Insights into Equine Endometritis: MicroRNAs Breakdown for Diagnosis and Treatment

Sana Asif[1], Talha Umar[2], Muhammad Qasim[1], Zulfiqar Ali[1], Muhammad Naveed Anwar[3], Muhammad Umar Zafar Khan[3], Huma Jamil[1], Muhammad Imran[4], Muhammad Imran[5] and Saqib Umer[1,*]

[1]Department of Theriogenology, University of Agriculture, Faisalabad, 38000 Punjab, Pakistan
[2]Department of Clinical Veterinary Medicine, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, PR China
[3]Institute of Microbiology, University of Agriculture, Faisalabad 38040, Pakistan
[4]Department of Pathology, University of Agriculture, Faisalabad, 38000 Punjab, Pakistan
[5]Department of Parasitology, University of Agriculture, Faisalabad, 38000 Punjab, Pakistan
*Corresponding author: saqib.umer@uaf.edu.pk

Abstract

Equine endometritis remains a leading cause of infertility and subfertility in mares, posing a major challenge to the reproductive efficiency of the equine industry. Traditionally diagnosed through clinical, bacteriological, and cytological evaluations, the condition often lacks early detection methods and targeted treatments. Recent advances in molecular biology have highlighted the pivotal role of microRNAs (miRNAs) in regulating immune responses, inflammation, and tissue remodeling within the endometrium. This chapter delves into the current understanding of miRNA expression profiles in healthy versus inflamed uterine tissues, emphasizing their diagnostic potential as non-invasive biomarkers. Furthermore, it explores the emerging therapeutic implications of modulating miRNA activity, offering promising avenues for the development of novel, precise, and effective treatments for both acute and chronic forms of endometritis.

Keywords: Infertility, Equine industry, Effective treatments, Artificial insemination

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Introduction

Acute and chronic endometritis has long been identified as a key cause of reduced fertility and subfertility in mares due to the inability to conceive, improper embryo development, and early embryonic mortality. Because of the inability to conceive, failure of embryos to develop properly, and early embryonic mortality associated with both acute and chronic endometritis, the condition has long been recognized as a major cause of reduced fertility and subfertility in mares. As a result, the equine breeding industry is significantly impacted financially (Gutjahr et al., 2000; Causey, 2006; LeBlanc & Causey, 2009). Additionally, it is one of the main issues that equestrian practitioners deal with (Traub-Dargatz et al., 1991). An infection of the endometrium is known as endometritis, and it can have a number of causes (Köhne et al., 2020). It can be separated into two categories: infectious and non-infectious, which frequently coexist. With the exception of the fact that bacteria are involved in the first type of endometritis, the clinical signs for these two types can be confused (Canisso et al., 2020). However, more recently, endometritis has been defined as a normal immunological reaction to semen entering the reproductive tract of a mare during breeding; this normal immunological response helps to remove excess spermatozoa, seminal plasma, and contaminating bacteria from the uterine lumen. If the mare fails to fix its biological endometritis before 48 hours of breeding, she may develop persistent-breeding induced endometritis (PBIE), a pathological condition. The PBIE is one such condition that can happen to the mare (Morris et al., 2020). Extraordinary advancements in molecular biology techniques over the past decade have led to the discovery of numerous key components involved in gene regulation under healthy, sick, and diseased conditions. As a result, a new era is emerging in the identification and management of endometritis (Pan & Chegini, 2008).

MicroRNAs (miRNAs), which have become significant regulators of gene expression, are among these key actors. MiRNAs are tiny, without coding RNA strands that regulate post-transcriptional gene regulation. They do it by completing or partially base pairing with the 3′-UTRs of target mRNAs to either block translation or degrade mRNA (Lim et al., 2003; Bartel, 2004). Reproductive diseases such as improper tissue regeneration and unsuccessful embryo implantation may be caused by changes in the production of mediators that promote inflammation (Jaiswal et al., 2006; Chapwanya et al., 2009; Ibrahim et al., 2015). A recent study found that the uterine transcriptome (mRNA) and miRNome patterns of cows with preclinical or diagnostic endometritis were altered, which significantly affected the uterine endometrium acceptance and homeostasis (Salilew-Wondim et al., 2016). The post-transcriptional mechanisms underlying the development of endometritis in mares remain unclear. To our knowledge, not much is known regarding the function of miRNAs in the equine endometritis pathogenesis. Therefore, more research is necessary to have a deeper comprehension of the chemical reactions that take place in mare endometritis. Prior research on both bovine and horse breeds have demonstrated this a useful model for studying endometritis is uterine cell cultivation and/or tissue specimens treated with LPS (Nash et al., 2008; Herath et al., 2009; Perrini et al., 2016). The purpose of this chapter is to examine the function of microRNAs in the identification and management of equine endometritis.

1. Understanding Equine Endometritis

One of the most frequent causes of infertility in horses is equine endometritis. In mares who are naturally resistant to persistent endometritis, local components of uterine defense mechanisms guarantee an efficient and quick clearance of an inflammation; however, mares who are unable to promptly clear the uterus of an inflammation are considered susceptible to persistent endometritis (Hughes & Loy, 1969). The two main causes of infertility are chronic endometritis generated by breeding and infectious endometritis either before or after breeding. Endometritis was identified by equine practitioners as the third most prevalent medical issue, and the occurrence of bacterial infection of the uterus in mares has been observed to range between 25 and 60% (Traub-Dargatz et al., 1991; Riddle et al., 2007; Frontoso et al., 2008; Nielsen et al., 2010). In equine reproductive science, the idea that mares are resistant to endometritis and susceptible to it, is well-established (Hughes & Loy, 1969; Peterson et al., 1969; LeBlanc & McKinnon, 2011). According to these investigations, the most common bacteria extracted from the uterus are *Escherichia coli* and Beta hemolytic streptococci (Riddle et al., 2007; Frontoso et al., 2008).

Subclinical endometritis has gained more interest as an underlying cause of mares' infertility tendency (LeBlanc & Causey, 2009; Overbeck et al., 2011; Buczkowska et al., 2014). Subclinical endometritis is poorly defined in literature. Mares who have positive cytology and bacteriology lack clinical evidence of endometritis (Overbeck et al., 2011; Buczkowska et al., 2014). Another way to describe mares who have subclinical endometritis is if they lack the normal clinical symptoms, which are major penetration of inflammatory cells into the lumen of their uterus and intrauterine fluid (LeBlanc & Causey, 2009). Subclinical endometritis could have very slight clinical manifestations that are evident only during certain stages in the estrus cycle (LeBlanc & Causey, 2009). Thus, it can be extremely difficult to diagnose subclinical endometritis through traditional methods. Failure to diagnose mares with subclinical endometritis because of inefficient methods and equipment leads to unnecessary delays in initiating proper treatment, which leads to unsatisfactory mare management (LeBlanc & Causey, 2009). Through the application of fluorescence in situ hybridization, it is possible to ascertain the deep endometrial site of *Streptococcus zooepidemicus* infection in mares suffering from recurring infections (Petersen et al., 2009). Non-observance of clinical manifestations and failure to diagnose mares suffering from subclinical endometritis could be because of the deep endometrial site of *S. zooepidemicus*.

Not a lot is known about bacteriological, cytological, and clinical parameters in mares submitted to artificial insemination (AI), both prior to and after breeding. The little research conducted on mares bred using AI in a commercial setting has mostly described the pre-breeding incidence of bacterial infections, their resistance to antibiotics, and the impact on pregnancy rates (Albihn et al., 2003; Frontoso et al., 2008; Nielsen et al., 2012). The majority of research has been conducted on thoroughbred mares where natural cover permitted (Hemberg et al., 2005; Riddle et al., 2007). Because the stallion's penis enters the vagina under natural conditions, infection of the reproductive tract is inevitable; however, artificial insemination (AI) allows for stricter hygiene protocols. Moreover, the majority of semen extenders contain antibiotics (Vieira et al., 2002). However, normal mares commonly display physiological inflammation during random mating or insemination. However, such inflammation disappears 48h after exposure to semen. If their physiological post-breeding endometritis is not resolved.

2. Understanding MicroRNAs

By base pairing with mRNAs, microRNAs (around 22-nucleotide RNAs) suppress gene expression post-transcriptionally. It is believed that miRNAs target more than half of all mRNAs, and that each miRNA can control numerous targets (Gurtan & Sharp, 2013). In line with their ubiquitous function, miRNAs control a wide variety of biological processes, such as apoptosis, differentiation, and proliferation. These small RNAs are especially important for development; if they are completely lost in the embryo, it results in death. It is becoming more and clearer that each miRNA affects several functionally related genes that make up the gene expression network, even though much research concentrates on binary miRNA-target interactions in describing phenotypes. MiRNAs are essential for many biological processes, including apoptosis, transcription, signal transduction, cell cycle, and proliferation of cells. Diseases are caused by the disruption of normal cellular activity caused by the dysregulation of miRNA function (Shah et al., 2009). The discovery of unregulated miRNAs and their goals opens up new avenues for veterinary illness diagnosis, prognosis, and treatment approaches. According to a study, specific miRNAs may have regulatory effects on basic equine endometrial cells and tissue explants that have been subjected to varying amounts of lipopolysaccharides (LPS). Following in silico analysis, RT-qPCR was used to assess eight miRNAs. It is interesting to note that following two doses of LPS, the expression of miR-155, −223 and −17 were dramatically elevated; in contrast, let-7a, −21, and miR-181b were significantly lowered in comparison to controls. Strangely, the low LPS dose was the only time when the expression of miR-24 and -532-5p was up-regulated. The authors proposed that the abnormal expression of miRNAs and the molecules they target, which are involved in controlling uterine homeostasis, was produced by LPS. These results validate the possible therapeutic benefit (Ibrahim et al., 2019).

3. MicroRNA-Based diagnostic and Therapeutic Approaches

Without a doubt, several mediators, including miRNA, which, depending on cell signaling, is in charge of activating or inhibiting specific target genes, govern appropriate uterine function. In addition to interfering with uterus function and potentially lengthening the ovarian cycle, uterine infection also affects female fertility (Pan & Chegini, 2008; Morris et al., 2020). Therefore, comprehending the molecular regulatory processes linked to the inflammatory immune response is essential to designing effective therapeutic medications to address the ongoing reproductive issues related to uterine inflammation (Kaminska, 2005). The free serum miRNA expression patterns are among the many molecular markers that may be useful as predictive and diagnostic markers for mares with endometrial inflammation. Several investigations have demonstrated the potential utility of miRNA as both prognostic and diagnostic biomarkers through contrasting the ways in which these molecules in healthy individuals and those in disease (Cosar et al., 2016; Guo et al., 2018; Condrat et al., 2020). In contrast to in order to manage healthy mares (both young and elderly), there was a significant over-expression of eca-miR-155, eca-miR-223, eca-miR-17, eca-miR-200a, and eca-miR-205 in sick mares, both young and old. These results are consistent with earlier research in cow that showed that host cells respond to infection by inducing the ,The mediators of the reactive immune reaction which help the body fight infection (Kasimanickam & Kastelic, 2016; Salilew-Wondim et al., 2016; Ibrahim et al., 2019). An imbalance between pro- and anti-inflammatory mediators may be linked to the aberrant miRNA expression that these inflammatory mediators may cause (Kamity et al., 2019; Mori et al., 2019). It's noteworthy to observe that the phrase pattern of serum microRNA was clearly influenced by mare age. In contrast, older sick mares expressed more of the genes eca-miR-155, eca-miR-223, eca-miR-200a, and eca-miR-205 than did younger sick mares or healthy control mares. This may be caused by a mare's increased fluid retention as she ages, which is then linked to observable alterations in the systemic immune response (Woodward et al., 2012). To the better of our understanding, this research is the first to examine the expression profiles in mare's serum during endometritis of eca-miR-155, eca-miR-223, eca-miR-17, eca-miR-200a, and eca-miR-205.

MiRNAs repress their target mRNAs by a post-transcriptional process that involves preventing translation or inducing mRNA destruction (Bronze-da-Rocha, 2014). This process regulates the development of the cell cycle, cancer, apoptosis, and cell proliferation. Creating treatments based on miRNA is essential for next studies (Kim, 2005a; 2005b). Given that miRNAs in their mature or immature form may be involved in the illness targeting elevated miRNAs with anti-miRNA oligos is one potential tactic (miRNA inhibitors) (Zhang, 2008). Instead of targeting a single protein, miRNA treatment targets many genes involved in the same pathway, which may be advantageous over conventional therapies (Krek et al., 2005; Sayed & Abdellatif, 2011; Caroli et al., 2013; Bronze-da-Rocha, 2014). Single-stranded antisense oligonucleotides are the building blocks of antimirs and antagomirs, oligonucleotides that target miRNAs. High-affinity nucleotide mimics, or miR-mimics (Bronze-da-Rocha, 2014). The miR-mimics are used to increase the amount of miRNA that is downregulated in certain diseases. In order to inhibit genes that resemble their natural counterparts, scientists have created artificial short nucleotide sequences known as "mimics." They can be identified by the RISC loading mechanism or the miRNA biogenesis apparatus (Fasanaro et al., 2010; Galasso et al., 2010; Caroli et al., 2013; Oliveira-Carvalho et al., 2013; Bronze-da-Rocha, 2014).

Conversely, when a specific miRNA is elevated and causes pathological conditions, antagonist miRNAs are employed. Modified antisense oligonucleotides (morpholinos) called antagomirs can increase the quantity of mRNA that a particular miRNA may target since they are specifically made to target the mature miRNA sequence (Weiler et al., 2006; Fasanaro et al., 2010; Galasso et al., 2010). One of the functions of antimiR during miRNA formation is to inhibit pre-miRNA and pri-miRNA export (Caroli et al., 2013). Additional possibilities for reducing miRNA function are provided by mechanisms referred to as erasers, masking, and sponges. Sponge prevents miRNAs from adhering to their intended target. Masking uses oligonucleotides that modify a specific miRNA target because they show complete complementarity to the target miRNA. Erasers were developed by scientists to remove miRNAs, that are oligonucleotides that include tandem repeats of the antisense sequence of the miRNA (Fasanaro et al., 2010; Galasso et al., 2010; Oliveira-Carvalho et al., 2013). When it comes to veterinary medicine, miRNA can be a crucial instrument for stopping the dissemination of illness and minimizing financial losses, especially for animals with significant economic worth. We believe that in the near future, miRNA identification in a hospital with relevance to the health of animals will be a reality.

A. Eca-miRNA-223

Exogenous and endogenous agonists affect Type 2 diabetes, inflammatory bowel disease, and atherosclerosis. The NOD-like receptor NLRP3 is bound by LPS, initiating a series of activation events that intensify tissue degradation (Davis et al., 2011; Lamkanfi & Dixit, 2012; Piras et al., 2017; Yu et al., 2017). In cattle uterine epithelial cells activated by LPS and endometritis,miR-223 was shown to be increased; however, when NF-κB was inhibited, the synthesis of miR-223 was considerably decreased (Zhao et al., 2018). To some extent, the inhibition of NF-κB and the impairment of inflammatory processes are dependent on the traditional NF-κB route activation or increased within cells miR-223 level. By acting as an NF-κB regulator, miR-223 expression may help limit NLRP3 activation and prevent inflammatory reactions. The potential application of miR-223 overexpression as a novel therapeutic for inflammatory conditions, such as subclinical endometritis, is indicated by its pharmacological stability (Gross et al., 2013; Zhao et al., 2018; Zhou et al., 2019).

B. Eca-miRNA-488

By regulating the generation of oxygen species that respond (ROS) and stimulating the AKT/NF-κB signaling routes within the lining of the uterus miR-488 prevents LPS-induced endometritis. Dose-dependent decreases in the expression of miR-488 in endometrial inflammation induced by LPS can imply an association between miR-488 and the immunological response stimulated by LPS (Zhang et al., 2019). The MiR-488 was negatively correlated with the production of pro-inflammatory cytokines, which repressed the expression of Rac1 and attenuated LPS-induced endometritis. For the complete understanding of the specific mechanism of regulation of Rac1/AKT/NF-κB signaling by miR-488 in endometritis and other inflammatory diseases, more research studies are recommended (Zhou et al., 2017; Zhu et al., 2019).

C. Eca-miRNA-643

Expression of miR-643 showed a maximum decrease in endometritis, while an expression of miR-215 display was not affected, according to previous research on endometritis and miRNAs. MiR-643 is down-regulated by LPS-treated HEECs, which can accelerate an onset of endometritis. Inflammatory cytokines, including IL-1β, TNF-α, and IL-6 generated and produced as a result of the activation of the NF-κB signal, which could lead to inflammation-related damage. X-linked apoptotic protein inhibitor and the zinc finger E-box binding site on homeobox transcription factor-1 are two of the numerous targets. Targets of miR-643 that have been identified. Just two of the several targets that have been located are these. Immunotherapeutic treatment for endometritis downsizing the miR-643 and the TRAF-6 gene to lessen inflammation response brought on by LPS (Zhao et al., 2020).

D. Eca-miRNA-148a

The highly conservative microRNA the miR-148–152 family includes miR-148a. It has been proven important for the control of immunity, inflammation, and tumor development (Wang et al., 2015). In this regard, it has been suggested that miR-148a may be responsible for preventing DSS-induced colitis in mice. Strikingly, the previous study also identified that LPS stimulated BEECs showed downregulation of miR-148a, suggesting some role of miR-148a in the pathogenesis of endometritis. Further work is required to explore the possible implications of this for the diagnosis and prediction of endometritis in mares (Wang et al., 2015). Essentially, to justify the recognized role of different receptors and ligands, one would need to explore study reports looking at the microRNA expression linked to endometritis in cows. The application of newer molecular therapies and diagnostics based on microRNA will deepen understanding of the cellular pathophysiology and etiology of endometritis, and lead to new approaches for its prevention, control, and treatment.

E. The Let-7 Family

Further investigation is required to understand the interaction between bacterial inflammation and the biological signaling pathway, as well as the miRNA of the Let-7 family, and its effect on endometrial function. However, MiR-let-7c is a prerequisite for inflammatory responses. Zhao et al. (2019) considered the pathogenesis of endometritis and discussed extensively the regulatory circuitry of let-7c. It was shown that by miRNA overexpression, activation of the NF-kB signal pathway was suppressed and thus reduced LPS-induced inflammation of the uterus and the resultant production of pro-inflammatory cytokines. After the injury induced by LPS, miR-let-7c enhanced the repair of the tissues (Zhao et al., 2019). Jiang et al.’s results suggest that let-7c neutralize inflammatory responses by acting as an anti-inflammatory through the suppression of pro-inflammatory cytokine release following LPS inflammation (Jiang et al., 2014). Thus, by operating, elements of the let-7c class of molecules may assist in the mitigation and prevention of endometritis in cattle, as they are like anti-inflammatory agents (Jiang et al., 2014; Zhao et al., 2019).

Conclusion and Future Perspective

It is possible to conclude that tracking or adjusting miRNA expressions is very helpful in the identification, assessment, and management of a wide spectrum of diseases, depending on the research analyzed and discussed. The likelihood of using this data will increase with our understanding of miRNAs and their function in equine health and disease. The idea of hiring a person to use a miRNA analysis for medical evaluation is appealing because of the miRNAs' exceptional stability in diagnostic samples, their apparent disease specificity, and the feasibility of relatively non-invasive sampling. Introducing miRNAs as a medication to treat equine ailments has a lot of potential. The use of miRNAs as a means of therapy to treat equine ailments shows great promise. Although elevated miRNAs have lately emerged as highly useful indicators for human viral, metabolic, genetic, and neoplastic disorders, domesticated animals haven't been thoroughly studied in this regard. Several miRNAs linked to human illness also have animal homologs. The subsequent considerable increase in information contrasts with the existing state of ignorance about the horse species. The majority of these illnesses have either not been explored at all or have been thoroughly examined for miRNA signatures on horses. However, miRNomics in equestrian medicine would probably be exploring this new ground.

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