Preventing postpartum uterine disease in dairy cattle depends on avoiding, tolerating and resisting pathogenic bacteria

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Abstract

Up to forty percent of dairy cows can develop metritis or endometritis when bacteria infect the uterus after parturition. However, it is unclear why other cows exposed to similar pathogens do not develop uterine disease. We suggest that resilient dairy cows prevent the development of uterine disease using the three complimentary defensive strategies of avoiding, tolerating and resisting infection with pathogenic bacteria. Avoidance maintains health by limiting the exposure to pathogens. Avoidance includes intrinsic behaviors to prevent exposure to pathogens or infected animals, perhaps signaled by the fetid odor of uterine disease. Tolerance improves health by limiting the tissue damage caused by the pathogen burden. Tolerance depends on controlling the tissue damage that pathogens cause in the endometrium by neutralizing bacterial toxins, enhancing tissue repair, and inducing adaptive metabolic responses. Resistance improves health by limiting the pathogen burden. Resistance relies on the immune system generating an inflammatory response in the endometrium to eliminate pathogenic bacteria. People who manage dairy cows can also help prevent uterine disease by using extended lactations, avoiding trauma to the genital tract, maintaining hygiene, and supplying appropriate nutrition during the transition period and after parturition to counter the metabolic stress of lactation. Developing new ways to prevent uterine disease depends on increasing our understanding of the mechanisms of avoidance, tolerance and resistance to pathogens in the postpartum uterus.

Keywords: Bovine; Uterus; Metritis; Endometritis; Resilience

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1. Introduction

Theriogenology published a set of definitions for postpartum uterine diseases in cattle in 2006 [1]. Since then, there has been increased understanding about the incidence and consequences of the main uterine diseases - metritis and endometritis - and their etiology and pathogenesis [2-6]. However, it is less clear why some dairy cows develop postpartum uterine disease, whilst others cows exposed to the same pathogens do not develop disease. We suggest that resilient dairy cows prevent the development of uterine disease by avoiding, tolerating and resisting infection with pathogenic bacteria (Fig. 1).

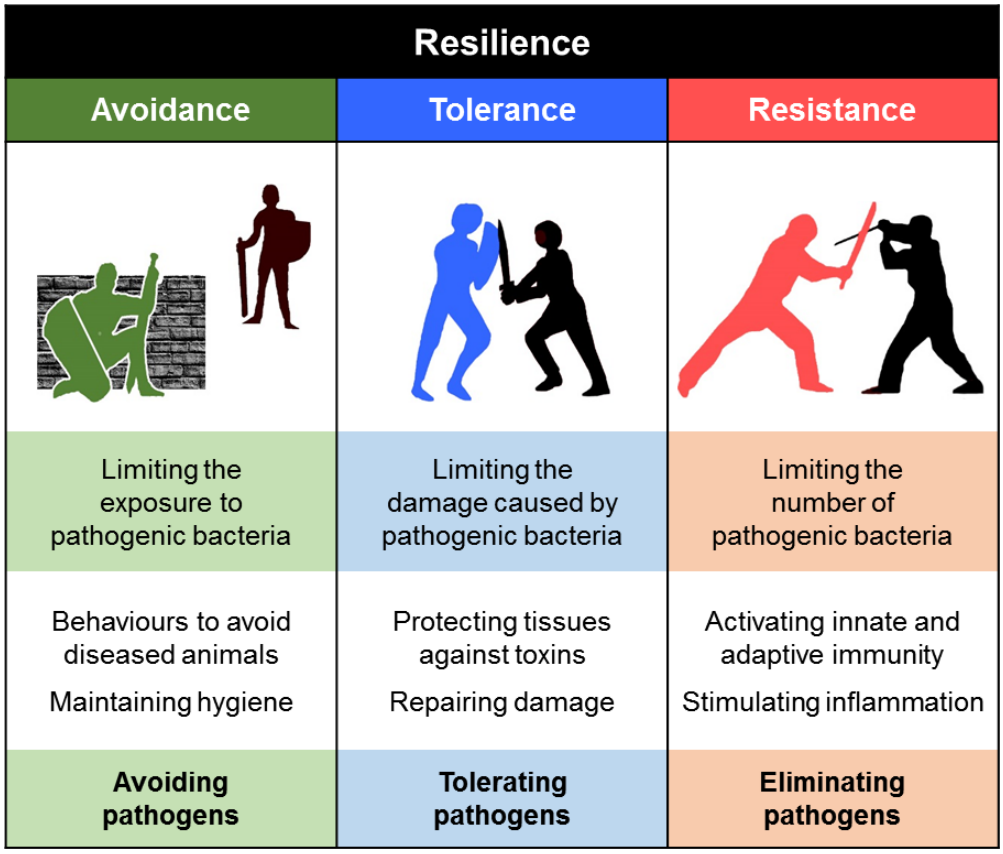

Figure 1. Resilient animals defend themselves against pathogens using the complimentary defensive strategies of avoidance, tolerance and resistance.

Avoidance is the ability to limit the exposure to pathogenic bacteria, most often using intrinsic behaviors. Tolerance is the ability to limit the tissue damage caused by the pathogen burden, without affecting the fitness of the pathogens. Resistance is the ability to limit the pathogen burden, which is usually the function of immunity, and leads to elimination of the pathogens. Resilience is the aggregate of these three complimentary defensive strategies - preventing disease and reducing the severity of disease [2, 7, 8]. We assume that the ancestors of modern dairy cows were highly resilient to postpartum uterine infections. However, genetic selection for milk

production and intensive farming have compromised the resilience of postpartum cows [9].

Metritis and endometritis commonly affect twenty to forty per cent of high-milk-yield *Bos taurus* dairy cattle [3]. We treat these animals because uterine disease causes pain, reduces milk yields, produces an unpleasant uterine discharge, and reduces fertility [10, 11]. However, the current reliance on antibiotics needs rethinking because, although antibiotic treatments help resolve the clinical signs, there is still reduced fertility and governments are concerned about the development of antimicrobial resistance [12]. The annual cost of the reduced fertility, lost milk production, and treatment was estimated to be €1.4 billion in the European Union and $650 million in the USA [3]. The economic impact may be even higher as a single case of metritis can cost farmers up to $410 in the USA [13]. Clearly, prevention of postpartum uterine disease would be better than cure, for both the animals and the economy.

Here we review the mechanisms that dairy cows use to prevent postpartum uterine disease. We only briefly discuss the etiology, pathogenesis, diagnosis and treatment of uterine disease because there are recent reviews available [2, 4, 6]. Instead, we focus on how preventing postpartum uterine disease depends on avoiding, tolerating and resisting infections with pathogenic bacteria.

2. The postpartum period

Optimal fertility in dairy cattle depends on completing several integrated physiological processes in the first five weeks after parturition: prompt involution of the uterus and restoration of a receptive endometrium; resumption of ovarian cyclical activity and ovulation of competent oocytes; and, control of pathogenic bacteria in the uterus.

Uterine involution involves reparative inflammation, remodeling the extracellular matrix, and regenerating the epithelium [14, 15]. There is concurrent return of ovarian cyclic activity, driven by coordinated endocrine programs in the hypothalamus, pituitary, ovary, and uterus [16-18]. Within days of parturition, the circulating steroid hormone concentrations associated with pregnancy decrease to basal values. Plasma follicle stimulating hormone concentrations increase about 7 days post partum, which prompts the emergence of a cohort of growing follicles in the ovary, with subsequent waves of growing follicles every 7 to 10 days. In normal animals, the first postpartum dominant follicle should ovulate, indicating the return of ovarian cyclic activity. However, uterine and other diseases, or the metabolic stress of lactation, can delay uterine involution and the return of ovarian cyclic activity [16, 19].

We propose that animals control (tolerate) rather than eliminate (resist) pathogenic bacteria in the postpartum uterus because healthy animals have an endometrial microbiota – a community of commensal, symbiotic and pathogenic microorganisms [20-22]. Even during pregnancy the microbiota includes uterine pathogens such as *Trueperella pyogenes, Fusobacteria* species and *Prevotella* species [20, 21]. After

parturition, the microbial community expands and fluctuates because vaginal and cervical dilation allow contamination of the genital tract with bacteria from the vagina, skin, feces and environment. The pathogenic bacteria cultured from animals with uterine disease include *Escherichia coli, T. pyogenes, Fusobacterium necrophorum*, and *Prevotella* and *Bacteroides* species [23]. Genomic techniques have identified additional phyla associated with uterine disease, including *Bacteroidetes, Fusobacteria, Proteobacteria* and *Firmicutes* [22, 24]. However, it is unclear whether specific keystone bacteria, combinations of bacteria, or the abundance of bacteria causes uterine disease. For example, uterine disease is associated with novel strains of *E. coli* [25, 26], but after three weeks postpartum, *Trueperella pyogenes* is the pathogen most associated with the severity of endometrial pathology [27, 28].

3. Uterine disease

The clinical definitions of postpartum uterine diseases are well established [1, 2, 29, 30]. Metritis commonly occurs within 10 days of parturition, and is characterized by an enlarged uterus, with a watery red-brown fluid to viscous off-white purulent uterine discharge, which often has a fetid odor. Clinical endometritis is defined by the presence of a purulent uterine discharge detectable in the vagina of cattle 21 days or more post partum. Subclinical endometritis is diagnosed when the proportion of neutrophils in endometrial flush or cytobrush samples exceeds operator-defined thresholds, usually about 5% of cells, and there are no signs of clinical endometritis. However, new knowledge is required to understand whether purulent vaginal discharge [31], associated with vaginitis or cervicitis, is independent of clinical or subclinical endometritis. In addition, future longitudinal studies should examine the interrelationships amongst the pathogens, and the progression of metritis, clinical endometritis and subclinical endometritis.

The incidence of metritis, including retained placenta, was 21% in a survey of 97,318 Holstein cows in the USA [32]; and, the incidence of endometritis was 19% in 19,870 Holstein cows in Germany [33]. Metritis is also important in purebred and crossbred *Bos indicus* dairy cows and buffalo; the incidence of metritis was 10.3%, 22.6%, and 9.7% in 1,609 Sahiwal lactations, 2,549 crossbred Holstein x Tharparkar lactations, and 1,604 Murrah buffalo lactations, respectively [34]. The greatest impact of uterine disease, is that there is reduced fertility even after successful treatment. For example, in a meta-analysis including more than 10,000 cases of metritis, disease increased the time to first insemination by 7.2 days, reduced conception rates to first insemination by 20%, and increased the calving-to-conception interval by 18.6 days [11]. The reduced fertility is caused by inflammation in the genital tract, disruption of ovarian follicle growth and function, and damage to oocytes [23, 35-37]. We argue that prevention of disease is far more important than developing new treatments for metritis or endometritis.

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4. Defense against pathogens in the uterus

Uterine disease provides an opportunity to study animal resilience because the incidence of disease varies amongst individual animals, breeds, levels of milk production, and farms, despite exposure of the uterus to similar bacteria. We suggest that preventing uterine disease depends on the integrated defensive strategies of avoidance, tolerance and resistance, which have different effects on the interaction of pathogen burden and health (Fig. 2).

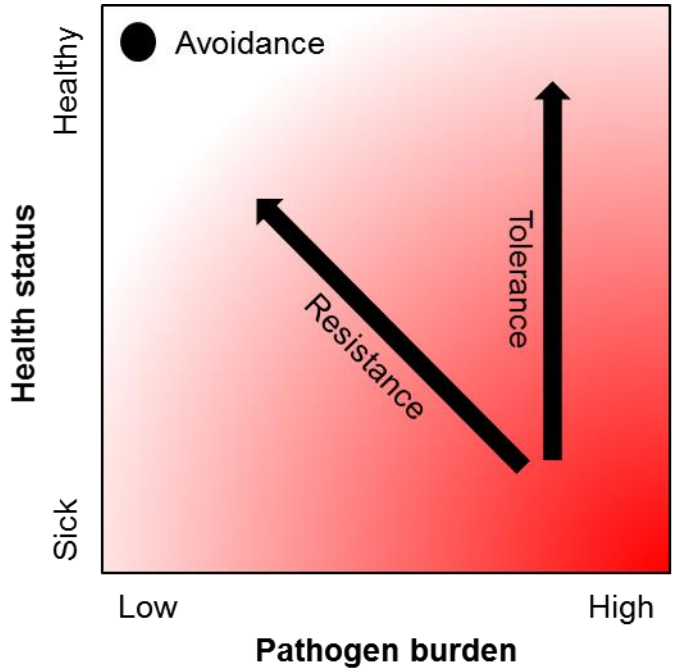

Figure 2. A conceptual framework for resilience to pathogens. A theoretical reaction norm graph of health status, where health increases vertically, against pathogen burden, where the pathogen burden increases horizontally [2, 7, 8]. Avoidance maintains health by limiting the exposure to pathogens. Tolerance improves health by limiting the tissue damage caused by the pathogen burden. Resistance improves health by limiting the pathogen burden.

4.1 Avoidance

Avoidance mechanisms that limit exposure to pathogens have the advantage over tolerance and resistance because there is no specific metabolic cost to counter the pathogens. Furthermore, the metabolic costs are low for avoidance mechanisms such as barriers and behavior. Examples of barriers are the vulva, vagina and cervix, along with the cervical mucus plug, which prevent bacteria ascending the genital tract during pregnancy. However, parturition, dystocia, and poor conformation of the vulva or vagina, then allow bacteria to breach these barriers and invade the uterus.

An example of an intrinsic behavior that can help animals avoid pathogens is that cows seek seclusion from herd-mates around the time of parturition [38]. Furthermore, cows with metritis have sickness behaviors, including inappetence, inactivity, and depression, which result in sick cows spending more time away from healthy herd-mates [38]. However, it is unclear whether metritis can be a transmissible disease, and whether pathogens spread more easily when there is a high density of postpartum animals, increasing their exposure to uterine pathogens.

Disgust is a behavior that many animal species use to avoid infection, although it is most evident in humans [39]. The fetid odor of metritis is a striking feature for humans, but it is not clear if herd-mates use this odor to avoid cows with metritis. However, cows avoid feces, presumably based on an averse odor [40]. Other animals also avoid the odor of pathogens or infection. The nematode *Caenorhabditis elegans*, which feeds on bacteria, learns to avoid the odor of pathogenic bacteria, whilst being attracted to odors from nonpathogenic bacteria [41]. Mice use the vomeronasal system to detect the odor of animals that have an infection or even an inflammatory response to LPS [42]. There is an urgent need to better understand the behavior of postpartum cows because intensive dairy production systems are likely to compromise the ability of postpartum animals to use avoidance behaviors [43]. If they are important, allowing cows to use behaviors that avoid infections may require a revaluation of current housing design.

4.2 Tolerance

Tolerance - which should not be confused with immunological tolerance - aims to benefit health by limiting the tissue damage pathogens cause without affecting the bacterial burden [2, 7, 8]. An advantage of tolerance over resistance mechanisms is that tolerance does not impact pathogen fitness, and therefore tolerance does not provoke the pathogen to develop counter measures, such as antimicrobial resistance. We propose that mechanisms for tolerance to pathogens in the uterus include barriers to bacterial infection, neutralization of bacterial toxins, repair of tissue damage, and adaptive metabolic responses (Table 1).

The physical barriers of the epithelial lining of the genital tract and the associated layer of mucus allow pathogens and host to coexist, as well as helping to avoid microbial invasion. Mucus also contains antimicrobial peptides, acute phase proteins, such as haptoglobin and serum amyloid A, and mucosal glycoproteins that neutralize bacteria or their toxins. These tolerance mechanisms are not all passive; there is increased expression of antimicrobial peptides and acute phase proteins in the inflamed endometrium and peripheral plasma of postpartum cows [44, 45]. The importance of the epithelial barrier is highlighted by the need to damage the epithelium when generating animal models of endometritis [46].

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Table 1. Potential disease tolerance mechanisms

Mechanism	Examples
Barriers to infection	Mucus Epithelium
Neutralization of bacterial toxins	Antimicrobial peptides Acute phase proteins
Repair of tissue damage	Cell membrane repair in response to damage caused by bacterial virulence factors, such as pore-forming toxins DNA repair in response to DNA damage caused by endotoxin Unfolded protein response to intracellular proteins damaged by bacteria
Adaptive metabolic responses	Autophagy in response to damage to cellular organelles by intracellular pathogens or changes in cell metabolism Hypoxia responses to the reduced oxygen availability in damaged tissues Oxidative stress responses to hemolysis or inflammation Reprogramming cell metabolism, such as using aerobic glycolysis to generate substrates and supply energy for tissue repair

Damage control and tissue repair help tolerate the presence of pathogens [47, 48]. However, damage, repair and regeneration of the endometrium is also a consequence of parturition [14, 15]. Crucially, loss of the epithelium allows pathogens to reach the underlying sensitive stroma. For example, stromal cells are ten times more sensitive than epithelial cells to damage by the pore-forming toxin pyolysin, which is the cholesterol-dependent cytolysin secreted by *T. pyogenes* [49]. However, it is possible to protect stromal cells against pyolysin *in vitro* by reducing the cellular cholesterol content using methyl-β-cyclodextrin (Fig. 3), or by inhibiting cholesterol synthesis using statins or squalene synthase inhibitors [49-52]. These findings could lead to products that protect the endometrium against pathogen damage.

At the cellular level, damage and pore-forming toxins also prompt cell stress responses: activating the mitogen-activated protein kinases, activating the unfolded protein response, activating caspase enzymes, suppressing protein synthesis, and inducing autophagy [50, 53]. These cell stress responses aim to repair damaged membranes and organelles, and to induce a quiescent dormant state [54]. In humans and mice, sickness behaviors, such as inappetence, activate catabolic pathways that support tolerance to pathogens [54]. However, in dairy cows, the additional metabolic stress of lactation reduces tolerance to uterine infections [2]

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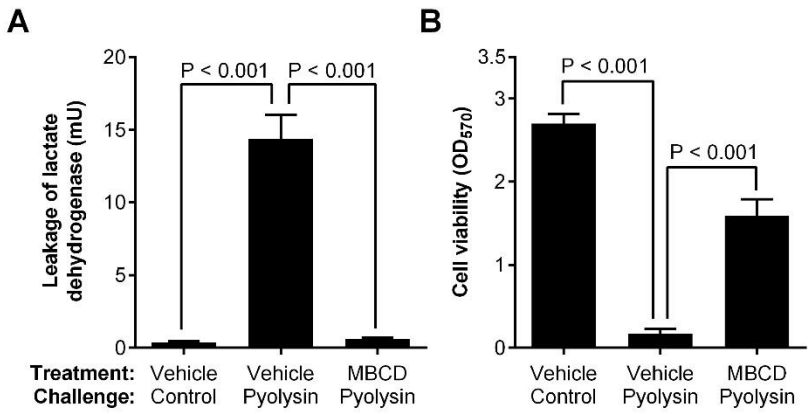

Figure 3. Reducing cellular cholesterol protects stromal cells against pyolysin. Bovine endometrial stromal cells were treated for 24 h in medium containing vehicle or methyl-β-cyclodextrin (MBCD) to depletecellular cholesterol, and then challenged for 2 h with control medium or medium containing the cholesterol-dependent cytolysin, pyolysin. The formation of cell membrane pores was determined by the leakage of lactate dehydrogenase (A) and cell viability was examined using a colorimetric assay for cell metabolic activity (B). Data are presented as mean + SEM (n = 4), and analyzed by ANOVA with Bonferroni post hoc tests. Redrawn from data published previously [51].

4.3 Resistance

If postpartum animals cannot avoid pathogens, as well as attempting to tolerate their presence in the uterus, animals must defend themselves by resisting the pathogens. The innate immune system provides a rapid response to pathogens [55, 56]. As uterine infections are polymicrobial, this innate immune response is ideal because it is non-specific and does not depend on prior exposure to pathogens. Instead, innate immune cells use pattern recognition receptors, such as Toll-like receptors, to recognize pathogen-associated molecular patterns, including bacterial DNA, lipopeptides, flagellin and lipopolysaccharide (endotoxin). This typically results in the secretion of antimicrobial peptides and inflammatory mediators, such as interleukin (IL)-1β, IL-6, IL-8 and prostaglandin E_2 [55, 57]. In the uterus, endometrial epithelial and stromal cells also have a role in innate immunity, generating inflammatory responses to bacteria [58, 59]. The inflammatory mediators cause vasodilation, attract and activate immune cells, and induce production of antimicrobial peptides, acute phase proteins and reactive oxygen species; all of which help counter bacteria in the tissue [2, 55, 57, 60]. The inflammatory mediators also suppress the hypothalamic-pituitary-ovary axis, and induce sickness behaviors, such as inappetence and lethargy [54]. The complement system, which promotes phagocytosis of pathogens by neutrophils and macrophages, provides additional non-specific defense [19].

A rapid and robust inflammatory response efficiently eliminates pathogens, whereas a delayed or blunted inflammatory response can lead to chronic inflammation [2, 54-

57]. Conversely, excessive or uncontrolled inflammation can cause tissue damage. Septic shock during metritis is an example of excessive and uncontrolled inflammation, and subclinical endometritis is an example of uncontrolled persistent inflammation. However, innate immunity is usually regulated to match the level of pathogen challenge. For example, feedback loops, such as STAT3 (signal transducer and activator of transcription-3), tune the immune response in endometrial cells [61]. Tissue damage, with the release of damage-associated molecular patterns also scales the immune response [62], with damaged endometrial cells releasing the intracellular cytokine IL-1α, which stimulates further inflammation [60]. Innate immunity also integrates with tolerance because the innate immune response also induces the production of mucins and antimicrobial peptides [63].

Adaptive immunity depends on prior exposure to specific antigens and takes longer to develop than innate immunity but is evident in the postpartum endometrium, which often contains lymphocytic foci of T cells and B cells [15, 27]. Furthermore, postpartum uterine disease is less likely if cows have increased levels of circulating antibodies, and vaccines containing components of *E. coli*, *F. necrophorum* and/or *T. pyogenes* can protect against metritis [64]. However, it is notable that the adaptive immunity induced by metritis is not sufficient to protect against disease after the next calving. Another feature of adaptive immunity, is that domestication and selective breeding have reduced the diversity of major histocompatibility complex antigens, and retained fetal membranes are more common in animals with reduced major histocompatibility complex antigen diversity [65]

Unfortunately, the biosynthetic demand to repair the endometrium after parturition and resist pathogens is at odds with the metabolic demand for lactation [5]. For example, cows in negative energy balance after calving have persistent endometritis, whereas animals with mild negative energy balance repair their endometrium by two weeks after parturition [19]. Even reduced feeding behavior before parturition predicts the development of metritis [66]. A widely accepted mechanism is that metabolic stress is associated with impaired neutrophil function around the time of parturition [5, 67]. However, deficiencies in glucose or glutamine also blunt the inflammatory response in the endometrium [68, 69]. Perhaps this interaction between metabolism and immunity is not surprising as mounting an immune response is energetically expensive; cows metabolize an extra kilogram of blood glucose in the first 12 hours after challenge with lipopolysaccharide [70]. Unfortunately, despite the association between metabolic stress and uterine disease, it is proving difficult to provide animal nutrition that supports resistance against pathogens.

5. Managing cows to prevent uterine disease

Whilst resilience to uterine infection depends on an animals' avoidance, tolerance and resistance strategies, good management can also help support animal resilience and prevent disease (Fig. 4). Conversely, and perhaps more important for animal welfare,

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suboptimal management of dairy cows can make cows susceptible to postpartum uterine disease.

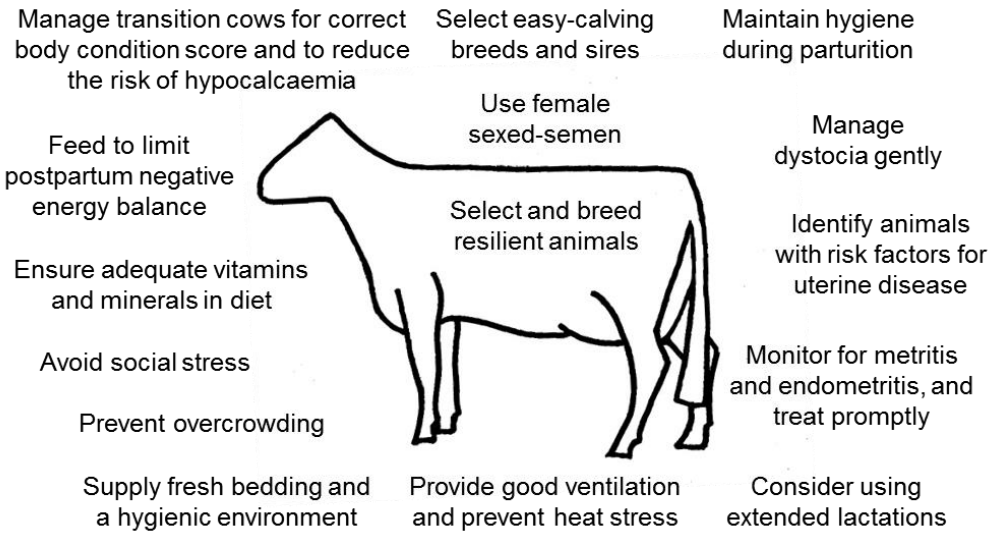

Figure 4. Management can help prevent postpartum uterine disease. Potential approaches that are currently available to farm managers that may help prevent postpartum uterine disease.

An obvious way to avoid postpartum uterine disease is to avoid parturition. Farmers already use extended lactations as a management tool to reduce the frequency of parturition, reduce the incidence of postpartum problems, and even improve herd reproductive performance [71]. Pharmaceutical induction of lactation also avoids parturition, but is rarely used and needs further development.

Another approach is to limit the impact of risk factors for uterine disease. The overarching risk factors for postpartum uterine disease are trauma to the genital tract followed by colonization with pathogenic bacteria. Trauma to the genital tract is associated with first parity, induction of parturition, dystocia, stillbirths, twins, male calves, and retained placenta [72-74]. Selection of easy-calving breeds and easy-calving sires within a breed could reduce the risk of parturient trauma to the genital tract. For example, using sexed semen to produce female calves from an easy-calving sire is a practical way of reducing the risk of uterine disease, and this would have a larger effect on preventing disease in a herd than eliminating retained fetal membranes [74]. Cows should have a clean, comfortable, quiet, and spacious environment to calve without stress. If there is need for intervention during parturition, farmers and veterinarians should ensure that they use aseptic techniques, they are gentle, and they maintain the cleanliness of the environment.

Prevention of uterine disease also requires appropriate nutritional management during the transition period and after calving [5]. Cows should be dried off at the target body condition score, fed a transition cow diet that provides the appropriate fiber, vitamins and minerals, and then feed a postpartum diet to limit the metabolic stress of lactation

[75]. After parturition, it is important to monitor animal health carefully, particularly for cows at risk of disease. Veterinary examination of the reproductive tract is useful to identify animals with disease that may need treatment [6]. However, the routine use of antimicrobials in animals at risk of uterine disease is no longer acceptable because of the risk of antimicrobial resistance.

Encouraging a return to ovarian cyclic activity by appropriate nutrition and reproductive management may also help counter uterine disease. Estrus and estradiol are associated with increased protection against uterine disease in cattle, and the induction of estrus is often used to treat endometritis [76]. Conversely, progesterone is used to suppress immunity when generating animal models of endometritis [46]. Interestingly, estrus and estradiol increase the expression of components of the complement system [77]. However, estrus and estradiol do not alter the innate immune response by endometrial tissues or cells in ex vivo studies [78], and it remains an open question as to how steroids influence the susceptibility of animals to uterine disease.

6. Future perspectives

Preventing endemic diseases is necessary to deliver sustainable intensification of dairy farming, as the dairy industry expands to help feed a projected world of 10.4 billion people by 2067 [79]. Preventing postpartum uterine disease depends on animals avoiding, tolerating and resisting pathogenic bacteria. Humans can also help to prevent disease in dairy cows. Improving resistance, particularly by developing vaccines, holds promise but does not overcome the issue that high-milk-yield dairy cows are prone to develop uterine disease. Instead, we suggest that the most urgent need for preventing disease is to better understand how to avoid and tolerate pathogens [2].

Improving avoidance or tolerance reduces the dependence on antimicrobials to kill pathogens, which helps reduce the danger posed by antimicrobial resistance. A more holistic approach is to breed resilient dairy cows that are more tolerant of uterine pathogens [9]. However, selection criteria for resilience to pathogenic bacteria should take into account the negative correlation between some tolerance and resistance genes [7, 8]. Therefore, we propose that developing new ways to prevent uterine disease depends on improving our understanding of the mechanisms of avoidance, tolerance and resistance to pathogens in the postpartum uterus.

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Declarations of interest

Declarations of interest: none.

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