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 develop metritis or endometritis when pathogenic bacteria infect the uterus after parturition. However, resilient cows remain healthy even when exposed to the same pathogens. Here, we provide a perspective on the mechanisms that dairy cows use to prevent postpartum uterine disease. We suggest that resilient cows prevent the development of uterine disease using the three complementary defensive strategies of avoiding, tolerating and resisting infection with pathogenic bacteria. Avoidance maintains health by limiting the exposure to pathogens. Avoidance mechanisms include intrinsic behaviors to reduce the risk of infection by avoiding pathogens or infected animals, perhaps signaled by the fetid odor of uterine disease. Tolerance improves health by limiting the tissue damage caused by the pathogens. Tolerance mechanisms include neutralizing bacterial toxins, protecting cells against damage, enhancing tissue repair, and reprogramming metabolism. Resistance improves health by limiting the pathogen burden. Resistance mechanisms include inflammation driven by innate immunity and adaptive immunity, with the aim of killing and eliminating pathogenic bacteria. Farmers can also help cows prevent the development of postpartum uterine disease by avoiding trauma to the genital tract, reducing stress, and feeding animals appropriately during the transition period. Understanding the mechanisms of avoidance, tolerance and resistance to pathogens will inform strategies to generate resilient animals and prevent uterine disease.

Keywords: Bovine; Uterus; Metritis; Endometritis; Resilience

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, etiology, pathogenesis and consequences of the main postpartum uterine diseases - metritis and endometritis [2-7]. It is less clear why some dairy cows develop postpartum uterine disease when infected with pathogenic bacteria around the time of parturition, whilst other resilient

cows remain healthy, even when exposed to the same pathogens. 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 complementary defensive strategies of avoidance, tolerance and resistance. The cartoon sets out the concept for each strategy and provides examples of the mechanisms that animals use to defend themselves against pathogens.

The scientific framework for understanding defense against pathogens has developed over the last 120 years. The origins of the study of resistance – often equated with immunology - are often based on the work of Metchnikoff and Ehrlich, which led to their shared Nobel Prize in 1908 "in recognition of their work on immunity." Among several Nobel Prizes awarded in the field of immunology, the most recent was in 2011, for discoveries about innate immunity and dendritic cells. The importance of tolerance was first recognized in plants, which have evolved mechanisms to counter the damage caused by pathogens and herbivores [8]. More recently, tolerance mechanisms were identified in animals and the role of tolerance in countering pathogens is an emerging area of animal research [9-12]. The role of avoidance behaviors in countering pathogens is also a relatively new field of research, with the recognition of pathogen-mediated avoidance behaviors in the last 25 years [13, 14]. Here we have adapted and applied the three defensive strategies of avoidance, tolerance and resistance, to help understanding of the prevention of postpartum uterine disease in cattle.

Avoidance is the ability to limit the exposure to pathogens [14]. Typically, this involves animals using intrinsic behaviors to avoid pathogens and reduce the risk of infection. Tolerance is the ability to limit the tissue damage caused by the pathogen burden [9]. Tolerance mechanisms include neutralizing bacterial toxins, protecting cells against damage, enhancing tissue repair, and inducing adaptive metabolic responses. Resistance is the ability to limit the

pathogen burden [10, 12]. Resistance is the function of immunity, which aims to kill and remove pathogen. Resilience is the aggregate of the complementary defensive strategies of avoidance, tolerance and resistance. Resilient animals prevent disease or restrict the severity of disease [7, 10, 12]. We assume that the evolutionary ancestors of cattle were resilient to postpartum uterine infections, but it is now common to read reports that twenty to forty per cent of modern dairy cows develop some form of postpartum uterine disease [2, 15-20].

We treat animals with uterine disease because the disease causes pain, reduces milk yields, and reduces fertility [21, 22]. However, the current use of antibiotics to treat uterine disease needs rethinking because, although antibiotics help resolve the clinical signs, there is still reduced fertility [23]. Furthermore, the use of antimicrobials in food-producing animals is increasing discouraged by governments that are concerned about the spread of antimicrobial resistance [24]. The annual cost of the reduced fertility, lost milk production, and treatment of metritis was estimated to be €1.4 billion in the European Union and $650 million in the USA [2]; a case of metritis costs farmers up to $410 in the USA [25]. Prevention of postpartum uterine disease would be better than cure, for both the animals and the economy.

Here we outline the role of bacteria in postpartum uterine disease and provide a perspective on the mechanisms that dairy cows use to prevent postpartum uterine disease. We encourage readers to consult comprehensive reviews if they require information on pathogens other than bacteria, or more detail about the pathogenesis, diagnosis and treatment of uterine disease [2-7]. Instead, we aim to bring new ideas to light and challenge readers to think about how resilient dairy cows prevent postpartum uterine disease. In particular, 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 cows 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 [26, 27]. There is concurrent return of ovarian cyclic activity, driven by coordinated endocrine programs in the hypothalamus, pituitary, ovary, and uterus [28-30]. The high concentrations of steroid hormones during pregnancy decrease to basal values within days of parturition. Plasma follicle stimulating hormone concentrations increase about 7 days after parturition, 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, disease or inadequate nutrition during the transition period (3 weeks before to 3 weeks after parturition) delays uterine involution and the return of ovarian cyclic activity [28, 31, 32].

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 [33-35]. For example, uterine pathogens such as *Trueperella pyogenes, Fusobacteria* species and *Prevotella* species have been identified in the uterus of healthy cattle, even during pregnancy [33, 34]. However, these data require caution after a study of 537 women found that despite the expectation there would be a microbiota, the placenta and amnion did not usually contain bacteria [36]; most of the nucleotide signals for bacteria were from contamination of samples or laboratory reagents. Irrespective of whether there is a microbiota in the bovine uterus during pregnancy, the microbial community blooms after parturition. The bacterial load expands massively and fluctuates widely, presumably because vaginal and cervical dilation and trauma allow contamination of the genital tract with bacteria from the vagina, skin, blood, feces and environment.

The pathogenic bacteria cultured from animals with uterine disease include *Escherichia coli, T. pyogenes, Fusobacterium necrophorum*, and *Prevotella* and *Bacteroides* species [37]. Genomic techniques have identified additional bacterial phyla that are more abundant in the uterus of animals with metritis compared with the healthy uterus, including *Bacteroidetes, Fusobacteria, Lactococcus, Proteobacteria* and *Firmicutes* [35, 38, 39]. However, prior to the development of disease there is evidence that many of the bacteria are common amongst animals that will or will not develop metritis. Using high-throughput metagenomic sequencing of the 16S rRNA gene on the Illumina MiSeq platform it was reported that, although the microbiota changed in uterine samples from the day of parturition to 6 days postpartum, cows that would subsequently develop metritis or maintain a healthy uterus had a similar uterine microbiota on the day of parturition [40]. The proportion of core bacterial genera shared between cows developing metritis and healthy cows was 77% at parturition, 79% by 2 days postpartum, and 60% by 6 days postpartum. Similar experimental approaches also independently reported that cows that remain healthy and cows that develop metritis share bacterial phyla and genera in postpartum uterine samples [41, 42]. Furthermore, apart from *Bateroides*, there was no significant difference in the abundance of the 15 most frequent bacterial genera in the uterus of healthy and metritis cows 0, 2 or 6 days postpartum [43]. As many pathogens infect both healthy and diseased uteri, it is unclear whether specific keystone bacteria, combinations of bacteria, failures in tolerance, or an increased abundance of bacteria causes uterine disease. For example, in the first weeks after parturition, strains of endometrial pathogenic *E. coli* are found in cows with uterine disease [44, 45]. Beyond three weeks after parturition, *Trueperella pyogenes* is the pathogen most correlated with the severity of endometritis and extent of the infertility [46-48].

3. Uterine disease

The clinical definitions of postpartum uterine disease are well established [1, 7, 49, 50]. Metritis commonly occurs within 10 days of parturition, and cows have an enlarged uterus, containing watery red-brown fluid to viscous off-white pus, which has a fetid odor. The severity of disease ranges from inapparent signs of metritis to pyrexia and inappetence in

animals with puerperal metritis, and even toxemia and shock in some animals. Clinical endometritis is characterized by the presence of pus in the uterus 21 days or more after parturition, usually with a purulent uterine discharge detectable in the vagina. Subclinical endometritis is diagnosed when there are no signs of clinical endometritis but the proportion of neutrophils in endometrial flush or cytobrush samples exceeds thresholds associated with reduced reproductive performance, which is usually about 5% of cells. New knowledge is required to understand how the etiology and pathogenesis of endometritis differs from purulent vaginal discharge caused by cervicitis or vaginitis [19]. Future longitudinal studies should examine the temporal interrelationships amongst the pathogens, and the progression of disease in the reproductive tract.

The lactational incidence of metritis was 21% in a survey of farm records of 97,318 Holstein cows in the USA [16]; although the farmers included retained fetal membranes when recording metritis, this incidence is the same as the 21% lactational incidence of metritis in 456 animals in Florida that were examined daily after parturition [51]. The lactational incidence of endometritis was 19% in a survey of farm records for 19,870 Holstein cows in Germany [17]; again, similar to the 17% lactational incidence of clinical endometritis in 1,865 cows in Canada examined in detail 20 to 33 days postpartum [52]. Metritis is also important in purebred *Bos indicus* cows, crossbred *Bos indicus* x dairy cows, and buffalo. For example, in 1,609 Sahiwal lactations, 2,549 crossbred Holstein x Tharparkar lactations, and 1,604 Murrah buffalo lactations, the incidence of metritis was 10.3%, 22.6%, and 9.7%, respectively [53]. Uterine disease is important because it causes infertility or reduces fertility, even after successful treatment of the disease. Even though farm records can have imprecise case definitions, in a meta-analysis of more than 10,000 cases, metritis 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 [22]. These data are similar to a precise case-controlled study of 1,865 cows in Canada, where clinical endometritis increased the time to first insemination by 5 days, reduced conception rates to first insemination by 8%, increased the median days open by 28 days, and cows were 1.7 times more likely to be culled for reproductive failure than cows without endometritis [52]. The reduced fertility is caused by inflammation of the genital tract, disruption of ovarian follicle growth and function, abnormal estrous cycles, and damage to oocytes [4, 15, 20, 37, 54, 55]. To avoid reduced fertility, we argue that prevention of disease is more important than developing new treatments for metritis or endometritis.

4. Defense against pathogens in the uterus

Uterine disease provides an opportunity to study animal resilience because, despite exposure of the uterus to similar bacteria, the incidence of disease varies amongst groups of animals, levels of milk production, breeds, and farms. We suggest that preventing uterine disease depends on the complementary defensive strategies of avoidance, tolerance and resistance. These are evolutionary ancient strategies used by plants and animals to counter infection or damage [8, 10, 12]. Avoidance, tolerance and resistance are integrated and complementary strategies, but they have different implications for the interaction between pathogen burden and health. This interaction between pathogen burden and health can be visualized using a reaction

norm plot (Fig. 2). Reaction norms for a population of animals also allow comparisons between different genotypes, breeds or environments [8, 10, 12, 56-58]. For example, dairy cows in straw yards are more able to avoid fecal pathogens than cows in cubicles [59]; dairy cows with lower milk yields are more able to tolerate uterine pathogens [7]; and, cows with higher cell mediated immune response are more able to resist uterine pathogens causing metritis [60].
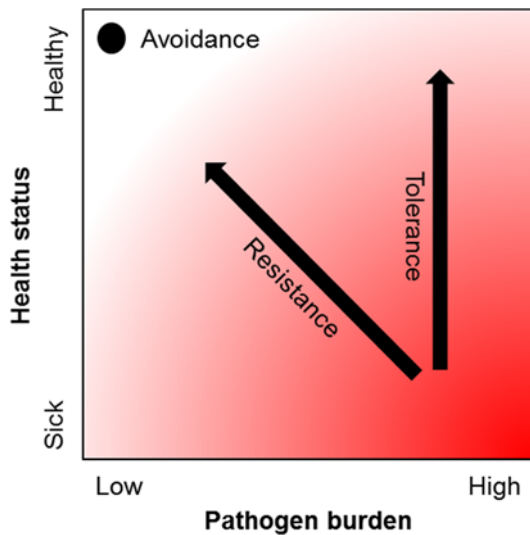
Figure 2. Reaction norms: a conceptual framework for understanding resilience to pathogens. A theoretical reaction norm plot of health status, where health increases vertically, against pathogen burden, where the pathogen burden increases horizontally [7, 10, 12]. Avoidance maintains health by avoiding exposure to pathogens. Tolerance improves health by limiting the tissue damage caused by the pathogen burden. Resistance improves health by limiting the pathogen burden. In the case of uterine disease, avoidance limits the quantity of pathogens reaching the uterus, tolerance limits the damage that pathogens infecting the uterus cause to the endometrium, and resistance reduces the quantity of pathogens that infect the uterus.

4.1 Avoidance

Avoidance mechanisms that limit exposure to pathogens have an evolutionary advantage over tolerance and resistance mechanisms because there is no direct metabolic cost to the animal of countering the pathogens. Furthermore, basal metabolism fuels the indirect metabolic costs of avoidance mechanisms such as barriers and behavior. Examples of physical barriers to infection that help avoid bacteria ascending the genital tract into the uterus are the vulva, vagina, cervix, and cervical mucus. However, calving, dystocia, and poor conformation of the vulva or vagina, breach these physical barriers and allow bacteria to invade the uterus.

Maintaining hygiene is an intrinsic behavior that has evolved over millennia in most species of animals [61]. The preference of animals to avoid feces, clean themselves, and prefer dry and clean bedding is such an intrinsic behavior that it often goes unremarked. For example, self-grooming and grooming each other is an intrinsic behavior used by many species to maintain hygiene, remove parasites, and for social interaction. Similarly, cows use grooming to maintain coat hygiene and limit stress [62, 63].

A greater understanding is needed about whether cows use intrinsic behaviors to avoid uterine pathogens. However, cows seek seclusion from herd-mates around the time of parturition, and cows with metritis display sickness behaviors, including inappetence, inactivity, isolation, and depression, which result in sick cows spending more time away from healthy herd-mates [64]. Other intrinsic behaviors to avoid pathogens, which have been studied in non-ruminants, are keeping away from other animals of the same species because they are most likely to harbor pathogens, and using disgust to avoid the odor of pathogens or infection [13]. For example, the nematode *Caenorhabditis elegans*, which feeds on bacteria, learns to avoid the odor of pathogenic bacteria, whilst being attracted to odors from nonpathogenic bacteria [65]. Bullfrog tadpoles avoid other tadpoles that are infected with a pathogenic yeast by detecting chemical signals from infected individuals [14]. Mice use the vomeronasal system to detect the odor of animals that have an infection or even an inflammatory response to the bacterial endotoxin lipopolysaccharide [66]. In the case of postpartum metritis, the fetid odor is disgusting to humans. Although cows use averse odors to avoid feces [59], it remains to be determined whether herd-mates use the fetid odor to avoid cows with metritis.

The value of avoidance behaviors for postpartum uterine disease depends on how transmissible uterine pathogens are, and how easily these pathogens spread when there is a high density of animals. Confined dairy cows often have a higher incidence of postpartum uterine disease than herds of free-ranging beef cattle or wild ungulates. There is an urgent need to better understand the behavior of postpartum cows because modern dairy production systems and buildings are likely to compromise the ability of postpartum animals to use avoidance behaviors [67]. If they are important, allowing cows to use behaviors that avoid infections may require a revaluation of current housing design and transition cow management.

4.2 Tolerance

Tolerance - not to be confused with immunological tolerance - aims to benefit an animal's health by limiting the tissue damage pathogens cause without affecting the pathogen burden [7, 10, 12]. An advantage of tolerance over resistance mechanisms is that tolerance does not exert a selection pressure on the pathogens that would provoke the pathogen to develop countermeasures, such as antimicrobial resistance. Tolerance mechanisms against uterine pathogens include functional barriers to bacterial infection, neutralization of bacterial toxins, repair of tissue damage, and adaptive metabolic responses (Table 1). For example, the epithelial barrier of the endometrium and the overlying mucus layer facilitate the coexistence of pathogens and host. However, these tolerance mechanisms are not always passive and some integrate with resistance. Mucus from the reproductive tract contains antimicrobial peptides, acute phase proteins, such as haptoglobin and serum amyloid A, and mucosal glycoproteins that neutralize bacteria or their toxins [68-70]. Furthermore, there is increased expression of antimicrobial peptides and acute phase proteins in the inflamed endometrium and the peripheral plasma of postpartum cows [71, 72].

**Table 1**. Potential disease tolerance mechanisms

Mechanism	Examples
Functional 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 energy for tissue repair

Damage control and tissue repair help tolerate the presence of pathogens [56, 73]. However, damage, repair and regeneration of the endometrium is also a consequence of parturition [26, 27]. The importance of the epithelial barrier for tolerating pathogens in the uterus is highlighted by the need to damage the epithelium when generating animal models of endometritis [74]. Crucially, loss of the epithelium allows pathogens to reach the underlying sensitive stroma in postpartum animals or when inducing disease. 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* [75]. However, it is possible, without impairing the intrinsic viability of stromal cells, to protect stromal cells against the damage caused by pyolysin *in vitro*, by reducing their cellular cholesterol content using methyl-β-cyclodextrin (Fig. 3), or by inhibiting cholesterol biosynthesis using statins or squalene synthase inhibitors [75-78]. These findings could lead to products that protect the endometrium against pathogen damage.

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 [76, 79]. These cell stress responses aim to help repair damaged membranes and organelles, and to induce a quiescent state [58].

Sickness behaviors, such as inappetence, also activate catabolic pathways that induce cell quiescence and support tolerance to pathogens in humans and mice [58]. However, dairy cows often struggle to meet the metabolic requirements of lactation (the metabolisable energy required to produce 40 liters of milk is 200 MJ/d; three times the energy needed for resting metabolism), and higher milk yields are associated with reduced tolerance to postpartum uterine infections [7]
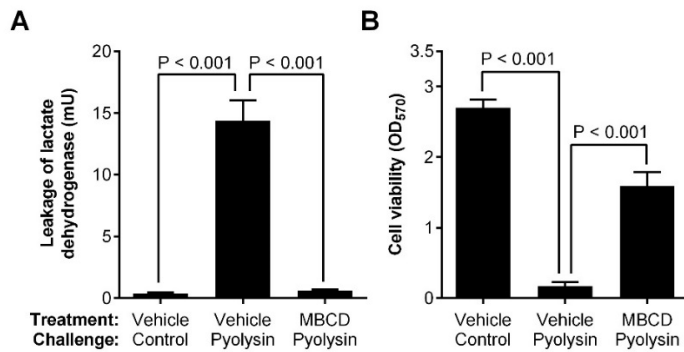
Figure 3. Reducing cellular cholesterol protects stromal cells against pyolysin. Bovine endometrial stromal cells were treated for 24 h in medium containing vehicle or methyl-β-cyclodextrin (MBCD) to deplete cellular cholesterol, and then challenged for 2 h with control medium or medium containing the 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 mitochondrial activity (B). Data are presented as mean + SEM (n = 4 animals), and analyzed by ANOVA with Bonferroni post hoc tests. Redrawn from data published previously [77].

4.3 Resistance

Whilst the research fields of avoidance and tolerance are still developing, there is extensive knowledge about resistance mechanisms against pathogens, and particularly the roles of innate and adaptive immunity. The innate immune system generates an immediate, non-specific response to pathogens that does not depend on prior exposure to pathogens [57, 80]. This innate immune response is ideal for uterine infections because they are polymicrobial and fluctuate during the postpartum period. 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). In the uterus, endometrial epithelial and stromal cells also have a role in innate immunity, generating inflammatory responses to bacteria, lipopeptides, and lipopolysaccharide [81, 82]. The immune response typically results in the secretion of antimicrobial peptides, cytokines such as interleukin (IL)-1β and IL-6, chemokines such as IL-8, and prostaglandin E_2 [80, 83]. These inflammatory mediators help counter bacteria in the tissue by inducing vasodilation, attracting and activating immune cells, and inducing the production of acute phase proteins and reactive oxygen species [7, 80, 83, 84]. The inflammatory mediators also suppress the hypothalamic-pituitary-ovary axis, and induce sickness behaviors, such as inappetence and lethargy [58]. The complement

system, which promotes phagocytosis of pathogens by neutrophils and macrophages, provides additional non-specific defense [32].

A general concept is that rapid and robust inflammatory responses efficiently control pathogens, whereas delayed or blunted inflammatory responses lead to persistent inflammation [7, 57, 58, 80, 83]. Interestingly, intrauterine infusion of IL-8 recombinant protein reduced the incidence of clinical metritis from 34% to less than 10% [18]. However, excessive or unrestrained inflammation causes immunopathology, such as toxemia and shock in some cases of metritis. Conversely, subclinical endometritis is an example of persistent or unresolved inflammation. Fortunately, innate immunity usually regulates the inflammatory response to match the level of pathogen challenge and tissue damage. For example, feedback loops, such as STAT3 (signal transducer and activator of transcription-3), regulates the IL-6 and IL-8 response to lipopolysaccharide in endometrial cells [85]. Tissue damage, with the release of damage-associated molecular patterns also scales the immune response [86]. Damaged endometrial cells release the intracellular cytokine IL-1α, which stimulates further secretion of IL-6 [84]. Innate immunity also integrates with tolerance because the innate immune response induces the production of mucins and antimicrobial peptides [87].

Adaptive immunity depends on prior exposure to specific antigens and takes longer to develop than innate immunity. Adaptive immunity is evident in the postpartum endometrium, with lymphocytic foci of T cells and B cells [27, 47]. 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 [88]. Unfortunately, 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 [89]. Another concern is that adaptive immunity is short-lived in the uterus. Spontaneous metritis does not protect against uterine disease after the next calving.

Unfortunately, the biosynthetic demand to repair the endometrium after parturition and resist pathogens is at odds with the additional metabolic demands of lactation [3]. For example, cows in severe negative energy balance after parturition have persistent endometritis, whereas animals with mild negative energy balance repair their endometrium by two weeks after parturition [32]. Even reduced feeding behavior before parturition predicts the development of metritis [90]. A widely accepted mechanism is that metabolic changes around the time of parturition impair neutrophil function [3, 91]. Furthermore, deficiencies in glucose or glutamine also blunt the inflammatory response in the endometrium [92, 93]. Perhaps this interaction between metabolism and immunity is not surprising. It is energetically expensive to mount an immune response, secrete inflammatory mediators, and repair tissue damage, and this often results in negative energy balance and negative nitrogen balance [94]. Not only do immune cells require metabolites, the immune response also reprograms cellular metabolism to deliver the immune response [58, 95]. Estimated energetic costs of an immune response range from a 15% to 30% increase in resting metabolic rate [94]. An example of the energetic cost of innate immunity in the whole animal is that cows metabolize an extra kilogram of blood

glucose in the first 12 hours after challenge with lipopolysaccharide [96]. Allocating additional metabolic resources to resistance during an infection requires trade-offs with other metabolically demanding processes that are not essential, which in many species is exemplified by reduced reproduction [58, 94]. The negative energy balance of lactation is one such trade-off in dairy cows, often made worse by an inadequate food supply or by sickness behaviors that reduce feeding. In an extreme example, challenging bees with lipopolysaccharide when they are starved increases mortality by 1.5 fold [97]. Despite evidence that negative energy balance is a risk factor for developing disease, and the metabolic cost of resisting pathogens, it is not clear how diets should be formulated for cows to optimize their immunity during the transition period [31].

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 (Fig. 4). Uterine disease is not inevitable; many well-managed dairy farms have high milk yields but a low incidence of postpartum uterine disease. Conversely, and perhaps more important for animal welfare, suboptimal management of dairy cows can make cows more susceptible to postpartum uterine disease.
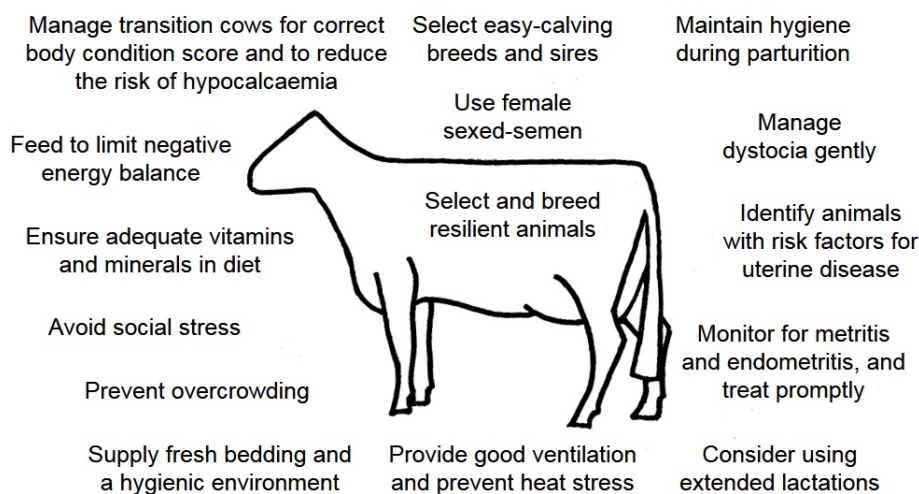
Figure 4. Management can help prevent postpartum uterine disease. Potential strategies that farmers can use to help prevent postpartum uterine disease.

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 in *Bos taurus* dairy cows, which reduces the annual incidence of postpartum problems. Whilst extended lactations are often thought to reduce herd reproductive performance, in some cases extended lactations improve performance [98]. Unfortunately, extended lactations are impractical in seasonally calved herds, and for *Bos indicus* and crossbred dairy cows that have less persistent lactation than *Bos taurus* cows. Pharmaceutical induction of lactation with estrogens and progesterone also avoids parturition, but the protocols need further development

to reduce the number of injections administered to each cow, and many countries prohibit the use of estrogens in food-producing animals.

Farmers can also try to control the risk factors for uterine disease. The predominant risk factors for postpartum uterine disease are trauma to the genital tract followed by colonization with pathogenic bacteria. Trauma to the genital tract is more likely in the first parity, after induction of parturition, or following dystocia, stillbirths, twins, male calves, or retained placenta [99-101]. Selection of easy-calving breeds and easy-calving sires within a breed could reduce the risk of parturient trauma to the genital tract, but may produce only short-term benefits if the smaller offspring are then used as replacement dairy cows. Using sexed semen to produce female calves, which are smaller than male calves, is a practical way of reducing the risk of uterine disease; mathematical modelling of the replacement of male calves by female calves estimates that this strategy would prevent more cases of clinical endometritis in a herd than eliminating an infrequent risk factor, such as retained fetal membranes [101]. 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 clean 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 [3, 31]. Cows should be dried off at the target body condition score, fed a prepartum cow diet that provides the appropriate fiber, vitamins and minerals, and then feed a postpartum diet with sufficient energy and protein to satisfy the metabolic requirements for lactation [102]. Farmers should also minimize stress, for example by avoiding transport or multiple changes in animal groups, which lead to social stress [103]. After parturition, it is important to monitor animal health carefully, particularly those cows that experience risks factors for uterine disease. Veterinary examination of the reproductive tract is important to identify animals with disease and decide on their treatment [6]. However, the routine use of antimicrobials in cows after parturition to try to prevent uterine disease is no longer acceptable because this metaphylaxis increases the risk of antimicrobial resistance, and reducing antimicrobial resistance is a priority for most human health agencies [24].

Encouraging a return to ovarian cyclic activity by appropriate nutrition and reproductive management during the transition period also helps counter uterine disease. Estrus and estradiol increase protection against uterine disease in cattle, and the induction of estrus is often used to treat endometritis [104]. Conversely, progesterone is used to suppress immunity when generating animal models of endometritis [74]. Interestingly, estrus and estradiol increase the expression of components of the complement system [105], but estrus and estradiol do not alter the innate immune response by endometrial tissues or cells in ex vivo studies [106]. It remains an open question as to how steroids influence the susceptibility of animals to uterine disease.

6. Future perspectives

In the present review, we suggest that resilient cows prevent the development of uterine disease using the three complementary defensive strategies of avoiding, tolerating and resisting infection with pathogenic bacteria. Preventing endemic diseases is important for delivering

sustainable intensification of dairy farming, which will be necessary to feed the projected world population of 9.4 billion people by 2050 [107]. Improving resistance, particularly by developing vaccines probiotics or biopharmaceuticals holds promise [18, 88]. Unfortunately, this does not overcome the problem that high-milk-yield dairy cows are intrinsically more prone to develop uterine disease than other cattle or domesticated species. Farmers should also be aware that suboptimal management of dairy cows increases the risk of uterine disease. The simplest piece of advice is for farmers, nutritionists, and veterinarians to pay attention to detail when designing buildings, managing dairy herds, planning and herd health programs.

A long-term solution to preventing uterine disease is to breed resilient dairy cows that not only have a robust immune system, but also tolerate uterine pathogens [108]. Phenotypic selection could be based on the occurrence and severity of clinical disease but this is subjective, and the time required to collect the data results in long generation intervals. Genomic selection for resilient animals has high potential but depends on identifying genomic markers of animal resilience, rather than just focusing on genes that determine resistance [108]. However, genomic selection requires care because there can be a negative correlation between resistance and tolerance genes [10, 12]. Improving avoidance or tolerance is another highly attractive strategy for preventing disease because, unlike resistance or using antimicrobials, avoidance and tolerance do not exert a selection pressure on the microbes. Improving avoidance or tolerance would also reduce the dependence on antimicrobials to kill pathogens, which helps reduce the danger to human health posed by antimicrobial resistance [24]. We propose that developing new ways to prevent uterine disease will depend on improving our understanding of the mechanisms of avoidance and tolerance of pathogens, to complement our knowledge about resistance in the postpartum uterus.

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Declarations of interest

Declarations of interest: none.

Author contributions

Martin Sheldon: conceptualization, writing – original draft, funding acquisition; Paula Molinari: writing – review and editing; Tom Ormsby: writing – review and editing; John Bromfield: conceptualization, writing – review and editing, funding acquisition.

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