

# Organoid Model For Equine Placentitis Research

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**This project's aim is to develop an in vitro model of equine placentitis to advance the study of this condition, ultimately improving diagnostics, treatments, and pregnancy outcomes.**



Placentitis, or inflammation of the placenta, is the most common placental abnormality in horses 1–5,10. Most cases are caused by a bacterial infection, primarily by *S. zooepidemicus*, which is believed to reach the placenta via the birth canal<sup>2,3,6,7</sup>. This type of placental infection is known as ascending placentitis. The resulting inflammation can trigger premature delivery or abortion in late-stage pregnancy<sup>1–5,10</sup>. Unfortunately, placentitis often develops subtly, with clinical signs appearing only in advanced stages, making effective treatment challenging.

Our current understanding of placentitis development and progression is primarily based on clinical cases, which typically represent more advanced stages of the disease, as diagnosis occurs only after symptoms become visible. Additionally, while experimentally induced cases are valuable tools, they rely on live animals, raising ethical concerns, limiting sample sizes, and incurring high costs. These constraints hinder the study of the early stages of the disease, particularly the initial infection triggers and the dynamic interactions between the horse placenta and the pathogen (host-pathogen). Consequently, these limitations restrict the development of early diagnostic tools and effective treatment strategies.

To address these challenges, we propose an innovative approach using placental organoids, which are miniature versions of the placenta grown in the laboratory. Over the past decade, organoid cultures have emerged as a vital tool to bridge the gap between in vitro and in vivo studies<sup>36–39</sup>. Organoids are complex, three-dimensional, self-organizing, and self-renewing multicellular structures that closely replicate the key functional, morphological, and molecular features of the original organ<sup>36–39</sup>. Even though organoids lack an immune system, they can still detect pathogens and respond to them, similar to the first line of pathogen detection in tissues such as the placenta<sup>55</sup>.

By replicating placentitis in a laboratory setting, we can explore crucial questions such as: How does the bacterium

initially infect the placenta? And how does the placenta respond on a molecular level during the early phases of infection? The insights gained from this research could contribute to the development of better diagnostic tools that can detect placentitis much earlier, allowing for prompt intervention before irreversible damage occurs.

Furthermore, by identifying specific genes, proteins, and pathways involved in the host-pathogen interaction, we may discover new therapeutic targets or test the efficacy of the current treatment strategies, leading to more effective and targeted treatments that reduce the need for broad spectrum antibiotics and improve outcomes for both mares and foals. To achieve our goal, we developed an equine-specific placental organoid model and, over the past two years, tested its similarity and function through several independent methods, such as RNA sequencing, single-cell RNA sequencing, hormone production, electron microscopy, protein assays, and fluorescent microscopy<sup>53,54</sup>. All these methods indicated a high degree of similarity between the in vitro model and the actual placenta. Additionally, we cultured *S. zooepidemicus*, the same strain used in most experimentally induced placentitis studies, analyzed its genome, transformed it with a fluorescent reporter, and tested its growth in the lab<sup>11</sup>. In addition to the equine placental organoids and microbial cultures, we evaluated the presence of the microbe in the placenta of both healthy<sup>21</sup> and placentitis-affected (preliminary data) horses and found an inactive form of *S. zooepidemicus* in the healthy placenta.

With this background, we aim to meticulously study the initial interactions of this bacterium with the placenta and the progression of placentitis in the lab. This study will allow us to get insights into the early disease process without the need for live animals, respecting animal welfare (principles of the three Rs)<sup>55</sup> while advancing scientific knowledge. In addition, the development of this organoid-based model has broader scientific implications, setting a foundation for similar studies in horses and other species.

**Importance to the Equine Industry:** Placentitis, the most common placental disorder in horses, poses a significant threat to equine reproduction<sup>1–5,10</sup>. Most cases result from *S. zooepidemicus* infections, believed to ascend through the birth canal and infect the placenta, causing inflammation that triggers premature birth or abortion in late pregnancy<sup>2,3,6</sup>. Placentitis progresses silently, with clinical signs appearing only in advanced stages, making early diagnosis and treatment challenging<sup>5–8</sup>. Beyond the health risks to foals, placentitis results in significant financial losses for the industry and emotional strain on horse owners.

Despite numerous studies on placentitis, many questions remain unanswered, particularly regarding how the infection begins and progresses. Our research aims to fill these gaps by creating an in-lab model of placentitis using placental organoids<sup>53,54</sup>. We will infect these organoids with *S. zooepidemicus* to study the initiation and progression of the disease. By analyzing gene expression and metabolite production in both the placenta and bacteria at different stages of infection, we aim to uncover new insights into their host-pathogen interaction over time.

We will compare these findings to in vivo cases of placentitis and conventional microbial culture techniques. This proposal will (1) identify how *S. zooepidemicus* initiates placentitis; (2) analyze the dynamic interaction between the microbe and placenta by identifying important genes and metabolites for the pathogenesis of placentitis; (3) identify gene expression patterns of the host to determine key genes involved in the host response to the pathogen. Overall, by simulating placentitis in a lab setting, our study aims to uncover critical details about the pathogens's behavior and the host's response. This research will pave the way for more effective diagnosis and treatments, ultimately reducing losses in the equine breeding industry

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