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Graduate Student Literature Review: Potential mechanisms of interaction between bacteria and the reproductive tract of dairy cattle*

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ABSTRACT

Although the presence of bacteria has been characterized throughout the reproductive tracts of multiple species, how these bacteria may interact with the host has yet to be described. Previous reviews have described how pathogenic bacteria interact with the reproductive tract to cause infections such as metritis. This review aimed to summarize the knowledge related to pathogenic and nonpathogenic bacteria in various locations of the bovine reproductive tract and the possible mechanisms underlying host–microbe interactions during gametogenesis and early pregnancy. Lactic acid bacteria such as *Lactobacillus* seem to be beneficial in multiple areas of the reproductive tract: they have been associated with increased oocyte quality when in follicular fluid and secrete reactive oxygen species that are beneficial during placental angiogenesis. However, other bacteria, including *Enterococcus*, *Staphylococcus*, and *Streptococcus*, may modulate T helper cells that inhibit maternal recognition of pregnancy. Available data on the reproductive microbiome focus on variations in microbial communities and their associations with reproductive performance. However, research on these host–microbiome interactions may provide more insight on how bacteria affect fertility.
Key words: reproduction, microbiome, host–microbe interaction

INTRODUCTION

In the late 1600s, Antonie van Leeuwenhoek discovered and characterized microscopic organisms, opening the door to our understanding of bacteria, protozoa, and fungi. Research by Louis Pasteur and Robert Koch in the 1800s added an understanding of microorganisms as sources of human infection and disease. In those early studies, microorganisms were isolated from infected animals and inoculated into healthy individuals that subsequently developed disease, demonstrating that microorganisms can be pathogenic. Bacteria were almost exclusively considered harmful until 1958. At that time, Ben Eiseman performed fecal enemas, an early form of fecal transplant, to successfully treat humans with *Clostridium difficile* infections (Eiseman et al., 1958). Since then, whether a microorganism is considered pathogenic or commensal depends on its interactions with its host environment.

Research in dairy cattle has demonstrated how bacteria can function in both a synergistic, commensal capacity and a harmful, pathogenic capacity, depending on the location within the host. Although some bacteria may not cause an inflammatory response in one location of the dairy cow, that same species can be detrimental in a different location. For instance, cows rely on metabolic functions of microbiota in the rumen to efficiently utilize nutrients from their diets while simultaneously providing an ideal environment for bacteria to survive. However, the presence of bacteria similar to those found in the rumen, like *Escherichia coli*, in the mammary gland or uterus causes inflammation, infection, and overall reduction in cow performance. Because of how sensitive the uterus is to infection, especially after calving, lack of infection is commonly seen as evidence that the upper reproductive tract is sterile.

Recent advancements in technology to detect bacteria in the gut have provided evidence contradicting the conventional wisdom of the sterile uterus. Instead of relying on culture-based techniques to isolate bacteria, sequencing of the highly conserved 16S rRNA region of bacterial DNA has provided the opportunity to identify microbiomes—the total bacterial community of a particular location—without needing specific culture parameters. This method has led to the discovery of a microbiome in the uterus of virgin heifers and pregnant cows, one that apparently does not negatively affect the dam or the pregnancy (Moore et al., 2017).

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The postpartum uterine microbiome of beef cattle was recently documented to vary based on pregnancy outcome (e.g., pregnant or not pregnant; Ault et al., 2019a,b). In humans, a *Lactobacillus*-dominated uterus is associated with increased success of in vitro fertilization (**IVF**; Moreno et al., 2016). These observations in cows and humans demonstrate that a reproductive microbiome exists and that it may affect reproductive performance. Understanding how bacteria interact with the reproductive tract could lead to development of new techniques to aid in dairy cattle reproductive performance. Therefore, the purpose of this review was to summarize the pathways that pathogenic bacteria utilize to influence fertility, delineate which commensal bacteria might be associated with reproductive traits in dairy cattle, and propose possible mechanisms that these commensal bacteria utilize to influence oocyte development, fertilization, and pregnancy recognition.

CURRENT KNOWLEDGE OF PATHOGENIC INTERACTIONS

Pathogenic Infections and Pathogenesis

Much of the current knowledge on bacterial interactions with the dairy cow uterus is limited to pathogenic bacteria. Metritis, or bacterial infection of the uterus, occurs soon after parturition, with the act of giving birth allowing for pathogens such as *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and *Bacteroides* spp. to enter the uterus and secrete pyolysin, leukotoxin, or LPS (Jeon and Galvão, 2018). Pyolysin, secreted by *T. pyogenes*, is a cholesterol-dependent, pore-forming exotoxin that binds to the cholesterol domain of endometrial cell membranes, allowing bacteria to enter cells and cause tissue damage (Sheldon et al., 2008). Leukotoxin, secreted by *F. necrophorum*, induces apoptotic cell death of leukocytes and can lead to necrosis of the endometrium at concentrations $\geq$625 U/mL (Narayanan et al., 2002). The endotoxin LPS, found in cell membranes of *E. coli* and *Bacteroides* spp., primarily induces immune response by binding to and activating toll-like receptor (**TLR**)-4, and leading to the recruitment of immune cells via release of cytokines and chemokines (Sheldon et al., 2008). These cytokines and chemokines attract macrophages and polymorphonuclear neutrophils, but their function is typically depressed during the early postpartum period (Sheldon et al., 2008). Endometritis, a less severe uterine infection, occurs about 20 d postpartum (Sheldon et al., 2008). Clinical cases of endometritis are caused by pathogens similar to those associated with metritis, but subclinical cases have been associated with the absence of typical pathogens and an increase of "commensal" bacteria, such as *Lactobacillus* or *Acinetobacter* (Wang et al., 2018). Subclinical cases are also associated with persistence of polymorphonuclear neutrophils in the uterus 21 d after parturition (Sheldon et al., 2008). Approximately 25 to 40% of animals will develop metritis within 2 wk postpartum and 20% of animals will develop clinical endometritis 3 wk postpartum or later (Sheldon et al., 2008). Although commensal or nonpathogenic bacteria dominate the uterus most of the time, fertility issues still persist in the dairy cow. Understanding how these commensal bacteria interact with the reproductive tract is critical to discovering the roles the microbiome plays during reproduction.

Postpartum infections can have long-term effects; even after symptoms have subsided, the uterine microbiome differs between healthy and metritic cows for up to 7 wk postpartum (Knudsen et al., 2016). Long-term effects of the microbiome could influence fertility in subsequent breedings; evidence in beef cattle has demonstrated differences in the microbial composition between pregnant and open animals 2 d before insemination (Ault et al., 2019a). A decrease in genera such as *Corynebacterium*, *Staphylococcus*, and *Prevotella* at −2 d was associated with cows that later became pregnant (Ault et al., 2019b). These differences in microbial composition between cows that will or will not become pregnant is helpful in understanding the extent to which uterine infection affects future fertility. Further research on how bacteria interact with the reproductive tract is needed to fully utilize the microbiome to benefit fertility.

Pathogens and Follicle Development

Postpartum uterine infections not only influence the reproductive tract shortly after birth, but also affect ovarian follicles during subsequent estrous cycles. Proper dominant follicle development and follicular fluid production are essential for production of high-quality oocytes for fertilization. Sheldon et al. (2002) observed that when uterine infections occurred, dominant follicles in subsequent estrous cycles were smaller and grew at slower rates compared with that in healthy animals. In turn, this led to a smaller corpus luteum (**CL**) diameter and reduced production of estradiol and progesterone (Sheldon et al., 2002; Williams et al., 2007). Reduced progesterone production may be beneficial in clearing infection, because progesterone can inhibit uterine eicosanoid production, reducing the immune response necessary to clear infection (Rawson et al., 1953; Hawk et al., 1964; Szekeres-Bartho et al., 2001). However, this reduces the likelihood of successfully maintaining a pregnancy. This inflammatory response is the main cause of poor follicular development. Increased inflam-

mation in the uterus increases LPS concentration in follicular fluid (Herath et al., 2009). Granulosa cells in ovarian follicles, which are responsible for estradiol production, contain TLR-4, the natural receptor for LPS (Herath et al., 2007). Binding of LPS to TLR-4 induces inflammation, reduces follicular steroidogenesis, and increases rates of meiotic arrest in the oocyte (Figure 1A; Herath et al., 2007; Bromfield and Sheldon, 2011). Therefore, bacteria present anywhere in the reproductive tract could be inducing an inflammatory response and lead to lower quality oocytes.

Pathogens, the Uterine Body, and Insemination

Another factor required for pregnancy success is a healthy, functioning endometrium. In ruminants such as cattle and sheep, the endometrium consists of aglandular caruncular areas that contain dense stroma and intercaruncular areas that contain uterine glands. These uterine glands secrete substances required for embryo survival and development (Gray et al., 2001b). A uterine gland knockout sheep has been developed, where the adult uterus does not contain any glands (Gray et al., 2001a). These adult knockout sheep were unable to sustain pregnancy and consistently experienced pregnancy loss (Gray et al., 2002). Uterine glands also interact with sperm during insemination. After insemination, some sperm enter uterine glands and induce an acute inflammatory response via the TLR-2 pathway (Akthar et al., 2020). This response is important in removing excess sperm and preparing the uterus for embryo implantation. Damage to glands may prevent

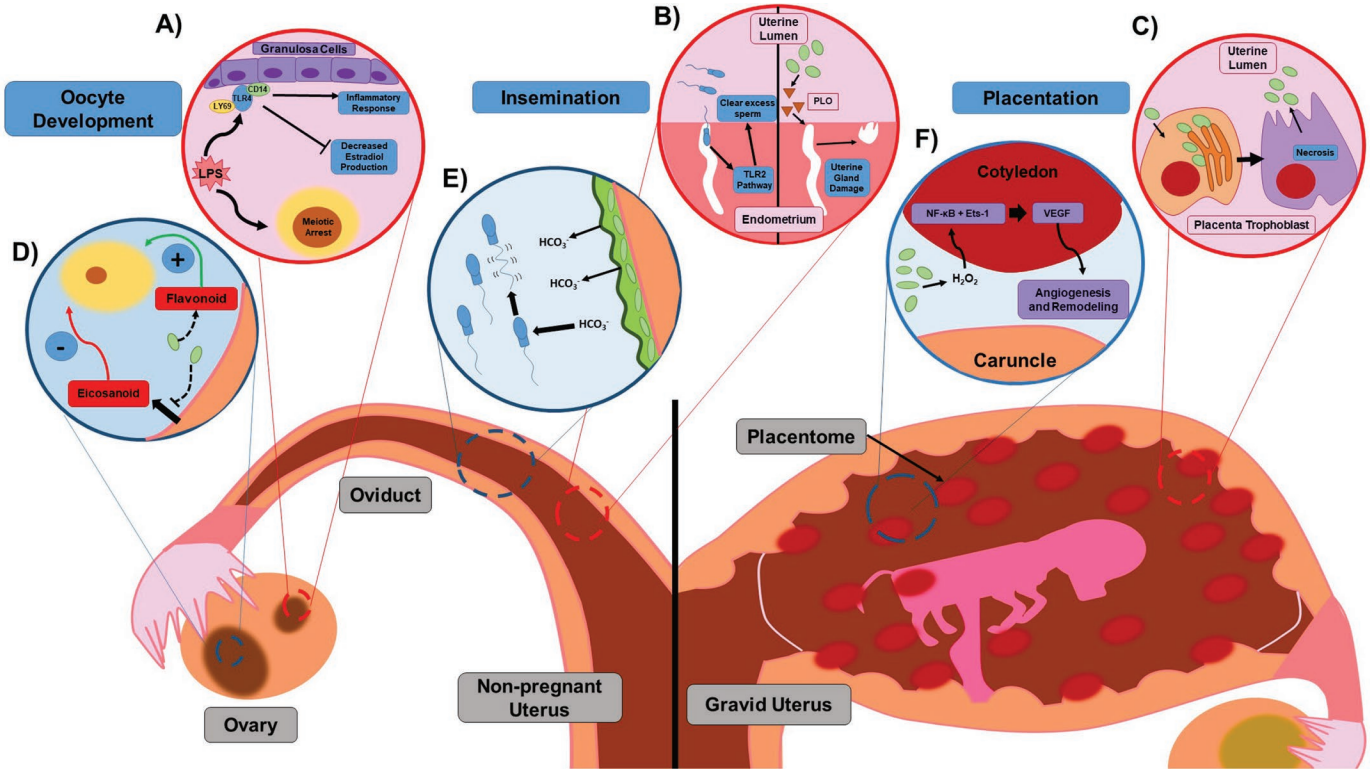

Figure 1. Summary figure showing the pathogenic (A–C) and possible nonpathogenic (D–F) interactions between the microbiome of the reproductive tract and the host. (A) Oocyte development: LPS from *Escherichia coli* in the uterus increases LPS in dominant follicles, causing meiotic arrest in the oocyte and binding to receptors on granulosa cells. This induces an inflammatory response and decreases estradiol production. (B) Insemination: Normally, some sperm will enter the uterine glands after insemination, inducing a response that prepares the uterus for the fertilized embryo. Uterine glands are damaged by pyolysin (PLO) from *Trueperella pyogenes*, which could hinder the response needed to clear excess sperm and allow embryo attachment. (C) Placentation: After being ingested, *Brucella*, *Listeria*, or *Campylobacter* travel through the bloodstream to placentomes in the uterus. They then invade placental trophoblasts and replicate, causing necrosis of the trophoblast and spreading the pathogen throughout the uterus. (D) Oocyte development: *Lactobacillus* identified in the follicular fluid could be secreting flavonoids, which have been associated with increased oocyte competency, while also mitigating the secretion of eicosanoids, which have been associated with decreased oocyte quality. (E) Capacitation: *Lactobacillus delbrueckii* within the uterus and vagina have the ability to produce HCO_3^- ion. This may enhance the ion gradient necessary for sperm capacitation, increasing the amount of fully capacitated sperm. (F) Placentation: The rate of angiogenesis is increased in the presence of reactive oxygen species (i.e., H_2O_2), which increase the expression of genes encoding transcription factors Ets-1 and nuclear factor (NF)-κB and increase production of the primary angiogenic factor vascular endothelial growth factor (VEGF). *Lactobacillus*, *Streptococcus*, and *Enterococcus* found in the uterus have been associated with increased pregnancy success; they produce H_2O_2, which could increase the rate of angiogenesis and increase placentation.

this response, leading to pregnancy failure. Defects or damage to endometrial glands could negatively affect fertility, both immediately after damage and in future pregnancies.

While tissue damage and inflammation are normal after parturition, uterine bacterial infections have the ability to further damage glands in the dairy cow's uterus. Endometrial glands in cows that experienced metritis had predominantly cuboidal epithelial cells compared with the columnar cells in healthy cows (Sicsic et al., 2018). Although these cuboidal cells may be able to secrete nutrients necessary for pregnancy, they may not function as efficiently as the typical columnar epithelia. Animals that experienced septic metritis, or foul-smelling vaginal discharge along with high fever and other signs of infection, had some destruction of endometrial glands (Sicsic et al., 2018). The specific bacteria responsible for this damage have not been identified, but it could be caused by secretion of pyolisin by *T. pyogenes*, which forms pores in endometrial cell membranes (Figure 1B; Carneiro et al., 2016). Depending on the extent of infection, this damage could have a long-term effect on fertility if the uterine glands are unable to recover.

Pathogens, the Placenta, and Abortions

The uterus is sensitive to subtle changes during gestation, and bacterial infiltration can cause pregnancy loss. Although not as common due to vaccine development, *Brucella abortus* is a pathogen known to cause abortions. Typically, *B. abortus* is ingested, where it grows in the lymph nodes before spreading to the udder and pregnant uterus via blood vessels (Anderson, 2007). It then invades trophoblasts in the placenta, causing inflammation of the placenta, necrosis of trophoblasts, and fetal death within 72 h (Figure 1C; Anderson, 2007). Other pathogens, including *Listeria monocytogenes*, *Salmonella* Dublin, and *Campylobacter* spp., function similarly, but time between infection and pregnancy loss can be up to 3 mo (Anderson, 2007). However, opportunistic bacteria are associated with 25 to 50% of pregnancy losses (Anderson, 2007). These opportunistic pathogens are typically found in low numbers in mucosal membranes, but disruptions to homeostatic conditions can lead to dysbiosis. Opportunistic bacteria associated with uterine disease and abortion have been found in endometrium and placentomes of pregnant cows using fluorescence in situ hybridization (Karstrup et al., 2017). However, because there were no signs of inflammation in observed animals, it is possible that bacteria normally exist in the uterus and placenta at low abundance. These bacteria could influence the microenvironment of the placenta in a nonpathogenic manner, but further research examining bacteria in the absence of inflammation or clinical infection is needed.

NONPATHOGENIC BACTERIA AND POTENTIAL INTERACTIONS

Nonpathogenic Bacteria, Oocyte Development, and Ovulation

Successful fertilization of an oocyte relies on the reproductive capabilities of both the bull and the dam. While a microbiome has been detected in the testes, vesicular fluid, and ejaculate of male humans, mice, and cattle (Moretti et al., 2009; Javurek et al., 2016; Alfano et al., 2018), this review will focus on the microbiome within the female. The literature suggests that bacteria present within the female urogenital tract influence key mechanisms involved in development of the ova, capacitation of sperm in the female, and fertilization of the ova. As discussed next, it appears that bacteria are capable of altering the environment for the sperm, oocyte, and embryo, therefore influencing the principal mechanisms required to support a pregnancy.

The environment in which an oocyte develops plays a key role in oocyte quality and its ability to be fertilized. Granulosa and theca cells within the follicular antrum create follicular fluid, which supports oocyte development (Fortune, 1994). Bacteria in follicular fluid could affect follicular fluid composition. In humans, *Lactobacillus* spp., *Actinomyces* spp., *Bifidobacterium* spp., and *Propionibacterium* spp. have been cultured from follicular fluid of ovaries but not from the vagina (Pelzer et al., 2013), demonstrating that live bacteria exist in the follicular fluid that are not contaminants from the vagina. Of these bacteria, only *Lactobacillus* spp. were positively associated with embryo transfer success during IVF, whereas other species were negatively associated with IVF outcomes (Pelzer et al., 2013). In the dairy cow, increased follicular fluid concentrations of flavonoids and phenolics have been associated with increased oocyte competency, whereas increased eicosanoid and docosanoid concentrations have been associated with lower quality oocytes (Guerreiro et al., 2018). Flavonoids and phenolics have an antioxidant property that helps protect the oocyte against oxidative stress and its negative effect on oocyte quality (Martins et al., 2016). Eicosanoids and docosanoids are intermediates in inflammation pathways, serving as markers for inflammation processes such as oxidative stress that have a negative effect on fertility (Jabbour et al., 2009). Within the gastrointestinal tract, *Lactobacillus* spp. have been shown to produce phenolics and suppress the production of eicosanoids (Fiander et al., 2005; Rodríguez et al., 2009). It is possible that the

species identified in human follicular fluid can perform either of these functions, thereby enhancing fertility (Figure 1D). To our knowledge, the bovine follicular fluid microbiome has not yet been studied in depth. Use of non-culture-based techniques of bovine follicular fluid samples, metabolomic screenings, or both could reveal new biomarkers of fertility.

Another important aspect to consider is the amount of bacteria necessary to influence the reproductive tract. Bacterial density in the bovine reproductive tract also influences the level of host response. Cows with a higher density of bacteria during postpartum infection had smaller follicles and CL as well as reduced concentrations of circulating estradiol and progesterone (Williams et al., 2007). Although commensal bacteria have been identified throughout the dairy cow reproductive tract, are they present at a high enough density to affect reproductive performance without causing dysbiosis?

Nonpathogenic Bacteria, Insemination, and Fertilization

Once sperm are placed within the female reproductive tract, they begin a process of final maturation called capacitation. During this process, sperm rely on the influx of bicarbonate (HCO_3^-) and Ca^{2+} through the plasma membrane to activate soluble adenylyl cyclase, cyclic AMP, and protein kinase A (Ickowicz et al., 2012). Activation of these enzymes then leads to the activation of the phosphoinositide 3-kinase (PI3K) pathway, which is responsible for exciting motility of sperm required for fertilization (Ickowicz et al., 2012). This sperm hyperactivated motility, characterized by asymmetrical beating of flagella, also enhances penetration through the zona pellucida for fertilization (Ickowicz et al., 2012).

An increase in extracellular HCO_3^- and Ca^{2+} increases the ability of sperm to undergo capacitation once in the female; conversely, a decrease prevents sperm from reaching full maturity (Visconti et al., 2011). Bacteria that have been identified in the uterus could influence this gradient for the sperm. For instance, in humans, a *Lactobacillus* spp.-dominated uterus ($\geq$90% relative abundance) is more likely to become and stay pregnant compared with a uterus not dominated by *Lactobacillus* spp. (Moreno et al., 2016). *Lactobacillus delbrueckii* has been commonly identified in the human vagina and is able to utilize carbonic anhydrase to convert H_2O and CO_2 into carbonic acid, which quickly disassociates into HCO_3^- and diffuses out of the cell (Li et al., 2015). Thus, *Lactobacillus* spp. present in the uterus could increase the amount of HCO_3^- in the reproductive tract, thus facilitating the capacitation process (Figure 1E). Although the phyla *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* have been detected in the virgin heifer uterus (Moore et al., 2017), functional assessment is still lacking and we do not yet know if, or how, uterine bacteria positively affect the capacitation process in dairy cattle.

Inflammatory Response During Early Pregnancy and Placentation

The conceptus implements mechanisms in early pregnancy to prevent the dam from treating it like a foreign body. During early pregnancy, interactions between the uterus and the conceptus are broadly defined as anti-inflammatory. Cytotoxic T cells and natural killer (**NK**) cells reside within the endometrium; these cells utilize major histocompatibility complex (**MHC**) class I molecules to detect whether antigens in the uterus are self or non-self (Bessoles et al., 2014). If the cell produces MHC class I molecules with peptides recognized as the host's, NK cells are not triggered to kill that cell (Bessoles et al., 2014). However, the conceptus does not produce these "self" MHC class I molecules during early pregnancy and is susceptible to triggering these NK cells. Natural killer cells seem to contribute toward pregnancy and placentation, as they modify maternal arteries to aid in placental angiogenesis and produce T-helper (**Th**) 2 cytokines during early pregnancy (Mori et al., 2016). What could be modulating the function of the NK cells to aid in pregnancy rather than hinder it? Although not fully understood, it is possible that increased production of indoleamine 2,3-dioxygenase (**IDO**) caused by the presence of the bovine embryo regulates the function of NK and cytotoxic T cells to suppress their inflammatory functions. The gene encoding IDO1, an immune response regulator that decreases inflammation, is stimulated by IFN-τ and has increased expression at the implantation site in mice (von Rango et al., 2007). Increased levels of IDO1 lead to increased activation of aryl hydrocarbon receptors (**AHR**). These AHR promote production of peroxisome proliferator-activated receptor-γ, which decreases proinflammatory cytokine production and improves immune tolerance, leading to recognition of the conceptus as "self" (Vacca et al., 2010).

Of the bacteria found in the uterus, *Lactobacillus* spp. have been positively associated with increased success of IVF in humans and so are potential mediators of IDO1 production and AHR activation. However, research shows conflicting information on how different *Lactobacillus* spp. may influence early pregnancy success. Within the mouse gastrointestinal tract, presence

of *Lactobacillus reuteri* inhibits the activity of IDO1 through its production of reactive oxygen species (**ROS**), leading to an overall increase in immune response by the mouse (Marin et al., 2017). *Lactobacillus reuteri* could be inhibiting IDO1 production at the site of implantation, reducing immune tolerance that aids in successful establishment of pregnancy. However, *L. reuteri* also produce indole-3-lactic acid, an AHR agonist, which converts $CD4^+$ T cells into $CD4^+CD8\alpha\alpha^+$ T regulatory cells (**Tregs**; Cervantes-Barragan et al., 2017). This suppresses the immune response and reduces inflammation (Cervantes-Barragan et al., 2017). Production of AHR agonists in the uterus by *L. reuteri* could increase the number of epithelial Treg cells that aid in immunosuppression and improve the ability of cow to become pregnant. Other species of lactobacilli have shown to affect the bovine endometrial production of inflammatory cytokines in vitro. Gärtner et al. (2015) cocultured bovine endometrial epithelial cells with 4 different *Lactobacillus* spp. *Lactobacillus vaginalis* and *Lactobacillus buchneri* did not affect expression of proinflammatory cytokines *IL1A*, *IL6*, and *IL8*, whereas *Lactobacillus amylovorus* and *Lactobacillus ruminis* increased expression of all genes with increased concentration of bacteria.

Of these proinflammatory cytokines, IL-6 seems to be important for pregnancy recognition and embryo development. Interleukin-6 can act in both a proinflammatory and anti-inflammatory manner, depending on the signaling method. If IL-6 binds to a membrane-bound receptor, the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway induces production of yes-associated protein for cell proliferation, tissue regeneration, and anti-inflammatory activities (Rose-John et al., 2006). If IL-6 binds to soluble receptors, a complex forms that can bind to nonspecific surface receptors and induce an inflammatory response (Rose-John et al., 2006). The bovine embryo has increased inner cell mass numbers and blastocyst development when cultured with IL-6, so it seems the embryo may primarily utilize the membrane-bound receptor (Wooldridge and Ealy, 2019). In humans, increased inner cell mass is associated with a decrease in early embryonic loss; therefore, increased IL-6 production induced by bacteria could be aiding in early pregnancy (Richter et al., 2001). Conversely, IL-6 is a key cytokine during the early phases of graft-versus-host disease, where blocking IL-6 receptors reduces the magnitude of inflammation (Belle et al., 2017). To fully understand the role of IL-6 during implantation and the effect bacteria may have on pregnancy success, research involving IL-6 in the presence of both the embryo and endometrium is needed.

Bacteria and Placental Attachment and Function

Once implantation has successfully occurred and placentomes have formed, energy delivered to the fetus is used primarily for growth. Fetal growth is regulated by the placenta, with the magnitude of vasculature of the placenta positively correlated with fetal weight (Echternkamp, 1993). The availability of certain nutrients affects placental growth and angiogenesis. A microbiome unique to the placenta has been identified in both humans and dairy cows in the absence of an inflammatory response (Aargaard et al., 2014; Moore et al., 2017). The bacteria present in the placenta have the capability to influence placental growth rate, angiogenesis, or transport of nutrients to the fetus.

Although the microbiome in the placenta has been detected, it is not as well characterized. In the dairy cow, research on the "nonpathogenic" microbiome in the reproductive tract is limited. Moore et al. (2017) observed that both the placentome (the site of placental attachment) and the intercotyledonary placenta in the pregnant dairy cow were dominated by the bacterial phyla *Firmicutes* (35 to 42% of the total microbiome) and *Bacteroidetes* (23 to 33% of the total microbiome). Results mirrored phyla present in the endometrium of the same cows (Moore et al., 2017). However, in that study, observations were limited and the category of "phylum" was too large a taxon to make any meaningful conclusions regarding the functions of identified bacteria. Aagaard et al. (2014) discovered a microbiome within the placenta of humans comprising primarily *Proteobacteria* and *Actinobacteria* and a greater level of *Tenericutes* compared with other locations in the body. Also, *E. coli* and *Bacteroides* spp. were abundant in the human placenta microbiome (Aagaard et al., 2014). However, that study did not characterize other locations of the reproductive tract, so the placental microbiome may not be completely distinct from the uterus, for instance. If the microbiome of the placenta and uterus are similar, *Lactobacillus* spp. could positively influence the placenta and its growth, as they have been positively associated with pregnancy outcomes (Moreno et al., 2016). More research into the placental microbiome of dairy cattle and reproductive outcomes seems warranted. That said, the importance of a placental microbiome has been questioned. While Aagaard et al. (2014) characterized a human placental microbiome, findings have not, to our knowledge, been replicated. This suggests either a low abundance microbiome in the placenta or contamination (Leiby et al., 2018). It is possible that a true, distinct placental microbiome does not exist, but that the placenta is instead another tissue in which bacteria in the uterus can

proliferate. Bacterial species, such as *F. necrophorum*, *T. pyogenes*, *Porphyromonas levii*, and other unspecified bacteria have been identified in the placentomes and endometrium of dairy cattle using fluorescence in situ hybridization (Karstrup et al., 2017). This provides evidence that even if the previously identified placental microbiome was due to reagent contamination, there are still bacteria within the bovine placenta that may influence placental angiogenesis.

Increased vasculature of the placenta causes greater fetal growth rate and improves placental ability to transport nutrients from dam to fetal calf. If angiogenesis is negatively affected, there is a negative effect on the size of the fetus that could even lead to a termination of the pregnancy (Kang et al., 2014). Reactive oxygen species, such as H_2O_2 and superoxide, are naturally produced by the dam when under metabolic stress through the oxidative phosphorylation pathway in the mitochondria (Starkov, 2008). Reactive oxygen species help control cellular development and function when adequately counterbalanced by antioxidants such as glutathione peroxidase (Iwaoka and Tomoda, 1994). During the first trimester of mammalian pregnancy, there is a dramatic increase in placental oxygen level when blood flow is established in the intervillous space, leading to increased oxidative stress and ROS production (Jauniaux et al., 2000). This spike in ROS throughout gestation could play a role in placental angiogenesis. Increased angiogenesis during periods of oxidative stress is most likely a response to meet the tissue's need for oxygen; by increasing vasculature in tissue, transport of oxygen and nutrients to the desired location is increased.

While not as well defined in the placenta, interactions between ROS and transcription factors that regulate angiogenesis and cellular differentiation have been studied in other blood vessels in cattle and humans (Pereira et al., 2015). Transcription factors such as E26 transformation specific oncogene homolog 1 (**Ets-1**) and nuclear factor kappa-light-chain-enhancer of activated B (**NF-κB**) increase rate of angiogenesis and trophoblast invasion. These transcription factors are also upregulated in the presence of ROS (Shono et al., 1996; Oikawa et al., 2001). When Ets-1 production is increased, it increases the production of vascular endothelial growth factor (**VEGF**), a protein that then increases angiogenesis (Hashiya et al., 2004). It is also thought that because Ets-1 is positively correlated with trophoblast invasion for placental attachment, increased Ets-1 production increases expression of matrix metalloproteinase 9 and urokinase-type plasminogen activator, both of which are known to be important for trophoblast invasion during placental attachment (Dittmer, 2003). Increased production of NF-κB occurs when intracellular ROS production increases, leading to an upregulation of downstream angiogenic factors (Gloire et al., 2006). Interactions between NF-κB and ROS also aid in the restructuring or formation of tubules for angiogenesis. When cells are exposed to H_2O_2, NF-κB has an increased capacity to bind to DNA and activate production of IL6 and IL8, which influence cellular differentiation and growth (Shono et al., 1996; Bonavia et al., 2012). Changes in the regulation of these transcription factors and their interactions with ROS could influence angiogenesis in the placenta and subsequently affect fetal growth. Several bacterial genera, including *Lactobacillus*, *Streptococcus*, and *Enterococcus*, have demonstrated an ability to produce H_2O_2 using NADH oxidase (McLeod and Gordon, 1922; Marty-Teysset et al., 2000). These genera have all been found in the oviduct, uterus, and vaginal microbiomes, with notable species including *L. delbrueckii*, *Lactobacillus crispatus*, and *Lactobacillus jensenii* (Antonio et al., 1999). If these species are present and produce appreciable quantities of H_2O_2 at the site of placental attachment in dairy cattle, the resultant exposure of placental and endometrial cells to ROS could increase production of transcription factors Ets-1 and NF-κB. The increased angiogenesis due to production of these factors would improve likelihood of a successful pregnancy (Figure 1F). If this is the case, there would be a positive association, functional relationship, or both between certain placental bacterial species and fetal growth rate. However, this might only occur at a lower bacterial density, with small increases in bacteria shifting the uterus into dysbiosis and allowing opportunistic bacteria to cause infection or pregnancy loss.

CONCLUSIONS

Commensal bacteria have been isolated from healthy female reproductive tracts of a variety of animals. In many but not all instances, the presence of these bacteria has been linked to favorable reproductive outcomes. Considerably less is known about presence and function of bacteria within healthy reproductive tracts of female dairy cattle compared with that of other animals. We have outlined pathogenesis of bacteria that induce uterine infections and potential ways that commensal bacteria may interact with reproductive host tissues in dairy cattle (Figure 1) and we have proposed future avenues of research. Uterine pathogens such as *E. coli*, *T. pyogenes*, and *F. necrophorum* can cause damage at the infection site and have negative influences during subsequent ovulations. *Lactobacillus* could be secreting flavonoids while mitigating the secretion of eicosanoids in follicular fluid to improve oocyte quality, disrupting the ion gradient necessary for successful sperm capaci-

tation. Alternatively, they may be producing H_2O_2 and increasing production of the primary angiogenic factor VEGF in the placenta. Further investigation of relationships between the reproductive tract's microbiome and specific reproductive performance traits in the dairy cow are needed to fully understand the role of the reproductive microbiome at all points in pregnancy. It also important to understand the bacterial density necessary to induce a physiologic response. Once these mechanisms are more thoroughly understood, strategies can be developed to utilize the microbiome to benefit fertility in the dairy industry.

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