibodies or unknown antibodies** (high risk of viral excretion in the absence of maternal antibodies). If the mother does not accept a caesarean section (risk of vertical transmission in vaginal delivery > 40%), it would be indicated to administer intravenous aciclovir (10 mg/kg every 8 h) to the mother during labour.
- Neonatologists should be aware of the history of maternal primary infection so as to perform cultures, neonatal follow-up, and consider administering intravenous aciclovir to prevent a serious neonatal infection.

5.4.2 Maternal recurrent infection during pregnancy:

- Recurrent lesions are usually milder and disappear within 7 days. Depending on the symptoms and the time since the start of the episode (recent onset of symptoms), consider administering aciclovir oral 400 mg/8 h for 5 days (aciclovir 200, 2 tablets/8 h) or valaciclovir oral 500 mg/12 h for 3 days, although treatment is generally not necessary.
- In women with recurrent episodes of genital-HSV infection during pregnancy, a **suppressive regimen with aciclovir oral 400 mg/8 h** (aciclovir 200, 2 tablets/8 h) or **valaciclovir oral 500 mg/12 h** is recommended **from 36 weeks**, to prevent an episode at the time of delivery.
- **Caesarean** section is only indicated **when** the patient has an **active episode** of genital HSV at the time of delivery or prodromal symptoms (vulvar pain, burning), regardless of the time of rupture of membranes. An episode of herpes at any other moment of pregnancy is not an indication for a caesarean delivery. Vaginal delivery in the presence of recurrent genital herpes lesions carries a very low risk of neonatal herpes (1-3%). In case of inevitable vaginal delivery or preference for vaginal delivery (after detailed counselling regarding neonatal risks and signing of the informed consent), invasive procedures should be avoided (invasive fetal heart rate monitoring, fetal blood sampling) and prolonged rupture of membranes. In this situation and in case of ≥ 35 weeks of gestation, active management with immediate induction of labour would be indicated without opting for an expectant management.
- Taking serial cultures to identify pregnant women with asymptomatic HSV excretion is not indicated.

- Neonatologists should be aware of the maternal history of recurrent genital herpes to perform cultures and follow-up of the newborn.

HSV treatment during gestation and mode of delivery

	PRIMARY INFECTION during pregnancy	**RECURRENT EPISODE** during pregnancy
ACUTE EPISODE TREATMENT Aciclovir oral 400 mg/8 h Valaciclovir 500 mg-1 g/12 h	yes 7-10 days	¿? 3-5 days
SUPPRESSIVE REGIMEN Aciclovir oral 400 mg/8 hValaciclovir 500 mg/12 h	yes > 36 wks until delivery	Yes > 36 wks until delivery
RISK OF INTRAPARTUM VERTICAL TRANSMISSION	40-50%	1-3%
CAESAREAN SECTION	If there is an infection at the time of delivery or within 6 weeks prior and there is either no presence or unknown IgG status.	If episode at the time of delivery.

5.4.1. Preterm PROM (< 34.6 weeks) in pregnant women with active HSV infection:

- In cases of **primary maternal HSV infection** at the time of delivery, the risk of vertical transmission is very high (> 40%). The case will be individualised based on the gestational age, considering, after discussion with neonatology, the most appropriate time for delivery. If an expectant management is decided upon, treatment with intravenous aciclovir (5-10 mg/kg every 8 hours) will be administered to reduce the risk of transmission before delivery. The duration of treatment with intravenous aciclovir will depend on the gestational age at the time of delivery and will be administered for a maximum of 7-10 days. Following that, an individualised oral suppressive therapy will be considered until the time of delivery. If the period from the onset of the primoinfection to delivery is more than 6 weeks (or if

specific homologous viral type-IgG is confirmed) and there are no lesions present at the time of delivery, a vaginal delivery may be considered.

- In pregnancies with recurrent maternal infection at the time of delivery, the usual treatment regimen with oral aciclovir or valaciclovir will be administered and the pregnancy will be monitored in the same way as for any pregnant woman with PPROM (see specific clinical guidelines). If lesions persist at the time of delivery, an elective caesarean delivery will be performed.

5.5. Risk of invasive procedures in pregnant women with HSV infection:

In pregnant women with recurrent herpes episodes, it is not contraindicated to perform a transabdominal invasive procedure (amniocentesis, chorionic villous sampling , or cordocentesis) in the presence of active genital lesions. It is recommended to avoid transcervical invasive procedures (chorionic villous sampling) until the resolution of genital lesions.

5.6. Neonatal considerations:

Newborns of mothers with genital herpes or suspected herpes infection should be isolated (except from the mother) to prevent infection of other newborns. In the case of clinical suspicion of neonatal infection, treatment with intravenous aciclovir should be started immediately.
Breastfeeding is not contraindicated.

6. SYPHILIS:

6.1. Pathogenesis and epidemiology:

Syphilis is a systemic infection caused by the *Treponema pallidum* spirochete that has two modes of transmission: sexual and transplacental. In recent years, there has been a significant increase in cases of syphilis, and it is essential that gestational screening allows for its detection to prevent an increase in congenital syphilis. In our environment, the epidemiological factors associated with a high risk of exposure are: drug use, sexual promiscuity, HIV infection, history of other STIs, teenage pregnancies, and pregnancies from areas with a high prevalence of infection (South America, Eastern Europe, Sub-Saharan Africa).
Syphilis does not confer long-term immunity and reinfections can occur

6.2. Clinical presentation of maternal infection:

The disease progresses through various stages:

6.2.1. Primary syphilis: the chancre appears at the site of inoculation after an incubation period of 2 to 6 weeks. It is a painless ulcer associated with regional lymphadenopathy and may be hidden in the vaginal, rectal, or oral mucosa. Without medical treatment, the ulcers disappear in 3-8 weeks.

6.2.2 Secondary syphilis: results from the dissemination of T. pallidum and is the period of maximum infectivity. It appears 3-8 weeks after the primary lesion. It presents with a maculopapular, papulosquamous or pustular skin rash with characteristic palmoplantar involvement or mucous membrane involvement. Occasionally, constitutional symptoms such as anorexia, fever, and arthralgias may appear. The clinical presentation of this stage resolves without treatment in 2-6 weeks, moving into a latent phase**.**

6.2.3. Latent syphilis: a subclinical period that is only detectable by serology. It is divided into:

- Early latent syphilis: < 1 year from the primary infection.
- Late latent syphilis: > 1 year or date of primary infection unknown.

6.2.4. Tertiary and quaternary syphilis: may appear after years of latent syphilis, includes syphilitic gummas, cardiac, auditory, and neurosyphilis abnormalities. It is uncommon since the introduction of penicillin but can occur in up to 1/3 of patients never exposed to treatment**.**

6.3. Diagnosis of maternal infection:

6.3.1. Clinical Diagnosis:

The infection should be suspected in the presence of any painless genital ulcer or an ulcer, regardless of location, which does not heal in 2 weeks.
In the presence of any generalised skin rash in a pregnant woman, a syphilis serology should be performed to rule out secondary syphilis.
The diagnostic suspicion should always be confirmed by serological study (reagin and treponemal tests) and, in the case of a visible lesion, also by microbiological testing.

6.3.2. Microbiological analysis of lesions (PCR):

In the case of a genital ulcer or suspicious lesion, a sample of exudate from the lesion should be obtained with a transport medium swab (liquid Amies) and keep the sample at 4°C. In the Microbiology laboratory, a specific PCR study or multiple PCR (T. Pallidum, H. Dukrey, and Chlamydia T) is conducted.

6.3.3. Serologic testing:

<u>Non-treponemal antibody test or reagin tests: VDRL and RPR</u>

They detect non-specific antibodies and reflect the extent of disease activity in titres. These tests are useful in assessing response to treatment and disease progression. They may become

negative over time, even without treatment, particularly in later stages. In up to 50% of correctly treated patients, positive titres may persist without indicating treatment failure.

False positive results: in some situations and in the absence of syphilitic infection, low positive titres (generally ≤ 1:8) may appear: pregnancy, intravenous drug users, autoimmune diseases, neoplasms, bacterial infections, viral infections (HIV, Epstein-Barr Virus (EBV), hepatotropic viruses), or mycobacterium infections, malaria, vaccinations.

False negative results: early stage of the disease (25%), HIV infection, prozone phenomenon due to excess antibodies (2% in secondary syphilis).

Treponemal tests: ELISA, TPHA, FTA-Abs

It consists of the determination of treponema-specific antibodies. They have high sensitivity and specificity. The TPHA and ELISA IgG tests are equivalent. Currently, the use of ELISA techniques is being generalised due to automation of the technique. If IgG are positive, the laboratory determines IgM. They are positive after primary infection before reagin tests, especially IgM (90% treponemal vs 75% VDRL). IgM persists for 2-3 months and IgG can remain positive for life in 85% of correctly treated patients.

6.4. Screening in pregnancy:

An ELISA IgG treponemal (or TPHA) test should be requested for all pregnant women at the first obstetric appointment. If the result is positive, the laboratory will directly determine the VDRL or RPR (activity evaluation) and, in the case of ELISA, treponemal IgM (temporal evaluation). If the VDRL is negative and there is no previous history of treatment, a second confirmatory treponemal test will be performed, which in our centre will be a line immunoassay (LIA). Serology should be repeated, ideally during third trimester at 28-32 weeks' gestation and at delivery, in cases where there is increased risk and in settings with a high prevalence of syphilis.

Interpretation of serological results:

REAGIN TEST	TREPONEMAL TEST	INTERPRETATION	MANAGEMENT
-	-	- No infection - acute infection (very recent infection)	If suggestive clinical symptoms or suspicion of contagion, repeat in 2-3 weeks
+	+	- confirmed or recently treated infection	Treatment if not previously treated

+	-	- Probable false positive (titres <1/8)	Repeat in 3 weeks for confirmation

-	+	- Old infection (treated or incomplete treatment) - Recent infection if IgM positive	Confirmation of the result (LIA) if no treatment history Treatment if no previous treatment or suspicion of incomplete treatment

Sexual partners will be referred to their GP or Sexual Health Clinic for testing.

In case of confirmed syphilis, genital cultures will be performed to rule out other STIs (see detailed cultures in Appendix 5).

Syphilis is a reportable disease.

6.5. Intrauterine transmission and congenital syphilis:

Intrauterine transmission of T. Pallidum can occur from 14 weeks on, increases with advancing gestation and is proportional to the degree of spirochetemia. The risk of transmission in the first 4 years of infection is high due to frequent spirochetemia. In the absence of treatment, the risk of intrauterine transmission is as shown in the table below:

Maternal syphilis	Vertical transmission
Primary/Secondary	50%
Early latent (<1 year)	40%
Late latent (>1 year)	10%
Tertiary/Neurosyphilis	10%

6.6. Congenital Syphilis:

If an infected pregnant woman does not receive penicillin treatment during pregnancy and intrauterine transmission occurs, the association with adverse perinatal outcomes is high and includes:

• 40% spontaneous miscarriage or perinatal death (more common in primary and secondary syphilis)

• 40% neonatal congenital syphilis with a high risk of premature birth, IUGR, and congenital

abnormalities, especially musculoskeletal.

• 20% asymptomatic or mild neonatal congenital syphilis, although these newborns can develop late congenital syphilis with more severe manifestations.

Ultrasound abnormalities of congenital syphilis may appear and are secondary to the effects of the infection itself and the fetal immune response. They usually do not appear before 18-20 weeks. The most common are:

- Hepatomegaly and splenomegaly (80%) (see normal values in Appendix 6)
- Signs of fetal anaemia (33%) (Doppler PVS-ACM > 1.5 MoM)
- Placentomegaly (30%). Defined as maximum placental thickness > 4 cm
- Polyhydramnios (12%)
- Fetal ascites/hydrops (10%)

Manifestations of congenital syphilis in the neonatal period: most guiding signs appear after the first week of life. 2/3 of infected newborns are asymptomatic at birth. If the infection is not diagnosed after birth (rhinitis, hepatomegaly, jaundice, rash, lymphadenopathy, bone lesions, etc.) and the appropriate treatment is not administered, 40% of newborns will develop late congenital syphilis, which is the evolutionary expression of intrauterine noxa and is irreversible despite treatment.

6.7. Maternal treatment:

Treatment with penicillin is very effective, especially when administered in the first and second trimesters. It is important to treat symptomatic cases as soon as possible, as well as asymptomatic cases diagnosed through gestational serological screening (regardless of serological titres), as long as there is no certainty that the patient has previously received correct treatment. In cases of diagnosis of **syphilis < 1 year** (primary, secondary, and early latent) during pregnancy, it is indicated to administer a 2nd dose of penicillin G benzathine as follows:

Primary or Secondary syphilis or early latent (< 1 year)
• Penicillin G benzathine 2.4 MU IM and repeat in 7 days (2 doses) (hospital admission for 1st dose administration if gestational age > 24 weeks)
Late latent syphilis (> 1 year) or inability to date the infection
• Penicillin G benzathine 2.4 MU IM/ week x 3 weeks (3 doses)

6.7.1. JARISCH-HERXHEIMER reaction:

Fever, headache, and myalgia in the first 24 hours of treatment due to treponemal destruction. It is common in pregnant women treated for primary or secondary syphilis from the second trimester of pregnancy (40%).

There may be a threat of premature labour, fetal distress, and cases of intrauterine fetal death have been described, but the benefit of treatment outweighs the risks. The pregnant woman should be advised to go to the emergency department in case of fever, uterine contractions or decreased fetal movements. In cases of primary and secondary infection > 24 weeks, the patient will be admitted for the administration of the first dose of penicillin and observation during 24 h. The treatment of the Jarisch-Herxheimer reaction is symptomatic with IV hydration, antipyretics, tocolytics, fetal well-being monitoring and, in severe cases, with corticosteroids.

6.7.2. Efficacy of treatment and response control:

A. **Maternal:** a new VDRL/RPR titre determination should be performed at 3 months, 6 months and at the time of delivery. Treatment is considered effective if, at 6 months, there is a 4-fold decrease in VDRL/RPR titres (equivalent to a decrease in 2 dilutions, for example from 1:16 to 1:4 or from 1:32 to 1:8). If this is not the case, a new HIV serology should be performed and the diagnosis of neurosyphilis should be considered. During pregnancy, there may not be enough time to evaluate the serological evolution before delivery. For this reason, it is considered advisable to repeat the same treatment regimen if the VDRL titres have not decreased at 3 months after treatment completion. In the case of active syphilis, or when the serological response has not been verified during pregnancy due to insufficient elapsed time, the patient will be sent for follow-up to the reference STI Clinic.

B. **Fetal:** Treatment during pregnancy with penicillin successfully treats fetal infection in most cases. There are situations with a higher risk of fetal impairment despite treatment:

   -Delivery before 30 days after treatment completion

   -Diagnosis and treatment in the 3rd trimester

   -Unfavourable evolution of antibody titres

   -Treatment following detection of ultrasound abnormalities

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6.7.3. Penicillin allergy:

Oral or intravenous desensitisation is recommended in a hospital setting. Alternative antibiotic regimens that are not contraindicated during pregnancy are not effective enough for treating maternal infection and preventing congenital syphilis (erythromycin, azithromycin) or have not been sufficiently tested (ceftriaxone).

Breastfeeding is not contraindicated.

6.8. Neonatal follow-up:

Whenever there has been a suspicion of maternal syphilis during pregnancy, it should be noted in the paediatric history so that neonatologists can properly assess the newborn and provide clinical and serological follow-up at the specific Paediatric Infectious Diseases Unit of their reference centre.

7. PARVOVIRUS B19:

7.1. Pathogenesis, clinical and epidemiological characteristics:

Parvovirus B19 is a respiratory transmitted DNA virus belonging to the *Parvoviridae* family, which causes epidemic erythema, also known as infectious erythema or fifth disease. It appears in the form of epidemics at the end of winter and beginning of spring, predominantly affecting primary school children. Common symptoms of parvovirus B19 (PV B19) infection include a mild non-specific prodromal illness that may consist of mild fever, malaise, headache, myalgia, nausea, and rhinorrhoea; typically beginning 6-12 days after initial infection. This is the most contagious period with a duration of 4 to 7 days. Due to the virus's special tropism for erythrocyte precursor cells, it produces a transient aplastic anaemia, to a variable degree, which is only severe in patients with chronic haemolytic disorders. At the end of the viremia phase, the characteristic macular rash appears, which begins and predominates on the cheeks, later extending to the trunk and proximal part of the limbs.

In adults, it is generally an asymptomatic infection, but it can cause fever, joint pain or arthritis (especially in women: 60%), affecting the knees, wrists, ankles, and proximal interphalangeal joints. Seroprevalence in pregnant women is 35-65%. The risk of infection in non-immune pregnant women is high: 50% after household exposure and 20-30% after occupational exposure (teachers). The incidence of infection during pregnancy is 1-2%, but it can reach 10-15% during seasonal outbreaks (especially at the end of winter and beginning of spring). Parvovirus B19 confers persistent immunity and reinfections have not been described.

7.2. Intrauterine transmission and fetal effects:

7.2.1. Vertical transmission/Fetal infection:

It is high, up to 30-40%, with a slight increase in the second trimester (55%), but the risk of fetal involvement is low and teratogenesis has not been described (see Table)

7.2.2. Fetal effects:

PVB19 can cause the following in the foetus: severe aplastic anaemia due to interference with erythropoiesis in the bone marrow and liver, thrombocytopenia, myocarditis, dilated cardiomyopathy, congestive heart failure, hydrops, miscarriages, and intrauterine death. The risk of fetal involvement depends on the trimester of pregnancy. The overall risk can reach 20% in

maternal infections before 20 weeks, but is much lower afterwards.

- **Fetal hydrops:** is the most frequent complication and is secondary to fetal anaemia. Myocarditis can also be involved in its pathogenesis. It appears globally in 4% of gestational infections but can reach 10-12% in infections occurring between 9 to 20 weeks, which is the period of predominantly hepatic fetal erythropoiesis and with a shorter lifespan of erythrocytes. Hydrops appears in 75% of cases during the first 8 weeks after maternal infection (between 2-8 weeks) and in 20% of cases between 8 and 12 weeks. However, there are some cases described up to 20 weeks after infection. The highest incidence of hydrops appears between 17 and 24 weeks of pregnancy. In the absence of treatment, fetal survival is 30% (spontaneous resolution of hydrops), but with treatment of anaemia, survival can reach 80-85%. Cases of subsequent neurodevelopmental delay and anomalies of cortical maturation (especially polymicrogyria) after fetal hydrops due to PVB19 have been described, probably due to hypoxic phenomena.
- **Intrauterine fetal demise:** a maternal infection before 20 weeks of gestation can also cause intrauterine fetal demise in the absence of fetal hydrops, in up to 10-15% of cases. The period of greatest risk is in maternal infections between 9 to 16 weeks of gestation. Although cases of intrauterine death in maternal infections > 20 weeks have been described in the literature, more recent studies have not been able to demonstrate it.

In the study of non-immune fetal hydrops, second trimester gestational loss and intrauterine fetal demise (refer to specific protocols), it is indicated to request a maternal serology for PVB19 and a molecular study (DNA-PVB19) in amniotic fluid, fetal blood or placental and/or fetal tissues.

Weeks at maternal infection	Fetal Hydrops	Intrauterine fetal demise without hydrops
< 9	<1%	4%
9-12	7%	11%
13-16	12%	9%
17-20	12%	2%
>20	<5%	<1%

Table: Probability of fetal hydrops and fetal death (in the absence of hydrops) according to gestational age at maternal infection by PVB19

7.3. Diagnosis of maternal infection:

It is generally an asymptomatic infection. Systematic screening for PVB19 during pregnancy is not recommended.

7.3.1. Serological diagnosis and maternal viremia:

- IgM: appears 3-4 days after the start of viremia (10-14 days after infection) and can last

3-4 months.

- IgG becomes positive at 7-14 days and persists for life.
- DNAemia persists after infection for several weeks (up to 4 months in 91% of patients)

7.3.2. Indications for requesting PVB19 serology during pregnancy (IgM and IgG):

- Compatible maternal symptoms. It is necessary to request serology in the case of non-vesicular exanthematous maternal rash.
- Compatible ultrasound abnormalities (see next section)
- Risk contact: Any household contact, "face-to-face" contact with an index case or same room > 15 min is considered a risk contact.
- Persistent thickened nuchal fold (> 16 weeks) in foetuses with normal karyotype/array

Pregnant women often seek consultation due to a history of **contact with an affected child**:

- If IgG + and IgM negative: past infection. No risk.
- If IgG and IgM negative: repeat 2-3 weeks after risk contact
- If IgG +/IgM positive or seroconversion in second determination (IgM is the first to become positive): indicates current infection. In these cases, fetal ultrasound follow-up should be carried out to rule out fetal anaemia or signs of hydrops (see point 7.5)

In seronegative (or serology unknown) pregnant women and an affected child, isolation of the index case is not necessary since transmission has probably already occurred at the time of consultation. In seronegative pregnant women who work in contact with children (teachers, daycare centres, etc.) it is advisable to keep them away from the workplace during the outbreak of infection.

7.4. Diagnosis of intrauterine infection:

The serological diagnosis or suspicion of maternal infection with PVB19, in the absence of ultrasound abnormalities consistent with fetal involvement or anaemia, does not justify the performance of amniocentesis for the detection of viral DNA in amniotic fluid.

In the context of the study of non-immune fetal hydrops (25% of hydrops with normal fetal anatomy are due to PVB19), PCR in fetal blood and amniotic fluid should be requested. Preferably, previous maternal serology should be available before the procedure, and PCR should be requested in cases of positive maternal IgG even if IgM is negative, as when fetal hydrops appears, IgM can be negative in 15-30% of pregnant women. Amniocentesis for the diagnosis of fetal infection could also be considered in selected cases with evidence of doubtful ultrasound findings (e.g. signs of cardiac dysfunction) and also in cases with difficulty in Doppler follow-up. As with other fetal infections, to obtain the maximum diagnostic sensitivity, amniocentesis should be performed from 4-6 weeks after maternal infection and always from 18 weeks of pregnancy.

In fetal infections due to PVB19, fetal blood IgM appears from 22 weeks, but only in 30% of

cases.

7.5. Diagnosis of fetal involvement and intrauterine therapy:

In infection with PVB19 during pregnancy, the most important thing is the early ultrasound diagnosis of **fetal anaemia**, preferably in the pre-hydrops phase.

7.5.1. Ultrasound follow-up:

The Doppler of the systolic velocity of the middle cerebral artery (VS-ACM) is the main method of follow-up. It is a sensitive and specific marker of fetal anaemia that allows early intervention in case of diagnostic suspicion. The frequency of control will be:

-Infection between 9-24 weeks: weekly control from 16 weeks and until 12 weeks have passed from maternal infection. Later, fortnightly control.

-Infection> 24 weeks: fortnightly control, since the risk of fetal anaemia is minimal.

The presence of hydrops signs (excess fluid in 2 or more cavities and/or subcutaneous oedema) should also be evaluated by ultrasound. In general, ascites predominates.

Other less specific ultrasound signs described are:

-Hyperechogenic bowel (isolated or prior to meconial peritonitis)
-Cardiomegaly or signs of cardiac dysfunction. A **fetal echocardiography** will be requested
-Alteration of the amniotic fluid (oligohydramnios or polyhydramnios)
-Placentomegaly (maximum placental thickness > 4 cm).

In foetuses surviving hydrops, a **neurosonography** will be requested together with an **intracranial MRI** at around 30-32 weeks to rule out cortical development abnormalities**.**

7.5.2. Fetal therapy:

An increase in the VS-ACM > 1.5 MoM or signs of fetal hydrops will indicate the need for cordocentesis in pregnancies of more than 18-20 weeks to assess the degree of fetal anaemia, and to indicate intrauterine transfusion if the haematocrit is < 30% (< -2 SD) (see specific clinical guidelines: 'Invasive Procedures' and 'Isoimmunisation'). More than one transfusion may be necessary before the anaemia is resolved. There have been described cases of persistent congenital aplastic anaemia of the Diamond-Blackfan type. In selected cases of fetal hydrops before 18-20 weeks with the inability to perform cordocentesis, it may be considered to perform an intraperitoneal transfusion without determining the degree of anaemia.

Breastfeeding is not contraindicated.

7.6. Neonatal follow-up:

It is recommended that, when there is a suspicion or confirmation of an intrauterine infection caused by PVB19, the newborn should undergo laboratory testing, which includes: a full blood count, IgG,

IgM PVB19, and PVB19 PCR. If there is a history of fetal anaemia or hydrops, it is advisable to refer to the reference Paediatric Infections Unit for neurological follow-up.

7.7. Investigation of PVB19 in cases of intrauterine fetal demise:

In addition to maternal serology (IgG and IgM) determination, in the investigation of second trimester pregnancy loss and intrauterine fetal demise, a PVB19 PCR in amniotic fluid should be requested. In the case of the inability to obtain amniotic fluid, a placental fragment (1 cm^3) will be submitted for PVB19 (DNA-PCR) analysis in the placental area near the umbilical cord and including fetal membranes. The sample should be sent to Microbiology in a sterile container with physiological saline.

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Sant Joan de Deu Code:	A-OBS-PC-0006-07

Appendix 1: MATERNAL SEROLOGICAL EVALUATION IN CASE OF ULTRASOUND ABNORMALITIES SUGGESTIVE OF FETAL INFECTION

In the presence of ultrasound abnormalities associated with fetal infection (e.g. Fetal hydrops, ascites, hyperechogenic bowel, intrahepatic or intracranial calcifications, microcephaly, hydrocephaly, destructive lesions of the central nervous system, Doppler VS-ACM suggestive of fetal anaemia, unexplained polyhydramnios (AFI > 25), or thickened placenta (> 4 cm)), the following serologies will be requested:

- Toxoplasmosis (IgG and IgM)
- CMV (IgG and IgM) (also if unexplained oligohydramnios (AFI < 5))
- Rubella (only in the absence of previous maternal serology or if the mother was non-immune)
- Syphilis (VDRL/RPR and Elisa treponema/TPHA)
- PVB19 (IgG and IgM) (especially in cases of suspicion of fetal anaemia, ascites, fetal hydrops)

It is not necessary to routinely request serologies for Herpes virus 1-2, Epstein-Barr, and Enterovirus. However, in selected cases of fetal myocardiopathy, it may be appropriate to request a Coxsackie serology.

If an acute infection (positive IgG and IgM) is confirmed or suspected, or if there are multiple ultrasound abnormalities present along with a positive IgG (especially for CMV, even if IgM is negative), the patient should be referred to the Infections Unit/Fetal Medicine Unit for evaluation and to assess the need for an invasive procedure.

Appendix. 2 EVALUATION OF INFECTIONS IN DIFFERENT CONDITIONS

A. Evaluation of infections in fetal hydrops:

1. Maternal serology:

- Toxoplasmosis (IgG and *IgM*)
- Syphilis (VDRL/RPR and treponemal Elisa/TPHA)
- Rubella (IgG and IgM)
- CMV (IgG and IgM)
- PVB19 (IgG and IgM)

2. Amniotic fluid PCR analysis for viral or parasitic DNA: (except in cases where maternal serology has already been performed and results for both IgG and IgM are negative)

- Toxoplasmosis
- CMV
- HSV-1 y 2
- PVB19
- Lymphocytic choriomeningitis virus
- Treponema Pallidum

B. Evaluation of infections in second trimester pregnancy loss and intrauterine fetal demise:

1. Maternal serology:

- Toxoplasmosis (IgG and *IgM*)
- Syphilis (VDRL/RPR and treponemal Elisa / TPHA)
- CMV (IgG and IgM)
- PVB19 (IgG and IgM)
- Rubella (only in the absence of previous maternal serology or if the mother was non-immune)

2. PCR analysis for viral or parasitic DNA in amniotic fluid, placental and fetal tissue:

The best sample for infection testing is amniotic fluid. Whenever possible, 5-10 cc should be sent to Microbiology for CMV and PVB19 (DNA-PCR) testing. If amniotic fluid is not available, a placental fragment (1 cm^3) should be sent to Microbiology for CMV and PVB19 (PCR) testing in the placental area near the umbilical cord including chorion frondosum and fetal membranes. The sample should be sent in a sterile container with physiological saline. In selected cases with a history of specific ultrasound abnormalities for any of the infections, or compatible maternal symptoms, or a history of exposure to risk, the suspicion of infection should be noted in the request for necropsy. The pathologist will send the tissues directly to Microbiology for testing.

- CMV
- HSV1-2
- Chickenpox
- Toxoplasmosis
- PVB19
- Syphilis

C. Evaluation of infections in foetuses with IUGR:

1. Maternal serology:

- Rubella (IgG and IgM) in the absence of previous serology or negative immunity in the 1st trimester
- Syphilis (VDRL/RPR and Elisa treponema/TPHA) in IUGR (excludes SGA)
- Malaria: if population at risk and IUGR (excludes SGA)

2. CMV evaluation:

- If invasive procedure for genetic study is indicated: add a sample for CMV PCR in amniotic fluid
- If invasive procedure not indicated: request maternal serology (IgG and IgM) only in IUGR (excludes SGA)
    - o IgG and IgM negative: infection is ruled out
    - o IgG and IgM positive: perform amniocentesis for CMV PCR
    - o Positive IgG with negative IgM: does not rule out fetal infection.

  Perform amniocentesis only if there is any other ultrasound marker of CMV infection (CNS or extra-CNS) except isolated oligohydramnios

D. Investigations for infections in polyhydramnios:

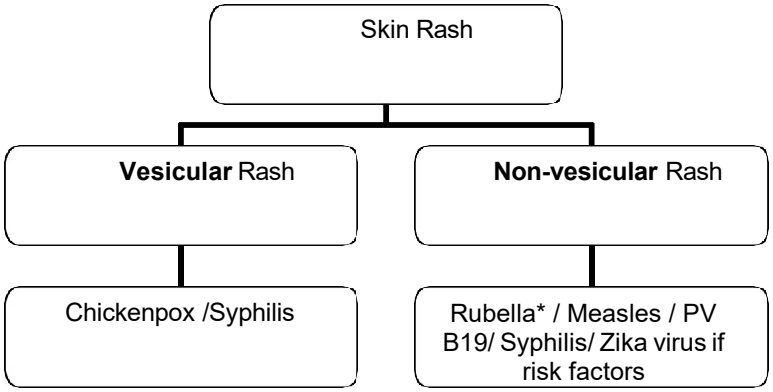
1. Maternal serology:

- CMV (IgG and IgM)
- Toxoplasmosis (IgG and *IgM*)
- PVB19 (IgG and IgM)
- Syphilis (VDRL/RPR and Treponemal Elisa/TPHA)

2. AF viral DNA determination (PCR):

In the absence of other ultrasound markers, the minimal association of isolated polyhydramnios with fetal infections does not justify this study.

E. Maternal serological evaluation in the presence of rash:



*To request maternal serology even if previous immunity known

For Rubella, Chickenpox and PVB19: to request IgG and IgM, for Syphilis: VDRL/RPR and Treponemal Elisa/TPHA, for Zika: IgG, IgM and PCR in maternal blood and urine (*detailed in the specific protocol)*

Annex

Appendix 5

Ultrasound measurement of the fetal liver from the 20th week.

(Vintzileos et al., Obstet Gynecol 1985; 66:177-80)

Gestational age (wk)	No. of measurements	Arithmetic mean (mm)	± 2 SD (mm)
20	8	27.3	6.4
21	2	28.0	1.5
22	4	30.6	6.7
23	13	30.9	4.5
24	10	32.9	6.7
25	14	33.6	5.3
26	10	35.7	6.3
27	20	36.6	3.3
28	14	38.4	4.0
29	13	39.1	5.0
30	10	38.7	5.0
31	13	39.6	5.7
32	11	42.7	7.5
33	14	43.8	6.6
34	11	44.8	7.1
35	14	47.8	9.1
36	10	49.0	8.4
37	10	52.0	6.8
38	12	52.9	4.2
39	5	55.4	6.7
40	1	59.0	
41	2	49.3	2.4

SD = standard deviation.
* Mean length ± 2 SD.

Annex

Appendix 6

Cut-off point to define placental thickness at risk of infection by CMV.

(From: La Torre et al., Clin Infect Dis 2006;43:994-1000)

Table 4. Cutoff values for maximal placental thickness, by week of gestation.

Comparison group, week of gestation	Cutoff value, mm	Area under ROC	Sensitivity (95% CI); no. of women	Specificity (95% CI); no. of women	PPV (95% CI); no. of women	NPV (95% CI); no. of women
Women with primary CMV infection with fetuses or neonates without disease vs. women with primary CMV infection with fetuses or neonates with disease						
16	≥32	0.83	0.86 (0.42–1.00); 7	0.86 (0.65–0.97); 22	0.67 (0.30–0.92); 9	0.95 (0.75–1.00); 20
20	≥40	0.84	0.69 (0.39–0.91); 13	0.88 (0.73–0.96); 40	0.64 (0.35–0.87); 14	0.90 (0.76–0.97); 39
24	≥40	0.90	0.86 (0.42–1.00); 7	0.81 (0.64–0.92); 36	0.46 (0.19–0.75); 13	0.97 (0.83–1.00); 30
28	≥51	0.77	0.71 (0.29–0.96); 7	0.89 (0.73–0.97); 35	0.55 (0.21–0.86); 9	0.94 (0.80–0.99); 33
32	≥54	0.86	0.80 (0.28–1.00); 5	0.92 (0.78–0.98); 37	0.57 (0.18–0.90); 7	0.97 (0.85–1.00); 35
36	≥61	0.94	1.00 (0.29–1.00); 3	0.82 (0.69–0.95); 22	0.43 (0.10–0.82); 7	1.00 (0.82–1.00); 18

NOTE. NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic curve.

Annex: INFORMED CONSENT INFORMADO

APPENDIX 7: TABLE SUMMARY OF SEROLOGICAL SCREENING DURING PREGNANCY

Serology	1st trimester (8-14 wks)	2nd trimester (24-28 wks)	3rd trimester (33-36 wks)	
HBV (HbsAg)	**X**		**X If risk factor and no immunisation**	In the event of a positive result, request an activity profile (HBeAg, quantitative HBV DNA). If risk factors and anti-HBs and anti-HBc are negative, initiate vaccination after 1st trimester (see specific protocol)
HCV (antiHCV Abs)	**X**			In the event of a positive result, request HCV RNA
HIV	**X**	**X If risk factors**	**X**	Couples with irregular condom use and patients with unknown HIV serology during gestation: request urgent HIV serology (rapid test or immediate ELISA) at the time of delivery.
CMV (IgG and IgM. If IgM positive, IgG avidity)	**x**			Universal screening at the time of 1st trimester blood test. In case of positive IgM, an alert will be generated to the clinician and the laboratory will automatically perform IgG avidity assay with the same sample. If the avidity is low or medium (depending on the weeks), initiation of valaciclovir is recommended until amniocentesis. If IgG is negative and IgM positive, it will be repeated after two weeks to differentiate between early infection or false positive IgM. In seronegative pregnant women, serology should not be repeated in the second or third trimester.
Syphilis (Treponemal test)	**X**		**X If risk factors**	If ELISA IgG Treponemal (or TPHA) is positive, the laboratory will directly determine VDRL or RPR (assessment of activity) and IgM treponemal determination (temporal assessment) will be requested. If Elisa is positive and VDRL negative and there is no previous history of treatment, a 2nd confirmatory treponemal test (LIA immunoassay) will be performed.
Rubella (IgG)	**X**			If protective titres (≥ 10 IU/ml), the IgM request is not necessary. If non-protective titres (< 10/ml) serology does not need to be repeated during pregnancy. Vaccination should be initiated in the postpartum period before discharge (1st dose) with the 2nd dose administered within 1-2 months at the reference GP or Vaccination Centre.
CHAGAS (IgG anti-Trypanosoma cruzi)	**X**			Target population: Latin American pregnant women, mother of the Latin American pregnant woman or a stay of over 1 month in any Latin American country (excluding Caribbean Islands). In case of a positive first ELISA test, the laboratory will automatically perform a confirmatory test (recombinant ELISA). In case of a discordant result, the laboratory will perform a third tie-breaking test (IFI).
Toxoplasma/ PVB19				Serological tests (IgG+IgM) are indicated if there is a clinical presentation compatible with maternal infection, a risk contact is identified, ultrasound markers compatible with fetal infection are found (see specific section) and CMV and PVB19 serology if the nuchal fold is above the 99th percentile (after 16 weeks) and with a normal karyotype/array CGH. In the case of CMV, serology should also be requested if there is severe and early IUGR (< p3 and < 28 weeks). If the IgG/IgM tests are positive, then Toxo: request IgG avidity assay. For PVB19, request viral DNA in the mother's blood.

Annex: INFORMED CONSENT INFORMADO

APPENDIX 7: TABLE SUMMARY OF SEROLOGICAL SCREENING DURING PREGNANCY (II)

Serology	1st trimester (8-14 wks) or 1st appt	2nd trimester (24-28 wks)	3rd trimester (33-36 wks)	
ZIKA	X			Indicated in: • Women who have travelled to an active Zika endemic area during pregnancy or two months prior • Women who have had unprotected sexual intercourse during pregnancy or two months prior with a partner who has travelled to an active Zika endemic area in the last three months (if the partner is male) or in the last two months (if the partner is female) The recommended diagnostic protocol will differ depending on the time elapsed since the risk exposure (< or > 2 weeks), the symptoms, and the previous exposure history to the current pregnancy: -**Exposure < 2 weeks WITH compatible symptoms**: PCR for Zika, Dengue and Chikungunya in serum PCR Zika in urine Zika*, Dengue and Chikungunya IgG/IgM serology -**Exposure < 2 weeks WITHOUT symptoms**: PCR for Zika in serum PCR Zika in urine Zika, Dengue IgG/IgM serology. For pregnant women with the possibility of repeated exposure to ZIKV or DENV previous to pregnancy, only request IgM Zika (and Dengue) **-Exposure > 2 weeks WITH history of compatible symptoms**: Zika*, Dengue and Chikungunya IgG/IgM serology If ZIKV IgG or IgM positive: ZIKV PCR in serum and urine -**Exposure >2 weeks WITHOUT SYMPTOMS:** Zika* and Dengue IgG/IgM serology. For pregnant women with the possibility of repeated exposure to ZIKV or DENV previous to pregnancy, only request IgM Zika* (and Dengue). If ZIKV IgG or IgM positive: ZIKV PCR in serum and urine *Positive ZIKV serology requires confirmation by neutralisation except if PCR positive.