ABSTRACT: Uterine and placental infections are the leading cause of abortion, stillbirth, and preterm delivery in the mare. Whereas uterine and placental infections in women have been studied extensively, a comprehensive examination of the pathogenic processes leading to this unsatisfactory pregnancy outcome in the mare has yet to be completed. Most information in the literature relating to late-term pregnancy loss in mares is based on retrospective studies of clinical cases submitted for necropsy. Here we report the development and application of a novel approach, whereby transgenically modified bacteria transformed with *lux* genes of *Xenorhabdus luminescens* or *Photorhabdus luminescens* origin and biophotonic imaging are utilized to better understand pathogen-induced preterm birth in late-term pregnant mares. This technology uses highly sensitive bioluminescence imaging camera systems to localize and monitor pathogen progression during tissue invasion by measuring the bioluminescent signatures emitted by the *lux*-modified pathogens. This method has an important advantage in that it allows for the potential tracking of pathogens in vivo in real time and over time, which was hitherto impossible. Although the application of this technology in domestic animals is in its infancy, investigators were successful in identifying the fetal lungs, sinuses, nares, urinary, and gastrointestinal systems as primary tissues for pathogen invasion after experimental infection of pregnant mares with *lux*-modified *Escherichia coli*. It is important that pathogens were not detected in other vital organs, such as the liver, brain, and cardiac system. Such precision in localizing sites of pathogen invasion provides potential application for this novel approach in the development of more targeted therapeutic interventions for pathogen-related diseases in the equine and other domestic species.

Key words: bioluminescence imaging, *lux*-modified pathogen, mare, uterine infection

INTRODUCTION

Premature birth is the leading cause of prenatal morbidity in humans, with an incidence of 12.5% (Behrman and Butler, 2006), of which 40% may be attributed to antenatal infection (Lettieri et al., 1993), with *Escherichia coli* being identified as the most common organism isolated from pregnant women (Lavanya and Jogalakshmi, 2002). In cattle, the rate of uterine infection has been estimated at 2.2 to 37.3% (Kelton et al., 1998), with *E. coli* being the most commonly isolated bacteria associated with postpartum uterine infections. Similarly, placental infection attributable to opportunistic pathogens such as *Streptococcus equi* ssp. *zooepidemicus* and *E. coli* are common causes of abortion, stillbirth, and premature delivery in horses (Whitwell, 1988; Giles et al., 1993; Hong et al., 1993a,b). Moreover, pathogen
progression during uterine infections and placentitis may involve invasion of endometrial and fetal tissues, leading to an increased expression of proinflammatory cytokines, resulting in the onset of premature delivery, fetal neurological damage, or both (Hillier et al., 1993; Duggan et al., 2001; Behrman, 2006). Although animal models, such as the rhesus monkey (Gravett et al., 1994) and rodents (Bennett et al., 2000; Terrone et al., 2001), have been used to understand pathogen-induced preterm labor in women, little is known about bacterial pathogenesis during uterine infections in the equid species. Thus, the intent of this paper was to discuss a novel approach that uses real-time bioluminescence imaging technology to elucidate the pathogenesis of uterine bacterial infections associated with preterm birth in the mare. An overview of uterine infections and background information on bioluminescence imaging, *lux*-modified bacteria, and their application in other living systems is provided in support of the discussion. Although this technology is used extensively in the field of biomedical research, mainly using the murine infection model, it is in its infancy in the field of animal science because of the significant logistical and biohazard challenges encountered.

**UTEROPLACENTAL INFECTIONS, PREGNANCY LOSS, AND NEONATAL MORTALITY**

It is well documented that uterine infection is highly correlated with idiopathic preterm labor (Hillier et al., 1993; Dudley 1997; Romero et al., 1998, 2010; Mazaki-Tovi et al., 2010), which is the leading cause of neonatal mortality and morbidity in humans (Gotsch et al., 2009; Shah et al., 2009). Moreover, infants that do survive have a greater likelihood of developing other disorders in early life (Butt et al., 2006; Pereira et al., 2007; Shah et al., 2009). Similarly, Giles et al. (1993) reported, based on the pathology records of horses, that 60% of fetal abortions, stillbirths, and foal deaths within 24 h of birth were associated with placental insufficiency, of which one-third were associated with placental infections. Among the many cases examined, the most common pathogens isolated from aborted fetuses or dead perinatal foals were the opportunistic *S. equi* ssp. *zooepidimicus*, *E. coli*, or both (Giles et al., 1993; Hong et al., 1993a,b). The etiology of uterine infection-induced preterm delivery in pregnant women, mice, and mares involves the release of proinflammatory cytokines, such as tumor necrosis factor α (TNFα), IL-1β, IL-6, and IL-8, among others, from placental and fetal tissues in response to infection (Dudley and Trautman, 1994; Pollard and Mitchell, 1996; Dudley, 1997; Mussalli et al., 1999; LeBlanc et al., 2002). The consequence of the inappropriate expression of these proinflammatory cytokines leads to the initiation of a preterm birth by increasing PG synthesis (Bennett et al., 2000; LeBlanc et al., 2002; McGlothlin et al., 2004).

As in humans, pregnancy loss during late gestation and the death of foals weakened by abnormal periparturient events constitute a large percentage of fetal and neonatal mortality in horses (Vaala and Sertich, 1994), and pluriparous mares are the most commonly afflicted (LeBlanc, 2010). Premature birth in horses usually results in the delivery of a nonviable afflicted because complete fetal maturation for successful extrauterine life does not occur until the final 5 to 7 d of development in utero (Silver and Fowden, 1994). Because of the expense of breeding contracts and the long gestation of the mare, late-term fetal death represents a major financial loss and time investment for the breeder. Early identification of placental insufficiency may make it possible to sustain the pregnancy through medical intervention. However, at this time, a full understanding of the pathophysiology of preterm birth is still lacking, necessitating the use of novel approaches to study pathogen invasion and progression in the pregnant mare. Such information may aid in the development of more targeted therapeutics for treatment of the afflicted mare and fetus.

Conventional methods, using in vivo models of infection to estimate bacterial numbers, are mainly based on harvesting and homogenization of infected tissues, serial plating of infectious agents, growing bacteria in cultures, and colony counting (Kuklin et al., 2003). This is accompanied by further microbiological tests to identify the bacterium of interest to reduce the risk of false positives. These processes require extensive work, time, and resources in monitoring bacterial pathogenesis in vivo in experimentally induced infection studies (e.g., placentitis). In addition, diagnostic tools that can accurately and reliably identify at-risk pregnancies do not readily exist. One approach to better understand the pathogenesis of uterine and placental infections is the use of modified pathogens with the *lux* gene as a photonic biomarker and real-time bioluminescence imaging technology.

**USE OF BIOLUMINESCENCE IMAGING TO MONITOR PATHOGEN PROGRESSION**

**Bioluminescence and Living Systems**

Biophotonics involves the fields of optical imaging, photonics, and biology, and deals with the interactions among light, biological matter, and its application to biomedical science. Nature has harnessed the photon (i.e., light) in many ways as a basic principle of life, whether through photosynthetic pathways in plants, as methods of communication among insects (e.g., the firefly), or in a multitude of other examples from across the natural world. Bioluminescence is described as the voluntary or involuntary emission of visible light in living organisms (e.g., bacteria, fungi, fish, insects, algae, and squid), mediated by an enzyme catalyst (Meighen,
with the Lux Gene bacterial pathogens, or the regulation and expression of ical responses in real time and over time, including the important and meaningful approach to studying biolog-

2004; Ryan et al., 2005; Zinn et al., 2008). Biolumines-

and rodents (Contag et al., 1995, 1997; Zhang et al., (Brandes et al., 1996), Drosphila son and Kay, 1995), (Willard et al., 1997, 1999, 2001), whole plants (Ander-

ologically relevant systems in situ, including single cells (Willard et al., 1997, 1999, 2001), whole plants (Anderson and Kay, 1995), Drosophila (Brandes et al., 1996), and rodents (Contag et al., 1995, 1997; Zhang et al., 2004; Ryan et al., 2005; Zinn et al., 2008). Bioluminescent imaging technology using luciferase reporter genes to monitor physiological events in vivo has become an important and meaningful approach to studying biological responses in real time and over time, including the efficacy of drugs for cancer therapy, the pathogenesis of bacterial pathogens, or the regulation and expression of specific genes during specific physiological events (Vo-Dinh, 2003; Klerk et al., 2007; Luker and Luker, 2008).

Genetically Modified Bacteria with the Lux Gene

Conventional models to localize or estimate bacterial populations and invasiveness in vivo typically require extensive sampling procedures at each sampling time point and considerable time and labor to establish cultures (Curbelo et al., 2010). A more recent approach has been the use of bacterial luciferases (e.g., photon-emitting indicators) to label pathogens and monitor their progression during infections in rodents (Contag et al., 1995). These luciferase-modified bacteria have been engineered by inserting a bacterially derived lux operon, which encodes for both the bacterial luciferase enzyme and a long-chain aldehyde substrate (Karsi et al., 2006), resulting in the emission of visible light that can be detected using highly sensitive imaging systems. The bacterial luciferase differs from the firefly luciferase in that it catalyzes the oxidation of a long-chain aldehyde and reduced flavin mononucleotide to cause light emission (Karsi et al., 2006).

The use of bioluminescence imaging allows biological processes associated with the infected state of eukaryotic cells to be monitored longitudinally, in vitro and in vivo, in real time and noninvasively (Contag, 2002). The lux operon has been modified for bacterial incorporation by using plasmids, via transposon mutagenesis and chromosomal insertion, and the inserted operon routinely carries antibiotic resistance selection markers for cultivation as monocultures under controlled environmental conditions (Herrero et al., 1990). For example, E. coli MM294 (no. 33625; American Type Culture Collection, Manassas, VA) has been modified with pAK1-lux plasmid, which contains an antibiotic-resistant gene cassette combined with the lux gene (Moulton et al., 2008, 2009c). The plasmid (11,904 bp) used in this instance is a broad-host-range cloning vector with numerous plasmid replicons containing the lux operon (Frackman et al., 1990). The lux operon itself is a cluster of genes (luxCDABE) isolated from a nematode symbiont bacterium Xenorhabdus luminescens and encodes for the bacterial luciferase and the biosynthetic enzymes for the proper substrate (Karsi et al., 2006). Alternatively, E. coli has also been conferred with bioluminescent properties by incorporating the lux genes of Photobacterium luminescens into its chromosome (e.g., lux operon in E. coli-Xen14, Caliper Life Sciences, Alameda, CA), which can then be selected for, using antibiotics to maintain pure cultures. In both cases (i.e., plasmid and chromosomal insertion), the lux operon system encodes all proteins required for photon emissions, including the luciferase, substrate, and substrate-regenerating enzymes (Luker and Luker, 2008). However, the chromosomal insertion, compared with plasmid integration, exhibits a continuous and stable luciferase expression over time and without antibiotic pressure; theoretically, the lux genes incorporated in the bacterial chromosome should be as stable as their native chromosome genes (Herrero et al., 1990). The use of bioluminescent bacteria, such as E. coli-pAK1-lux or E. coli-Xen14, coupled with bioluminescence imaging technologies, may represent an efficient model for achieving a greater understanding of the pathogenesis of uterine and placental infections in domestic species. Some studies have established posi-

tive relationships between bioluminescent signals and bacterial populations for gram-negative bacteria (r = 0.99; Kadurugamuwa et al., 2005), whereas other investig-

tors have described and validated bioluminescent bacterial models, both in vitro (Moulton et al., 2006, 2009c) and ex vivo (Moulton et al., 2009a,d; Curbelo et al., 2010). More recently, studies have reported the use of bioluminescent reporters and biophotonic imag-

ing technology to study mechanisms of pathogenesis of such pathogens as Staphylococcus aureus (Kuklin et al., 2003), Aspergillus fumigatus (Brock et al., 2008), Liste-

teria monocytogenes (Konjufca and Miller, 2009), E. coli (Foucault et al., 2010), and Mycobacterium tuberculosis

1993). In general, the reaction that produces light in-

volves a luciferase enzyme and a specific substrate that is oxidized to emit light (Doyle et al., 2004). In the case of firefly luciferase, the chemical reaction requires D-luciferin, as well as O2, ATP, and Mg2+, which are readily available in living cells. As shown below, the resultant exergonic reaction oxidizes luciferin, produc-

ing oxyluciferin, adenosine monophosphate, inorganic phosphate, CO2, and energy in the form of light, which can be detected and quantified using highly sensitive imaging equipment:

\[
\text{d-luciferin} + \text{O}_2 + \text{ATP} \xrightarrow{\text{luciferase}} \text{oxyluciferin} + \text{adenosine monophosphate} + \text{inorganic phosphate} + \text{CO}_2 + \text{light (photons)}.\]

The use of bioluminescence imaging in experimental models of human conditions has become a key diagnos-
tic and research tool for understanding physiological systems in normal and disease states that were not previously attainable with other detection systems. The last 10 to 15 yr has seen unprecedented adaptations of this technology for investigating a variety of physi-

ologically relevant systems in situ, including single cells (Willard et al., 1997, 1999, 2001), whole plants (Anderson and Kay, 1995), Drosophila (Brandes et al., 1996), and rodents (Contag et al., 1995, 1997; Zhang et al., 2004; Ryan et al., 2005; Zinn et al., 2008). Bioluminescent imaging technology using luciferase reporter genes to monitor physiological events in vivo has become an important and meaningful approach to studying biological responses in real time and over time, including the efficacy of drugs for cancer therapy, the pathogenesis of bacterial pathogens, or the regulation and expression of specific genes during specific physiological events (Vo-Dinh, 2003; Klerk et al., 2007; Luker and Luker, 2008).
Bioluminescence Imaging of Pathogens in Domestic Species

Recent applications of bioluminescent paradigms in domestic animal models, including swine and sheep (Moulton et al., 2009a,d), and the development of methods to facilitate deep tissue photon capture (e.g., optical clearing techniques; Moulton et al., 2006) across tissues (i.e., skin and intestine) indicate that in vivo imaging, as opposed to ex vivo or postmortem imaging, may be feasible in the near future by using minimally invasive detection procedures. Investigators have demonstrated the benefits of using genetically modified bacteria with the lux operon and bioluminescence imaging technology to improve our knowledge of the pathophysiology of diseases that affect farm animals economically. For instance, Moulton et al. (2009d) monitored in real time in situ pathogen progression of bioluminescent Salmonella in the gastrointestinal tract of swine. Studies have also evaluated fetal lambs delivered to bioluminescent E. coli-infected ewes ex vivo (Moulton et al., 2009a,b) and detection of bioluminescent E. coli within the excised bovine reproductive tract (Curbelo et al., 2010). Others have used the same approach for noninvasive disease monitoring (Edwardsiella ictaluri) in living fish (Karsi et al., 2006). Through further development of the paradigm outlined in the studies reported above, applications might be extended to provide a more resolved model for understanding the progression of events (e.g., bacterially induced endocrine changes, bacterial invasiveness of the fetal environment, or both) that leads to preterm delivery and pregnancy failure in mares, and may have applications for evaluating therapeutic interventions, with the goal of reducing antenatal mortality (Ryan et al., 2010b).

Bioluminescence Imaging of Pathogens in the Mare

A small number of studies using experimental infectious conditions have been undertaken to better understand placental pathogenesis and more appropriate therapeutic regimens of late-term uterine infections that lead to abortion or preterm delivery in the mare (McGlothlin et al., 2004; Morris et al., 2007; Ryan et al., 2009; Bailey et al., 2010). However, the application of bioluminescence imaging technology and the ability to modify pathogens with the lux gene provides investigators a unique opportunity to study the etiology of preterm delivery in the mare. The infectious experimental pony mare model developed by McGlothlin et al. (2004) was a starting point for our group to explore this approach. It has been proposed that the use of bacteria modified to express light and bioluminescent imaging technology may offer animal and veterinary researchers a useful means for understanding the pathogenesis of infectious pathogens leading to uterine infections and preterm delivery in the mare (Ryan et al., 2010b). Initial in vitro feasibility studies were performed with excised reproductive tracts from mares sent to necropsy to determine the ability of the imaging system to detect light from photon-emitting lux-transformed bacteria through uterine tissue. These studies followed the FASS (2010) Guide for the Care and Use of Animals in Agricultural Research and Teaching and were approved by the Mississippi State University Institutional Animal Care and Use Committee. Figure 1 is an example of photonic images derived from the ex vivo equine reproductive tract with bioluminescent E. coli-lux-filled balloons placed in the body of the uterine horns. These images demonstrate that it is feasible to detect the presence of the pathogen through the uterine wall and that the intensity of light emissions corresponds to the number of light-emitting bacteria placed inside the uterine horn.

To explore the practicality of infecting mares in vivo with light-emitting pathogens to better understand preterm delivery in mares with uterine infections, Ryan et al. (2010a) inoculated pony mares at approximately 300 d of gestation, via ultrasound-guided transvaginal injection into the amniotic cavity, with light-emitting E. coli-pAK1-lux. Mares maintained pregnancies from 24 to 48 h postinfection and then aborted fetuses. Intact fetuses were recovered immediately after abortion and were imaged for detection of lux-expressing bacteria. Images of an aborted fetus after bioluminescence imaging are shown in Figure 2a and 2b. The blue color represents the presence of light-emitting pathogens that have contaminated the amniotic fluid and, thus, the hair coat of the fetus. Yellow-red colors, indicative of greater bacterial colonization, can be detected in the region of the nares and sinuses and in the abdominal region representing sites along the gastrointestinal tract (Figure 2b). Representative bioluminescent images are shown in Figure 3 of the contaminated fetal membranes of aborted fetuses and the localization of light-emitting pathogens in the nares, indicating that the pathogens are inspired by the fetus. To determine the presence of pathogens in specific organs, bacteria externally coating the fetuses were removed with a bleach bath.
to avoid contamination of the internal tissues. Subsequently, the heart, lungs, liver, bladder, gastrointestinal tract, and brain were removed and imaged. Imaging of excised tissues revealed heavy contamination not only of the lungs, but also of the gastrointestinal tract and kidneys, indicating that the pathogens were not only

Figure 1. Illustration of intrauterine photonic detection. An empty uterus from a mare (panel A) and small balloons filled with 25 mL of Luria-Bertani broth containing *Escherichia coli*-pKA1-lux ($5 \times 10^6$ cfu/25 mL, panel B; or $4.5 \times 10^6$ cfu/25 mL, panel D) were imaged separately before insertion of the balloons into the uterus to acquire background measurements from the uterus (autobioluminescence) and total light emission from *E. coli*-pKA1-lux-filled balloons using an XR/MEGA-10Zero imaging system (Stanford Photonics Inc., Palo Alto, CA). All photonic images were acquired for 60 s and the results are expressed as relative light units per second (RLU/s). After imaging of the 2 balloons filled with light-emitting bacteria, the balloons were inserted separately into the empty uterus (panels C, E, and F) and reimaged. In these examples, the balloons filled with *E. coli*-pKA1-lux emitted 234.6 and 158.4 RLU/s (panels B and D, respectively), whereas the uterus on its own emitted 0.29 RLU/s. When the balloons were inserted into the uterus, the light emission values recorded were 32.6 (panel C), 0.46 (panel E), and 1.8 (panel F) RLU/s, yielding a transference of photonic emission of 13.9, 0.46, and 1.81%, respectively. The images shown in panels B to F are equivalent intensity of pseudocolor used for representation purposes.

Figure 2. Photonic emission from *Escherichia coli*-pAK1-lux bacteria on the surface of a fetal foal delivered preterm. Photonic image showing the presence of *E. coli*-lux on the amniotic surface (arrow) of the fetal foal in the lateral position (panel A) with no emitting bacteria observed on the chorionic surface of the placental membranes (lower arrow). Photonic-emitting bacteria on the skin surface of the fetal foal lying in the ventral position (panel B). Note the increased light emissions on the lower abdomen (arrow; gastrointestinal tract) and the nares and sinuses (arrow) of the preterm foal.
Figure 3. Photonic emission from *Escherichia coli*-pAK1-lux bacteria on fetal membranes of a preterm foal. Evidence of heavy contamination of the intact fetal membranes and amniotic fluid with light-emitting bacteria (panel A) and the exposed fetus after rupture of the amnion (panel B). After a 10% bleach bath to remove bacteria from the external surface of the fetus, light-emitting bacteria could still be detected in the nares and mouth region of the foal (panel C; detected via the skin surface).

Figure 4. Photonic emission from *Escherichia coli*-pAK1-lux bacteria in excised organs from a preterm foal. The exposed cranial cavity of the fetal foal is shown in panel A. Note the presence of light-emitting bacteria in the nares and sinuses (arrows) but their absence from the cranial cavity and brain. Panel B shows the excised heart and lungs with light-emitting bacteria present only in the lungs but absent from the heart (arrow), whereas panel C shows the excised gastrointestinal tract with evidence of light in the stomach, small intestines (arrows), and large intestines.

Figure 5. Presence of *Escherichia coli*-Xen14 light-emitting bacteria on the surface of obstetrical examination sleeves. Aliquots of cervical discharge were collected using examination sleeves from mares inoculated with *E. coli*-Xen14 on d 295 of gestation on d 3 (approximately 43 h), 5 (approximately 91 h), and 10 (approximately 211 h) after preterm delivery.
inspired but also ingested as the fetus consumed amniotic fluid (Figure 4).

An observation of particular interest was the presence of pathogens in the nares and sinuses of the fetus; however, no pathogens were observed in the brain when the cranial cavity was exposed (Figure 4a). This is an interesting observation because of reports in the human literature that infants born to mothers diagnosed with chorioamnionitis are at increased risk of cerebral palsy (Grether and Nelson, 1997; Grether et al., 1999) and that increased fetal blood concentrations of IL-1β, IL-8, IL-9, TNFα (Nelson et al., 1998), and interferons (Grether et al. 1999) have been reported in children with cerebral palsy, indicating a pathogen-induced increase in systemic proinflammatory cytokines. Other studies have demonstrated an association between intrauterine infections, increased fetal blood and neural tissue concentrations of proinflammatory cytokines, and incidence of cerebral tissue damage (Yoon et al., 1997, 2003; Duggan et al., 2001). Similar findings were observed in experimental studies with pregnant rats (Cai et al., 2000; Rodts-Palenik et al., 2004). Whereas several studies have reported a greater incidence of septicemia in neonatal foals born to mares with uterine infections (Raisis et al., 1996; Gayle et al., 1998; Stewert et al., 2002) and that foals with increased serum TNFα activity had greater mortality rates (Morris and Moore, 1991), no light-emitting bacteria were detected in the fetal foal brain (Ryan et al., 2010a). Nevertheless, it is not clear at this point whether hypoxic ischemia encephalopathy (i.e., neonatal maladjustment foal syndrome) observed in foals that endure difficult deliveries might also be a consequence of in utero compromise of the fetus caused by placental insufficiency associated with uterine infections (i.e., placentitis). Recently, Wilcox et al. (2009) reported severe brain histopathology associated with placental insufficiency in a cloned foal consistent with that seen in preterm infants (Yoon et al., 1997, 2003; Duggan et al., 2001) and in experimentally infected animal models, including rodent pups (Cai et al., 2000; Rodts-Palenik et al., 2004) and lambs (Moulton et al., 2009a). Our experience with preterm foals born to dams experimentally infected during late gestation to induce placentitis (Ryan et al., 2009; Christiansen et al., 2010) supports the observation of Wilcox et al. (2009), but the cerebral histopathology on such foals has yet to be completed. Applications that include the use of photon-emitting pathogens as a rapid biosensor of bacterial movements enable investigators to better understand the pathogen progression leading to preterm delivery and possibly improve on targeted antibacterial therapies in model systems (LeBlanc, 1997; Bailey et al., 2010; Christiansen et al., 2010).

The ability to assess the postpartum clearance of bacteria after abortion and therapeutic intervention is another potentially valuable application of this technology (P. L. Ryan, D. L. Christiansen, K. Moulton, and F. K. Walters, unpublished data). A pilot study was performed in which 2 mares were infected intracervically (McGlothlin et al., 2004) with commercially available E. coli-lux-Xen14 (1.2 × 10⁷ cfu; Caliper Life Sciences, Hopkinton, MA). After preterm delivery, mares were treated for 10 d postpartum with trimethoprim sulfaemethoxazole (30 mg/kg of BW) twice daily for 10 d immediately after preterm delivery.

Figure 6. Clearance rate of pathogens in cervical discharge samples obtained from mares after preterm delivery. Bioluminescent images of culture plates at 24 h after plating of 10-μL aliquots of discharge samples obtained on d 3 to 5 and d 10 from mare 2 (panels A to D) and mare 11 (panels E to H). Mares were placed on antibiotic treatment with orally administered trimethoprim sulfaemethoxazole (30 mg/kg of BW) twice daily for 10 d immediately after preterm delivery.
fluid discharge samples were collected daily for culture from d 3 to 7 postpartum and again on d 10. Sterile examination sleeves used to recover uterine discharge samples revealed heavy contamination of light-emitting bacteria (Figure 5), but contamination levels declined with time. Light-emitting bacteria were detected from uterine cultures of both mares on d 3 to 7 postpartum; however, contamination was observed to decrease markedly (6.0 × 10⁸ to <1.0 × 10³ cfu) over time and was negative by d 10 postpartum, consistent with the observed decrease in light emissions over the same period of time (Figures 6 and 7). These studies are the first reported attempts to use lux-modified bacteria and bioluminescent imaging technology to better understand an economically important disease process in the horse. Although controlled hypothesis-driven studies are needed, the application of this technology in large domestic species is promising, notwithstanding the logistical and biological hazard challenges associated with such experimental animal models.

SUMMARY AND CONCLUSIONS

Recent applications of biophotonic paradigms in other domestic animal models [e.g., swine (Moulton et al., 2009d), sheep (Moulton et al., 2009a)] and the development of methods to facilitate deep-tissue photon capture (e.g., optical clearing techniques; Moulton et al., 2006) across tissues indicates that in vivo imaging, as opposed to ex vivo or postmortem imaging, using minimally invasive detection procedures may be feasible in the near future. Through further development of the paradigm outlined in the present report, applications might be extended to provide a more resolved model for understanding the progression of events (e.g., bacterially induced endocrine changes, bacterial invasiveness of the fetal environment) that lead to preterm delivery and pregnancy failure. Additionally, use of this technology may facilitate the development and evaluation of novel specific tissue-targeted therapeutic strategies to reduce or prevent antenatal mortality in equid species. In addition, the postpartum clearance of bacteria after abortion and subsequent therapeutic intervention are potentially valuable investigative applications for this technology.

LITERATURE CITED


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