From Ultrasound to PCR:
A Modern Approach to Prenatal Pathogen Detection

Ph.D. Thesis

Nándor Tűzkő, M.D.
Department of Obstetrics and Gynecology
Saint Margaret's Hospital

Supervisor:

Attila Keresztúri, M.D., Ph.D.
Department of Obstetrics and Gynecology
Albert Szent-Györgyi Medical School
University of Szeged

Director of Doctoral School of Clinical Medicine:
Lajos Kemény, M.D., D.Sc.
Director of Reproductive Health Programme:
Gábor Németh, M.D., Ph.D.
.

University of Szeged
Faculty of General Medicine
Department of Obstetrics and Gynecology
Albert Szent-Györgyi Medical Centre
Szeged, 2025.

1 Publications

I.

Somogyvári, Ferenc; **Tűzkő, Nándo**r; Keresztúri, Attila; Párducz, László; Szécsényi, Mária; Endrész, Valéria; Ábrók, Marianna; Ardizzone, Caleb M.; Burián, Katalin; Virók, Dezső Péter

Comparison of the inhibitory effects of Lactobacillus supernatant and coculture on Gardnerella vaginalis

BMC RESEARCH NOTES 18 : 1 Paper: 346 , 7 p. (2025)

II.

Tűzkő, Nándor; Bartek, Virág; Simonyi, Atene; Harmath, Ágnes; Szabó, István; Virók, Dezső Péter; Beke, Artúr

Associations between Fetal Symptoms during Pregnancy and Neonatal Clinical Complications with Toxoplasmosis

CHILDREN (BASEL) 11 : 9 Paper: 1111 , 13 p. (2024)

III.

Tűzkő, Nándor; Bartek, Virág; Beke, Artúr; Ács, Nándor

A méhen belüli toxoplasmafertőzés magzati ultrahangeltérései és a fertőzésnek kitett újszülöttek postnatalis tünetei

MAGYAR NŐORVOSOK LAPJA 88 : 2 pp. 85-94. , 10 p. (2025)

2 Abbreviations

BV	bacterial vaginosis
ET	embrio transfer
G. vaginalis	Gardnerella vaginalis
ICSI	intracytoplasmic sperm injection
Ig	immunglobulin
IUGR	intrauterine growth restriction
IVF	in vitro fertilisation
OD_{600}	optical density at 600 nanometers
PCR	polymerase chain reaction
PTB	preterm birth
SGA	small for gestational age
SNI	serious neurological injury
SUA	single umbilical artery
TOP	termination of pregnancy
TORCH	Toxoplasma, Other (including syphilis and varicella), Rubella, Cytomegalovirus (CMV), and Herpes Simplex Virus (HSV).

3 Introduction

Infections during pregnancy represent a significant clinical concern due to their potential to adversely affect both maternal and fetal health. For medical health professionals, understanding the complexities of these infections is essential, yet often challenging due to the wide range of pathogens involved, their varied modes of transmission, and the nuanced approaches required for diagnosis, treatment, and prevention. Pregnancy-associated infections such as those caused by *Toxoplasma gondii*, *Listeria monocytogenes*, *Cytomegalovirus*, *Rubella virus*, *Herpes simplex virus*, *Treponema pallidum*, and *Gardnerella vaginalis* can result in severe complications ranging from miscarriage and premature labour to congenital abnormalities and neonatal infections.

The TORCH panel is a crucial screening tool during pregnancy that tests for five infectious pathogens: Toxoplasma, Other (including syphilis and varicella), Rubella, Cytomegalovirus (CMV), and Herpes Simplex Virus (HSV). Detection of these infections is vital as they can cause severe congenital anomalies, fetal growth restriction, and other complications [Alsamarai et al., 2013; Fitzpatrick et al., 2021]. Routine screening helps to identify pregnant women at risk and enables early interventions to mitigate adverse neonatal outcomes [Al-Hakami et al., 2020]. Despite its importance, the interpretation of results can be complex, and the diagnostic yield may vary, highlighting the need for careful consideration in clinical practice [Shqara et al., 2023; Lopez-Moreno et al., 2021].

One of the main difficulties for medical health professionals lies in grasping the unique immunological environment of pregnancy. The maternal immune system is modulated to tolerate the fetus, which can increase susceptibility to certain infections and alter typical immune responses.

Recent research into the modulation of the immune system during gestation has highlighted the complex interplay between immune tolerance and immune responses, which are necessary for maintaining both maternal health and fetal development (Fig 1.).

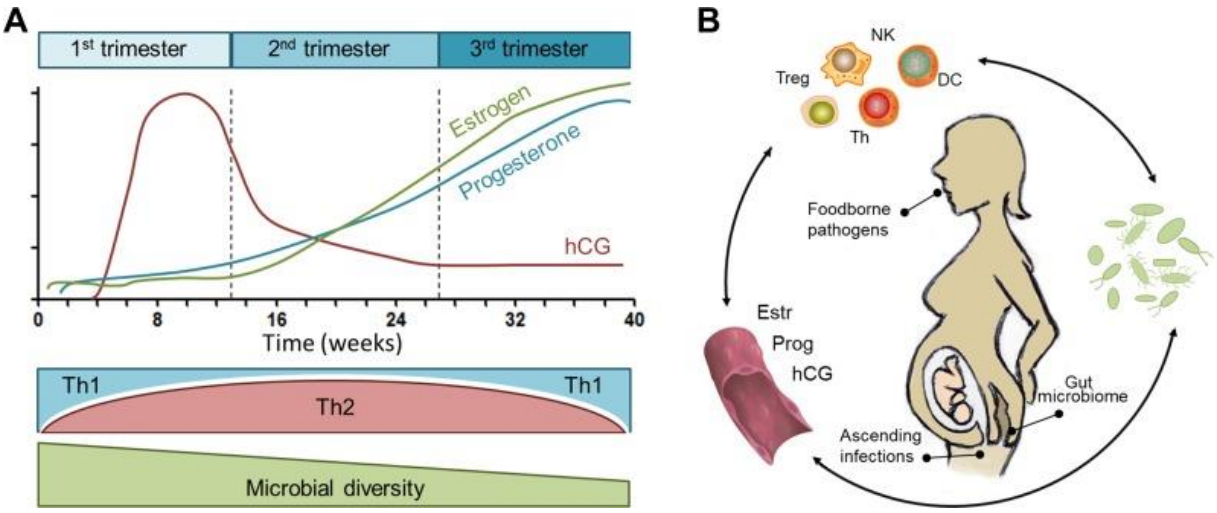

Figure 1: A. Hormonal changes during pregnancy in immunological adaptation (left side). B. Impact of the maternal gut, oral and vaginal microbiome present relationship with immunomodulation during pregnancy (right side).

Moreover, studies analysing the impact of the maternal microbiome depict an intricate relationship with immune modulation during gestation. Understanding the impact and mechanisms of these infections during pregnancy is essential for early diagnosis, prevention, and therapeutic intervention to reduce the risk of vertical transmission and ensure optimal maternal-fetal health.

3.1 Toxoplasmosis

Toxoplasma gondii, an obligate intracellular parasite, is the causative agent of toxoplasmosis and a member of the TORCH complex—a group of pathogens known to cause congenital infections [Voekt et al 2017]. Although often asymptomatic in immunocompetent individuals, toxoplasmosis during pregnancy can lead to severe fetal complications such as hydrocephalus, intracranial calcifications, chorioretinitis, and even miscarriage [Belanger et al 2025, Kochanowsky et al 2018]. The risk of fetal transmission increases with gestational age, but the severity of outcomes is usually greater when infection occurs earlier in pregnancy.

Timely diagnosis and treatment are essential to prevent serious complications [Bollani et al 2022]. The severity of congenital toxoplasmosis depends on the week of gestation, and whether the pregnant woman has had the infection before [Weiss et al 2009].

Guerina et al. found that 40% of the confirmed cases of intrauterine infection were found to be free of abnormalities on routine neonatal examination [Guerina et al., 1994], but later, ophthalmological or neurological abnormalities were revealed. Therefore, early diagnosis of these subclinical abnormalities could reduce the extent of subsequent damage.

3.2 Gardnerella infection

Gardnerella vaginalis (G.vaginalis) is a facultative anaerobic bacterium commonly associated with bacterial vaginosis (BV), a condition characterized by the disruption of normal vaginal flora, particularly the depletion of protective *Lactobacillus* species. BV is a common condition during pregnancy that can lead to severe complications if left untreated. The pathogenesis of Gardnerella vaginalis in BV involves the production of virulence factors, such as sialidase and vaginolysin, which are associated with its ability to invade the vaginal epithelium and form biofilms [Rosca et al., 2022]. These virulence factors complicate the effective treatment, contributing to recurrence and difficulties in management. Moreover, the immune response in pregnant women may be compromised during BV, further exacerbating the risk of intrauterine infections. The complex interactions between hormonal changes during pregnancy and the immunomodulation associated with *G. vaginalis* suggest that a multifaceted approach is necessary for effectively managing BV to reduce potential complications [Bornes et al.,2021].

4 Aims of studies

4.1 Toxoplasmosis

The aim of our study was to investigate which are the ultrasound abnormalities most commonly associated with active Toxoplasma infection and the long-term complications of the infection. We aimed to investigate whether there is an association between expected complications and prenatal ultrasound abnormalities.

4.2 Gardnerella infection

Screening the antimicrobial activity of vaginal probiotic candidates against relevant microorganisms, including *G. vaginalis*, is a crucial step in the selection process. A common method involves testing the cell-free supernatants of lactobacilli in liquid cultures of pathogens, such as *G. vaginalis*. While this method is relatively simple, it fails to replicate the natural interactions between lactobacilli and *G. vaginalis* that occur *in vivo* within a shared microenvironment. Coculture methods, in which *Lactobacillus* strains are incubated with *G. vaginalis*, can address this issue. Although these methods are well-established, they require considerable manual work, making them less suitable for screening. Our goal was to develop a cost-effective, rapid, reproducible screening method that requires slight manual labour.

5 Hypothesis

5.1 Toxoplasmosis

1. The review of literature and our prenatal ultrasound investigation can present a sensitive and specific ultrasound marker for detecting active Toxoplasma infection.
2. The fetal ultrasound alterations will indicate later neonatal complications. This allows us to predict the appearance and extent of neonatal infectious complications with a non-invasive method.

5.2 Gardnerella infection

1. The direct qPCR will be effective for measuring the antimicrobial activity, as a screening method in bacterial vaginosis.
2. Cocultures that may reflect to *in vivo* microbial interactions better should also be used to evaluate *Lactobacillus*-mediated inhibition of *G. vaginalis* growth.

6 Material and methods

6.1 Toxoplasmosis

Following a thorough literature review that encompassed all prenatal ultrasound markers, we designed a Toxoplasma infection study. In our prospective study, we analysed cases of recent maternal Toxoplasma infections confirmed by serological testing at the Department of Obstetrics and Gynecology, Semmelweis University, Hungary, between 1996 and 2020.

We included in the study those pregnant women who applied for genetic counselling at the department and who were confirmed to be infected with Toxoplasma by serological testing and who requested amniocentesis. Those who had a miscarriage before amniocentesis or did not request amniocentesis were excluded from the study.

As for Toxoplasma detection we applied serological tests, ultrasound investigations, amniocentesis with DNA isolation, fluorescent PCR and DNA fragment analysis.

6.2 Gardnerella infection

Two *L. crispatus* (*L. crispatus*-200, *L. crispatus*-202), two *L. gasseri* (*L. gasseri*-212, *L. gasseri*-224), one *L. jensenii (L. jensenii*-241*)*, and one *G. vaginalis* isolate were obtained during a routine microbiology diagnosis of vaginal swab samples (Department of Medical Microbiology, University of Szeged, Szeged, Hungary).

As for Gardnerella infection tests we applied bacterial stain, growth kinetics, direct qPCR, inhibition of *G. vaginalis* growth by *Lactobacillus* supernatant and coculture.

7 Results

7.1 Toxoplasmosis

Based on our literature review covering all prenatal ultrasound markers, the most frequent prenatal ultrasound signals suggestive of congenital toxoplasma infection according to the available literature were hydrops fetalis, ascites, pericardial effusion, pleural effusion and hyperechogenic bowel. As other abnormalities, brain parenchymal anomalies, ventriculomegaly, hepatosplenomegaly, SUA and SGA were present.

In the study, 133 cases of ultrasound abnormalities were detected during pregnancy, while in 105 cases no abnormalities were found during ultrasound examinations. Figure 2. demonstrates the distribution of ultrasound abnormalities.

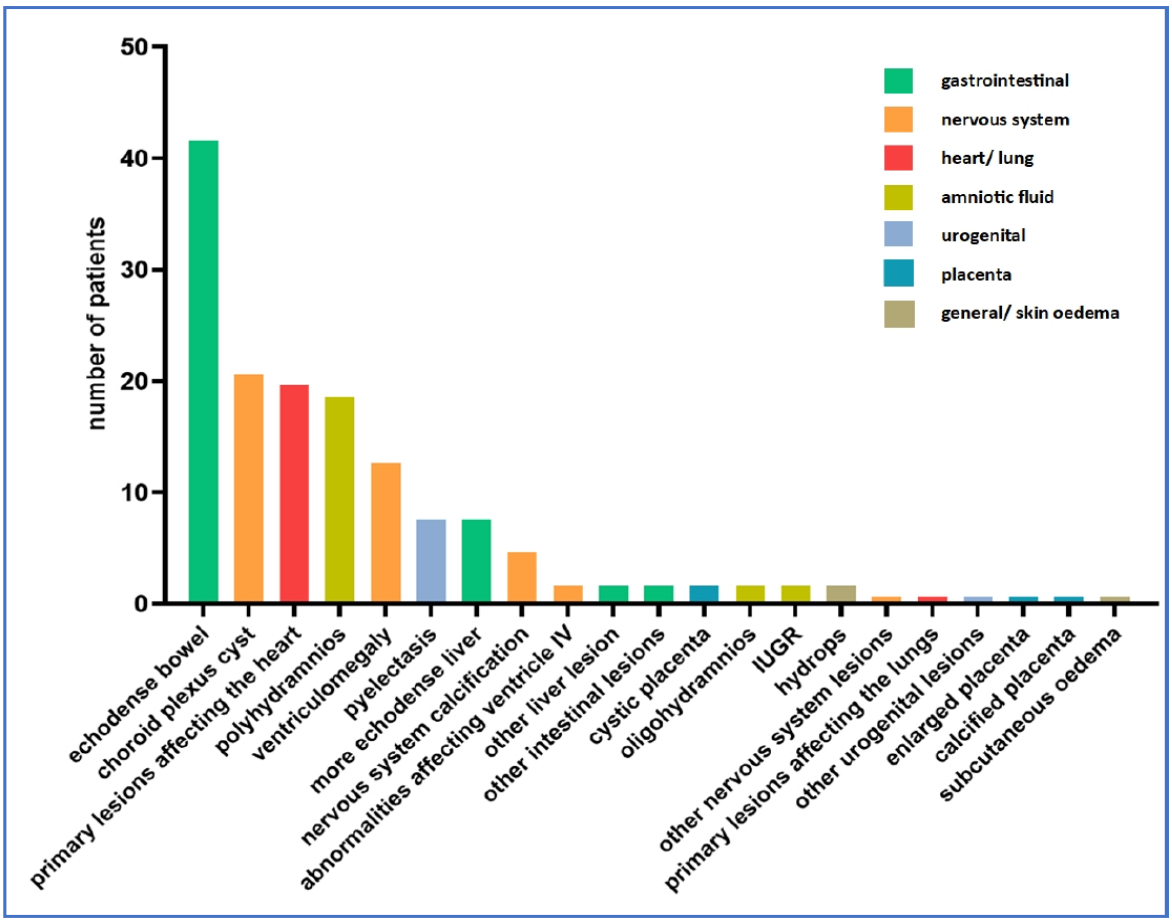

Figure 2 . Distribution of ultrasound abnormalities in toxoplasmosis (n = 105 cases).

Serological testing of maternal blood showed IgG positivity in 234 out of the 238 cases with positive IgM, whereas four cases had borderline (equivocal) IgM. From amniotic fluid sampling, eight cases of Toxoplasma were detected by PCR, and negative results were obtained in 230 cases. Of the positive PCR samples, five pregnancies were aborted, and three pregnancies were carried to term. In all three of the latter cases, a mature baby was born. Although the positive amniocentesis PCR rate was significantly lower, it can be seen that when the amniocentesis sample was negative, a higher proportion of pregnant women chose to be carried to term.

Neonatological follow-up was performed in 139 cases and 117 cases had no abnormalities during the follow-up, whereas 22 cases had detectable complications probably related to Toxoplasma infection. Figure 3 shows the distribution of neurological and other abnormalities detected in the follow-up period.

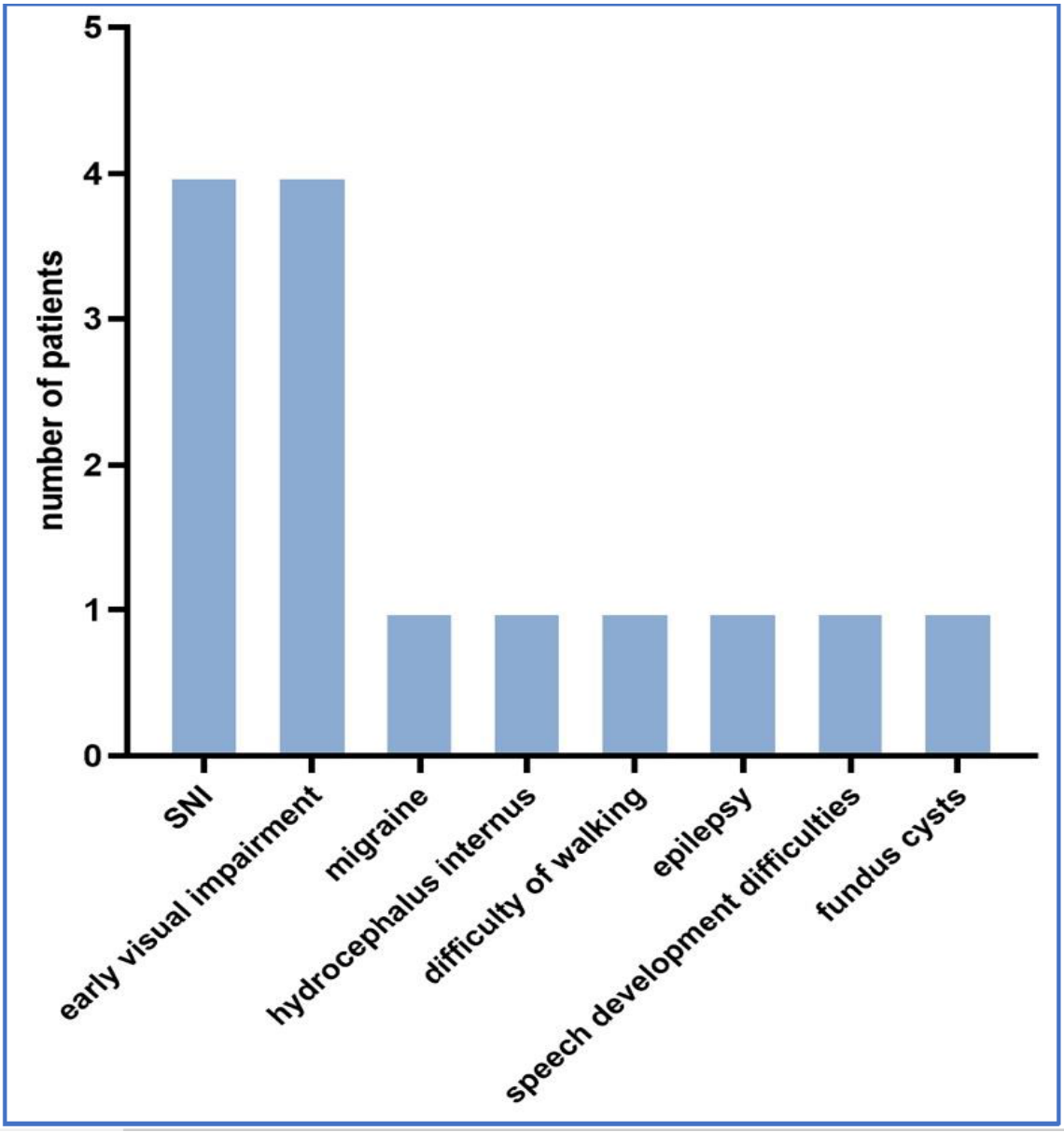

Figure 3. Distribution of neurological and other abnormalities detected during follow-up in Toxoplasmosis

7.2 Gardnerella infection

To compare the ability of MRS and NYC-III media to support *G. vaginalis* and *Lactobacillus* growth, we cultured both bacteria in parallel in each medium (Fig. 4 A). These data indicate that MRS may not support the growth of a low amount of *G. vaginalis*. *Lactobacillus* growth was also tested in the two media (Fig. 4B). Interestingly, only *L. gasseri*-224 grew better in the well-established *Lactobacillus* medium MRS than in NYC-III. *L. crispatus*-200 exhibited similar growth kinetics in both media, and the remaining three isolates grew better in NYC-III than in MRS. Altogether, these experiments showed that NYC-III supports the growth of both *G. vaginalis* and the *Lactobacillus* strains and is suitable for coculture experiments.

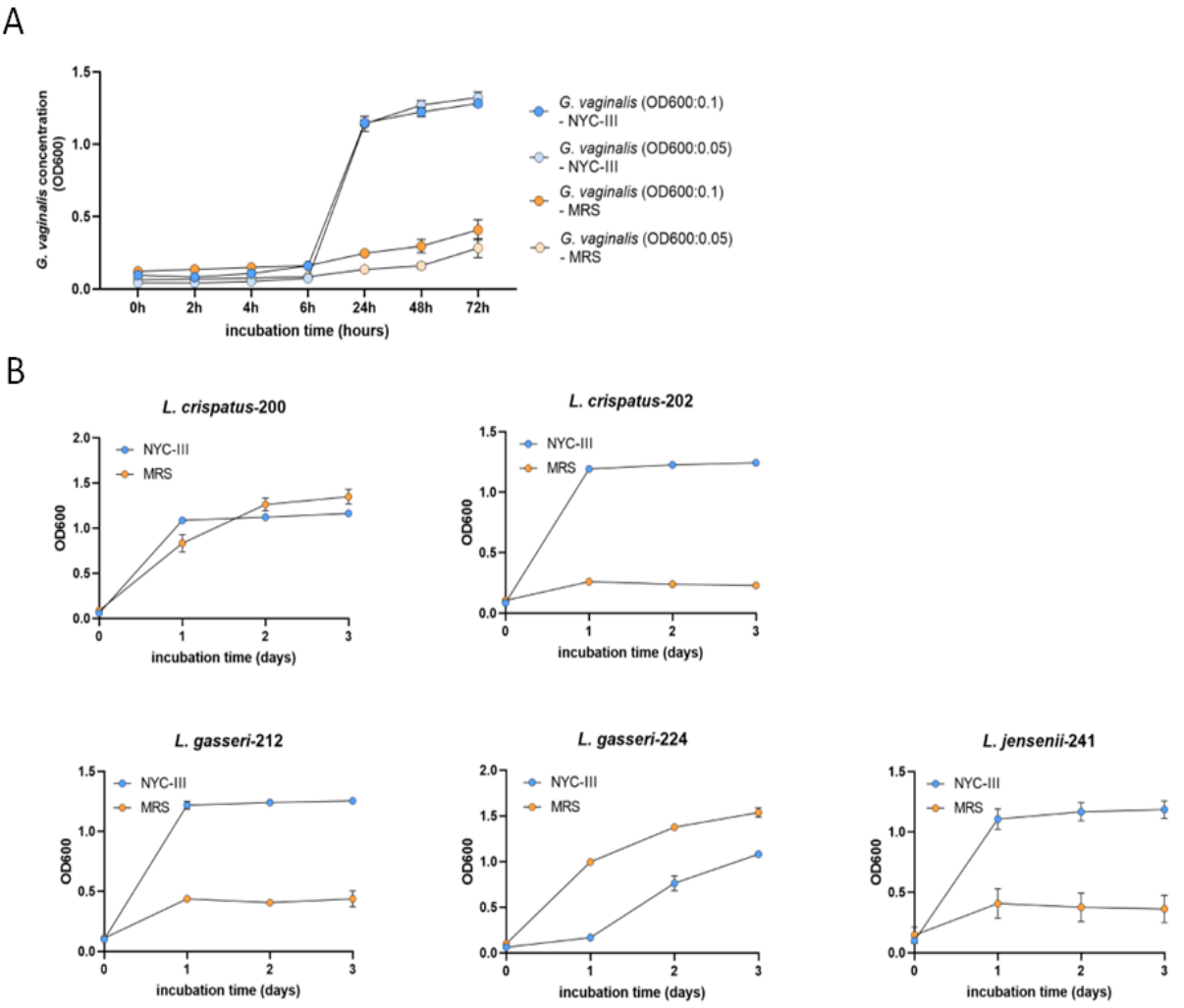

Figure 4. Growth kinetics of G. vaginalis and Lactobacillus spp. in MRS and NYC-III media. (A) G. vaginalis growth in MRS and NYC-III media. G. vaginalis was propagated in MRS or NYC-III media for 72 h at 37°C with 5% CO_2. (B) Lactobacillus spp. growth in MRS and NYC-III media. Lactobacillus strains were propagated in MRS or NYC-III media for 72 h at 37°C with 5% CO_2. OD_{600} was measured at each timepoint (n=4). Data are presented as mean ± SD.

Cell-free supernatants from each strain were tested at 50%, 25%, and 12.5%. At 50%, three strains reduced *G. vaginalis* growth to approximately 28.2-31.9% of the control, while *L. gasseri*-224 and *L. gasseri*-212 were less effective, only reducing growth to 48.6-63.9%. At 25%, *L. jensenii*-241 and *L. crispatus*-202 exhibited the greatest inhibition, limiting *G. vaginalis* growth to 52.5% and 57%, respectively. At 12.5%, all strains restricted growth to approximately 80% of the control.

For coculture experiments, we tested three initial *Lactobacillus*: *G. vaginalis* ratios: 10:1, 1:1, and 1:10. The direct qPCR method was employed to monitor *G. vaginalis* growth. *L. jensenii*-241 and *L. gasseri*-224 significantly inhibited *G. vaginalis* growth at all three inoculum ratios.

L. crispatus-200 and *L. crispatus*-202 showed marked growth inhibition only, when there were either 10-fold more *Lactobacillus* or equal amounts of *Lactobacillus* in the coculture samples initially. *L. gasseri*-212 was the weakest inhibitor, exhibiting significant inhibition only, when there was a 10-fold increase in *Lactobacillus* in the cocultures initially.

8 Conclusions

Screening for prenatal infections is important during pregnancy, because the earlier the exposure is, the more serious the consequences are.

In toxoplasmosis the most common ultrasound abnormalities affect the nervous system and the gastrointestinal system. In case of suspicion, in addition to indirect methods, it is recommended to carry out a PCR test, helping the pregnant woman to make a decision regarding pregnancy.

We suggest 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), then initiation of drug therapy is recommended until amniocentesis. If IgG is negative and IgM positive, then 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

Regarding the severity and appearance of complications, we did not find any prenatal factors with which we could prove a significant correlation. While ultrasound and PCR testing

remain indispensable tools in managing toxoplasmosis during pregnancy, further research is needed to identify additional factors that may contribute to the variability in clinical outcomes. A multidisciplinary team experienced in pregnancies complicated by toxoplasmosis must carry out the follow-up and care.

BV screening is important during gestation. Our study suggests that cocultures that may reflect to *in vivo* microbial interactions better should also be used to evaluate *Lactobacillus*-mediated inhibition of *G. vaginalis* growth. Our direct qPCR method enables rapid and quantitative measurement of antimicrobial activity in cocultures. Lactobacillus therapy, an intimate wash that uses probiotics containing Lactobacillus strains, is generally considered safe during pregnancy. Our study suggests potential benefits, such as reduced risk of overgrowth of G. vaginalis and bacterial vaginosis. This way the maternal health can be improved.

9 Limitations

9.1 Toxoplasmosis

A limitation of our study is that in the study period prenatal MRI was not widely used, and therefore in many cases ultrasound diagnosis was not confirmed. Moreover, the time of follow-up was variable in the reports.

9.2 Gardnerella infection

Our qPCR method was tested using the NYC-III medium. While the direct qPCR method performed well with this medium, employing a more diverse range of media would better demonstrate the robustness of the method. Another limitation is the number of Lactobacilli strains that we tested. Analyzing additional strains would clarify whether the difference in inhibitory activity mediated by cell-free supernatant versus coculture is common among Lactobacilli.

10 New findings and results:

10.1 Toxoplasmosis

1. We recommend a universal toxoplasmosis screening at the time of maternal blood test in the 1st trimester.
2. The ultrasound screening is important in fetal life, but it is not considerably specific to diagnose fetal infections.
3. We did not find any specific prenatal ultrasound features, with which we could prove significant screening results for Toxoplasmosis.
4. Amniocentesis is an important diagnostic tool for fetal toxoplasmosis. It allows for the detection of Toxoplasma gondii DNA in the amniotic fluid using PCR, which can confirm or rule out fetal infection. This information is crucial for guiding treatment decisions for both the mother and the fetus.

10.2 Gardnerella infection

1. *Lactobacillus*-mediated inhibition of *G. vaginalis* growth was proven.
2. The direct qPCR was effective for measuring the antimicrobial activity.
3. We compared the inhibitory effects of *Lactobacillus* cell-free supernatants and those from *Lactobacillus*-*G. vaginalis* coculture on *G. vaginalis* growth. The cocultures that may reflect to *in vivo* microbial interactions better should also be used to evaluate *Lactobacillus*-mediated inhibition of *G. vaginalis* growth.
4. Lactobacillus therapy improves maternal health by reducing the risk of *G. vaginalis* overgrowth and bacterial vaginosis.

11 References

- Al-Hakami, A., Paul, E., Al-Abed, F., Alzoani, A., Shati, A., Assiri, M.,Chandramoorthy, H. Prevalence of toxoplasmosis, rubella, cytomegalovirus, and herpes (TORCH) infections among women attending the antenatal care clinic, maternity hospital in Abha, South Western Saudi Arabia. Saudi Medical Journal, 2020;41(7), 757-762. https://doi.org/10.15537/smj.2020.7.25121
- Alsamarai, A. and Aljumaili, Z. Seroepidemiology of toxoplasma, rubella, cytomegalovirus and herpes simplex virus-2 in women with bad obstetric history. Part i: toxoplasma and rubella infections. Our Dermatology Online, 2013;4(4), 522-535. https://doi.org/10.7241/ourd.20134.135
- Belanger, B.G., Lui, F. Embryology, Teratology TORCH. . In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan (updated 24 Jul 2024) Available from: https://www.ncbi.nlm.nih.gov/books/NBK545148/
- Bollani, L.; Auriti, C.; Achille, C.; Garofoli, F.; De Rose, D.U.; Meroni, V.; Salvatori, G.; Tzialla, C. Congenital Toxoplasmosis: The State of the Art. Front. Pediatr. 2022; 10, 894573. doi: 10.3389/fped.2022.894573
- Bornes, S.; Camarès, O.; Paquet-Gachinat, M.; Philippe, V.; Jacques, R.; Caroline, D.; Adrien, N. Rapid Quantitative PCR Assay for the Detection of the Three Vaginal Pathogens Candida,Gardnerella and Atopobium as well as the Commensal Lactobacillus Genera. Int. J. Microbiol. Biotechnol. 2021, 6 (3), 71–77. doi: 10.11648/j.ijmb.20210603.12
- Fitzpatrick, D., Holmes, N., & Hui, L. A systematic review of maternal torch serology as a screen for suspected fetal infection. Obstetrical & Gynecological Survey, 2022;77(7), 398-399. https://doi.org/10.1097/01.ogx.0000852748.18194.82
- Guerina, N.G.; Hsu, H.W.; Meissner, H.C.; Maguire, J.H.; Lynfield, R.; Stechenberg, B.; Abroms, I.; Pasternack, M.S.; Hoff, R.; Eaton, R.B.; et al. Neonatal serologic screening and early treatment for congenital Toxoplasma gondii infection. The New England Regional Toxoplasma Working Group. N. Engl. J. Med. 1994, 330, 1858–1863. doi: 10.1056/NEJM199406303302604.
- Kochanowsky, J. A. and Koshy, A. A. Toxoplasma gondii. Curr. Biol. 2018, 28, R770–R771. doi: 10.1016/j.cub.2018.05.035.
- López-Moreno, A.; Aguilera, M. Vaginal Probiotics for Reproductive Health and Related Dysbiosis: Systematic Review and Meta-Analysis. J. Clin. Med. 2021, 10, 1461, doi:10.3390/jcm10071461.
- Rosca, A.S.; Castro, J.; Sousa, L.G.V.; França, A.; Cavaleiro, C.; Salgueiro, L.; Cerca, N. Six Bacterial Vaginosis-Associated Species Can Form an In Vitro and Ex Vivo Polymicrobial Biofilm That Is Susceptible to Thymbra Capitata Essential Oil. Front. Cell. Infect. Microbiol. 2022, 12, 824860, doi:10.3389/fcimb.2022.824860.
- Shqara, A.R., Bussidan, S., Glikman,D.,Rechnitzer, H., Lowenstein,L., Wolf,M.F.Clinical implications of uterine cultures obtained during urgent caesarean section. ANZ-JOG 2022;63(3):334-351. https://doi.org/10.1111/ajo.13630
- Voekt, C.A.; Rinderknecht, T.; Hirsch, H.H.; Blaich, A.; Hösli, I.M. Ultrasound indications for maternal STORCH testing in pregnancy. Swiss Med. Wkly. 2017; 147: w14534. doi: 10.4414/smw.2017.
- Weiss, L.M.; Dubey, J.P. Toxoplasmosis: A history of clinical observations. Int. J. Parasitol. 2009;39:895–901. doi: 10.1016/j.ijpara.2009.02.004.