Detection & Treatment of Endometritis in Dairy Cattle

Use of Metricheck & Metricure in New Zealand

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Abstract

Of the three major postpartum uterine diseases affecting dairy cattle (metritis, endometritis, and pyometra), endometritis is the most detrimental due to its high prevalence, negative impact on fertility, and diagnostic difficulty. Clinical signs are subtle or completely absent, making endometritis challenging to detect. The dynamics of uterine inflammation and involution are vital to consider in the context of the number of days postpartum since timing directly impacts the sensitivity and specificity of endometritis diagnostics.

Timely identification of endometritis is vital since the subsequent failure or delay in pregnancy decreases DIM (days in milk), limits recovery time between calving and mating season, and increases culling risk. Metricheck was developed in New Zealand as a diagnostic alternative to more arduous cytology based techniques to identify eligible cows for treatment, particularly with Metricure, an intrauterine cephapirin. Several studies have demonstrated Metricure's pharmacological efficacy and ability to mitigate the delay in days open linked to the chronic inflammation of endometritis (Kasimanickam, 2005b; Dohmen, 1995). These results are similar to those achieved with either prostaglandin analogues (Kasimanickam, 2005b). However, differences in production systems, product availability, and drug regulations limit the translatability of Metricheck, Metricure, and prostaglandins between New Zealand's grazed herds and the United States' confinement systems.

Finally, no matter the context of use, distribution of metaphylactic treatment for endometritis must be reconciled with the insensitivity of available diagnostics. This paper outlines the need for additional research to refine the definition, diagnostics, and therapeutics for subclinical and clinical endometritis.

Key Words: Endometritis, Dairy, Bovine, Metricure, Metricheck
Abbreviations: DIM = Days in Milk, CM = Centimeter, IL = interleukin, LPS = lipopolysaccharide, PGF = prostaglandin $F_{2\alpha}$

Introduction

The fundamental challenge of the inflammatory condition endometritis is determining when an animal transitions from a normal, progressive resolution of postpartum inflammation via uterine involution to a pathological chronic inflammatory condition. Within the first 2 weeks postpartum, >90% of all cows harbor intrauterine inflammation inducing bacterial contaminants (Bondurant, 1999). The presence of bacteria is not always pathogenic, and in >90% of individuals common contaminants are cleared by 5 to 6 weeks without impairing fertility (Bondurant, 1999; Hussain, 1990). Bondurant, 1999, notes that active bacterial infection may or may not be a necessary cause of endometritis, but prior or ongoing chronic infection with pathogenic bacteria is a component of inflammation. Pathogens such as *Arcanobacter pyogenes* and *Prevotella melaninogenica,* when present at 49 DIM account for 48% of the

variance in polymorphonucelear cell counts (Gilbert, 2007 in Galvao, 2009). As is noted in Figure 1 and by Gilbert, 2005; as time passes and an increasing number of animals resolve their inflammation. By 35 DIM, the percentage of cows with clinical endometritis plateaus at about 14% (McDougall, 2007). This lingering population most likely indicates the individuals who have not and will not resolve their uterine inflammation spontaneously. The exact proportion of cows with persistent inflammation cannot be predicted with certainty given the weak sensitivity of available testing methods and an evolving case definition. The true prevalence of chronic endometrial inflammation may be close to 25%, the median prevalence reported in this paper's references, but will be highly dependent on whether clinical and subclinical endometritis are considered separate pathologies.

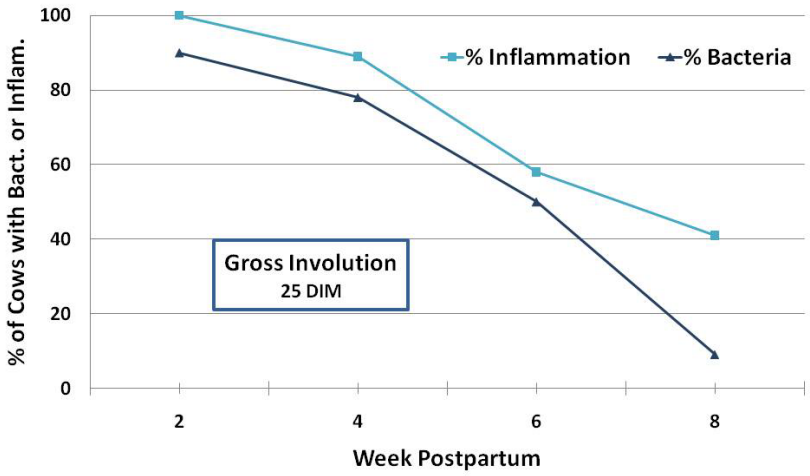

Figure 1: Involution, Inflammation, and Implications
(Gilbert, 2005; Runciman, 2008; Bondurant, 1999; Hussain, 1990; Murrow, 1966)

In both definitions delay to onset of cyclicity and infertility is likely due to the impact of bacterial products such as LPS and host generated inflammatory mediators which alter the endocrine environment of the reproductive tract. The bovine ovary is uniquely subject to uterine origin inflammatory products due to the concentrating effects of the uterine-ovarian blood flow countercurrent exchange system. Inflammation and infection of the endometrium negatively correlate with follicular development by deprecating the ability of the endometrium to produce and release PGF (Sheldon and Dobson, 2004 in Runciman, 2008). This suppression of follicular development and luteolysis delays onset of cyclicity and promotes a progesterone dominated environment conducive to continued bacterial survival (Shrestha, 2004 in Runciman, 2008). These delays in cytological uterine

involution play a direct role in infertility, as a uterine environment dominated by inflammation and its products is not suitable for implantation and survival of a conceptus.

The pathogenicity of endometritis is determined by the intensity of the host's response to the postpartum bacterial challenge. Intuitively an immune response is indicated against the bacterial contaminants, but a vigorous inflammatory response will reduce fertility relative to the proportion of pro-inflammatory cytokines (ex. IL-1) to anti-inflammatory mediators (ex. IL-10, Prostaglandin E_2) (Herath, 2009). Therefore, a balanced immune response is as important as an adequate immune response when considering the long term effects on fertility.

The following sections outline the diagnostic methods available for identifying bovine endometritis as well as the clinical approaches used to address endometritis associated fertility in the production systems of New Zealand and the United States.

Signalment

Infertility is a multifactorial issue that affects all dairy herds. In particular, cows with endometritis are 1.7 times more likely to be culled for reproductive failure than herdmates (LeBlanc, 2002a). In the United States, cows calve on average every 13 to 13.5 months. Elongated and variable intra-herd calving intervals are manageable because forages are grown, harvested, and stored. Unlike confinement herds, New Zealand's seasonally grazed cattle must adhere to a strict 12 month calving interval. In this system, all forage is garnered from pasture; therefore peak milk production must be synchronized with peak pasture availability. The allotted time for uterine involution between calving and mating is dictated by when a cow calves within the calving season. Cows that calve earlier in the calving season will have a longer interval for recovery until planned start of mating, than those that calve later. Fertility at onset of the mating season is essential because earlier pregnancies are more valuable pregnancies. In confinement systems cows are dried off based on when they are due to calve thus cows are always milked for their 'full' lactations. In seasonally grazed systems, all cows are dried off at the same time

when the grass is no longer available. Late bred cows will calve later the following year, but still will be dried off with the rest of the herd prior to fulfilling their full lactation length. In addition to production losses, these late bred cows will have a shorter calving to mating season interval, reducing their fertility and increasing their risk for culling from the herd.

Periparturient Uterine Disease Differentials

Unlike endometritis, other postpartum uterine diseases such as pyometra and metritis have succinct, easily diagnosable presentations.

Metritis rarely occurs beyond the first 2 weeks after parturition. It is characterized by an enlarged uterus with fetid discharges. Unlike endometritis, which never affects the cow's immediate mortality or milk production and systemic signs of illness, metritis penetrates all uterine layers allowing for systemic manifestations such as fever, depression, and decreased milk production. Metritis has a modest lactational incidence rate of 10.1% (Kelton, 1998). This population has a 15-20% relative reduction in fertility rate (Lee, 1989; Eicker, 1996).

Pyometra occurs only after a cow begins cycling, typically >33 DIM, and is characterized by purulent material in the uterus with a closed cervix in the presence of a corpus luteum; whereas endometritis occurs independent of P4 levels (Bondurant, 1999). Since there is minimal uterine inflammation with pyometra and the bacterial proliferation is dependent on progesterone influence, an 80% cure rate with PGF can assure a good reproductive future (R.O. Gilbert 2010 Lecture Notes). Pyometra has a minimal lactational incidence rate of 4% (R.O. Gilbert 2010 Lecture Notes).

Endometritis is far less discrete. It occurs at some undefined period of time after 21 DIM. It is characterized by minimal to absent non-specific, variable gross clinical signs such as vaginal discharge. Although the inflammation is limited to the endometrium, endometritis increases median days open by 32 days compared to healthy cohorts (LeBlanc, 2002a; McDougall, 2007; Gilbert, 2005). As noted above,

the prevalence of endometritis is difficult to pinpoint, but has been estimated at up to 53% (Gilbert, 2005).

Variation in inter-herd prevalence indicates that endometritis, like most other transition cow issues, is as much a management disease as it is a pathological disease (Barlund, 2008). Risk factors for endometritis such as dystocia and twins put additional strain on a cow's immunological resources (Cheong, 2011; Potter, 2010). These resources have already been tapped if metabolic and macronutrient needs are not sufficiently met, resulting in retained fetal membranes, metritis, hyperketonemia, and hypocalcemia (Walsh, 2011). Macro-management breakdowns then lead to micro-physiological consequences. If the immune system is unable to clear uterine contaminants in a timely manner, chronic inflammation becomes endometritis and the reproductive consequences outlined above occur.

Disease Definition

Achieving consensus on a definition for endometritis is the rate limiting step to interpreting diagnostic and therapeutic data from clinical trials. Unfortunately, what is seen microscopically is not consistently manifested as overt clinical signs. The pathological definition is simply: inflammation of the endometrium. However, postpartum inflammation and involution are dynamic processes. The diagnostic techniques outlined in the following section define clinical endometritis as purulent discharge in the vagina and subclinical endometritis as a percentage of PMN on uterine cytology adjusted for DIM at sampling; ex. >5% PMN at 35 DIM (Gilbert, 2005). However, it is important to note that all cutoffs values were calculated for statistical significance based on pregnancy status at 120 to 150 DIM. Since failure to conceive is a multi-factorial issue, none of these diagnostic tools will achieve 100% sensitivity (LeBlanc, 2002a).

Diagnostic Techniques

There are several strategies for diagnosing endometritis including: trans-rectal evaluation, uterine biopsy, uterine cytology, and vaginal gross inspection.

Despite being the predominant method of diagnosing uterine disease, palpation per rectum fails to account for normal variation in uterine involution and is of little or no value for diagnosing endometritis once the vaginoscopic score is known (LeBlanc, 2002a; Runciman, 2008). On average a cow's uterus has returned to pre-pregnancy size (< 5cm diameter) by 25 DIM signaling completion of gross involution irrespective of her cytological status (Morrow, 1966). Ultrasound per rectum is capable of quantitatively measuring uterine horn diameter, location of the reproductive tract, thickness of the uterine wall, and cervical diameter. However, none of these measures can be significantly correlated with endometritis, again due to normal variation in uterine involution. In particular, fluid accumulation and endometrial edema associated with estrus distorts data too much to establish sensitive cutoff values (Barlund, 2008).

Uterine biopsy is the gold standard technique; however, unlike in horses, uterine biopsy compromises fertility and therefore negates any diagnostic value (Bonnett, 1993). Alternatively, exfoliative cells can be sampled from the uterine lining via Cytobrush or uterine lavage. The disposable sampling tip of the Cytobrush is passed within a protective sheath through the cervix into the uterus. The sample is obtained by brushing the tip against the luminal wall of the uterus; collected cells are rolled onto a glass slide for evaluation. Uterine lavage samples a similar population of cells. A catheter is passed through the cervix into the uterus to permit introduction and collection of a small amount of fluid. The recovered fluid and cellular debris is then centrifuged to concentrate material for microscopic analysis. Cytobrush is the preferred technique for several reasons. First, there is a degree of inter-operator error with uterine lavage. Seventeen percent of attempts in Kasimanickam, 2005a, resulted in failure to recover a sufficient amount of fluid for analysis. Second, cells collected via lavage tend to be

more distorted than those from Cytobrush, most likely due to either chemical properties of the transport media or physical trauma during centrifugation (Kasimanickam, 2005a).

Gross inspection of the vaginal vault via vaginoscopy or Metricheck is far less invasive, requires less training and equipment than cytology, and can rapidly and easily be implemented in the field. Vaginoscopy is performed by visualizing the vaginal vault via an illuminated vaginal speculum. Instead of visualizing material in situ, the Metricheck device retrieves material from the vaginal vault for evaluation. The Metricheck device was developed in New Zealand for rapid evaluation of large cohorts of cows. The design has evolved from a squash ball to its custom hemispheric design on the end of a 30cm rod with a non-ergonomic but practical handle.

Several scoring schemes have been developed to grade the discharge collected or visualized by gross inspection. Typical grades are as follows: (0) clear mucous/no discharge, (1) flecks purulent material, (2) mucopurulent, (3) purulent, and (4) fetid. Grading schemes can be simplified to dichotomous categories since any degree of purulence negatively affects fertility and all grades respond to therapeutics (Runciman, 2008). A typical New Zealand farmer will present cows 3-4 weeks fresh; evaluate and treat, then recheck positives 2 weeks later. Several years ago when first adopted, cows were Metrichecked at 5-6 weeks postpartum. As would be expected, fewer animals were draughted as dirty or treatable. The adjustment to the evaluation window may have occurred for two reasons. First, there is a rigid timetable for the breeding season, therefore there is a great deal of pressure to intervene as early as possible to maximize the potential recovery time. Second, additional cows can be detected if the evaluations are done earlier. However, since resolution inflammation postpartum is a gradual process, the proportion of dirty cows will always be higher if detection is done earlier thus increasing the population receiving treatment. Therefore treatment may not always be warranted since many of these cows checked early will clear the inflammation if given additional time. Barlund, 2008 found that

vaginoscopy is only appropriate for diagnosis after the 4th week postpartum. They did not evaluate the Metricheck device.

Diagnostic Technique	Sensitivity	Specificity	Kappa Value
Cytobrush	12.9%	89.9%	
Uterine Lavage	14.3%	84.0%	0.74 (p<0.01)
Vaginoscopy	7.1%	87.4%	0.52 (p<0.01)

Table 1: >8% PMN = positive for endometritis; Se/Sp based on pregnancy status at 150 DIM; Kappa values in comparison to Cytobrush (Barlund, 2008)

Table 1 outlines the relationship between three selected techniques: Cytobrush, uterine lavage, and vaginoscopy (Barlund, 2008). The cytological measures were validated based on their ability to predict pregnancy status at 120-150DIM; in this study endometritis was defined as >8% PMN when sampled between 28 and 41DIM. The cytological techniques, Cytobrush and uterine lavage, have a high specificity (84.0-89.9%), low sensitivity (12.9-14.3%), and exhibit a high level of agreement (kappa = 0.74, p<0.01). Vaginoscopy, the gross technique, has a similar specificity (87.4%) but only half the sensitivity (7.1%) of the cytological techniques.

As expected, the sensitivity of all techniques is poor, and merely represents the proportion of infertility for which endometritis is responsible. Unexplainably, despite the low sensitivity large numbers of cows are being draughted for treatment.

Gross techniques such as vaginoscopy have been shown to miss up to 9% of cows with uterine disease and may falsely diagnose cervicitis or vaginitis, or mis-diagnose neutrophil influx and mucus production associated with estrus as pathogenic inflammation; given these errors the similar specificities in Table 1 may actually be due to chance, as is reflected in the modest kappa values (0.52, p<0.01) between vaginoscopy and cytological techniques (Kasimanickam, 2005a; Barlund, 2008; Kasimanickam, 2005b; Bondurant, 1999).

Despite similar theoretical approaches, differences also exist between vaginoscopy and Metricheck. In a group of high risk cows, there was extremely low levels of agreement (kappa = 0.27) when ordinal values (0-4) were used. Kappa values improved (kappa = 0.45) if only dichotomous, positive versus

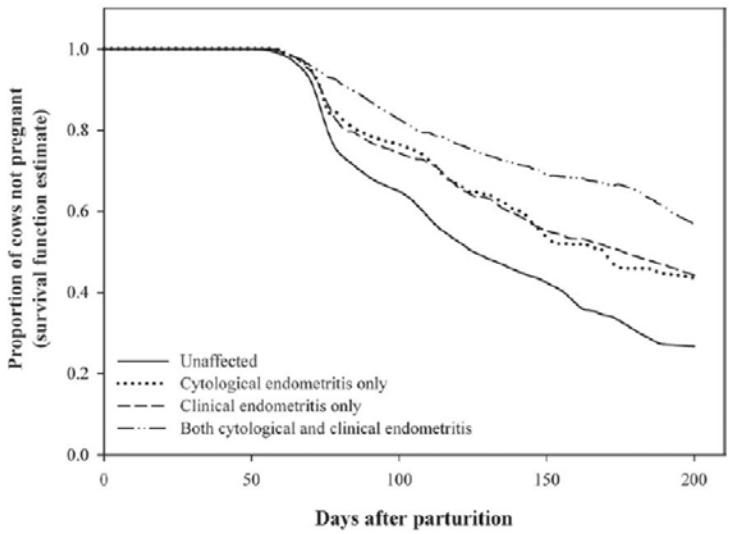

Figure 2: Survival Analysis for cows evaluated at 35+/-3 DIM Clinical = mucopurulent vaginal discharge (Metricheck) Subclinical = >6% PMN (Cytobrush); (Dubuc, 2010)

negative, measures were applied. It is important to recognize that 74% of the time Metricheck and vaginoscopic scores were identical, yet there is still minimal agreement (McDougall, 2007).

Figure 2 shows that there is a summative effect on fertility for cows with both clinical and subclinical endometritis. If endometritis is truly a single entity, a spectrum from subclinical to clinical, the effects on fertility would not be cumulative. This leaves a nagging question: what are we diagnosing? If infertility as a marker for endometritis is insensitive, but infertility is the most clinically apparent and relevant manifestation of this disease(s), where do we go from here?

McDougall, 2007 points out: the clinical importance in the differences in test functionality will depend on the biological and economic costs of false negative and positive diagnoses. Clinically, the therapeutic approach will not change as these techniques are successful in identifying an at risk population responsive to metaphylactic treatment. Scientifically, defining the disease target is fundamental to justifying use of reproductive hormones and antibiotics.

Therapuetic Options

There are two main treatment options for endometritis: intrauterine cephalosporins and intramuscular prostaglandins or prostaglandin analogues. Intrauterine tetracycline and lavages with cleansing agents such as chlorohexadine and betadine are contraindicated since they incite additional inflammation. Intrauterine penicillins are not appropriate for treatment of uterine contaminants since many of these organisms synthesize penicillinases and thus render the antibiotic useless. Paraenteral

ceftiofur protocols (IM, IV, SQ) achieve minimum inhibitory concentrations within the endometrium to kill the primary uterine pathogens, such as *Escherichia coli, Arcanobacter pyogenes, and Prevotella melaninogenica*, and therefore are effective for the treatment of the bacteria suspected to be the inciting inflammatory factor (Gilbert, 2007; Bondurant, 1999; Witte, 2011). However, multi-day, multi-administration, expensive antibiotic protocols are not practical for metaphylactic treatment and constitute extra-label usage.

Metricure (500mg cephapirin) is an intrauterine antibiotic suspension commonly used in New Zealand but not yet approved in the United States. Metricure is similar to the ToDay and Cefalak mastitis treatments (both 200mg cephapirin) available in the United States. Cephapirins are 1st generation cephalosporins, meaning their bacteriocidal properties against gram negative bacilli make them more broad spectrum than naturally occurring beta lactams such as penicillin. Beta lactam antibiotics, penicillins and cephalosporins, act by inhibiting bacterial cell wall synthesis (Plumbs). Animals are evaluated for treatment based on Metricheck results performed at 3-4 weeks postpartum. Each Metricure tube comes with a disposable plastic pipette and rectal sleeve. The pipette is carefully passed through the cervix, into the uterus for direct intrauterine delivery of the Metricure suspension. Cows are typically re-evaluated in 2-3 weeks when the next cohort of fresh cows is eligible to be Metrichecked. Anecdotally, in less than 2 years, a practice in the Waikato region of New Zealand has achieved a 90% Metricheck adoption rate among its clients who treat between 5 and 30% of their herd with Metricure each year and despite a meager 50% cure rate (Personal Communication, Dr. Jess Spatz-Shelgren). Even with such a seemingly low cure rate, return on investment estimates done by a kiwi practitioner are between 4:1 and 7:1 (Provided by Dr. Jess Spatz-Shelgren).

Prostaglandin usage is widespread in many managerial systems of the United States. Producers choose to use synchronizations protocols to compensate for poor heat detection or to more easily implement artificial insemination in their herds. The most popular protocol, PreSynch-OvSynch, involves several injections of PGF prior to the end of the voluntary waiting period, or minimum days in milk at first breeding. Use of the PreSynch-OvSynch protocol has been shown to negate the infertility caused by subclinical and clinical endometritis (Kasimanickam, 2006). This suggests that some component of the synchronization protocol has a protective effect. As shown in Figure 3, a single dose of the prostaglandin analogue Cloprostenol is as effective as Metricure in mitigating the fertility effects of subclinical endometritis (Kasimanickam, 2005b).

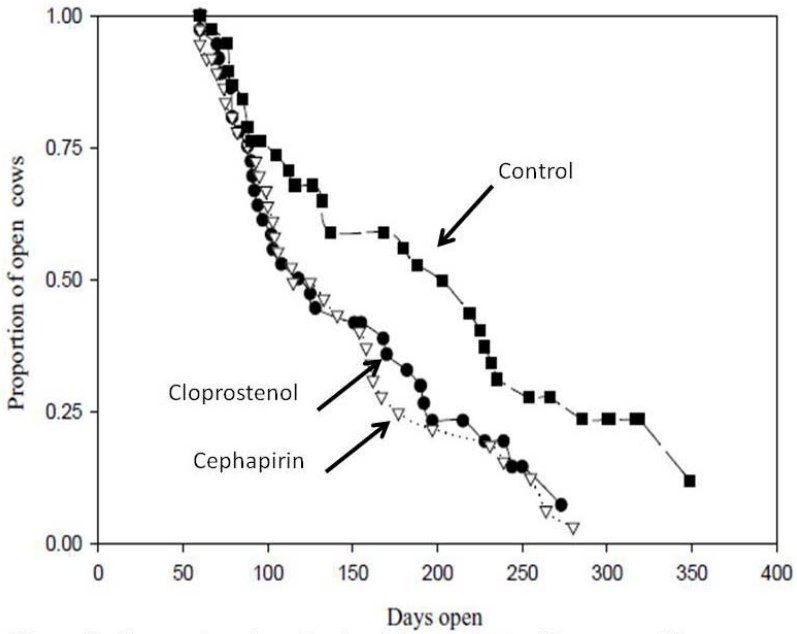

Figure 3: Cloprostenol vs. Cephapirin vs. Control in cows with subclinical endometritis (Kasimanickam, 2005b)

Although the improvements in fertility with Metricure and prostaglandin analogues are identical, these results are not additive. In Galvao, 2009a; intrauterine ceftiofur had no effect on the prevalence of subclinical endometritis or fertility in cows already receiving PGF. The above results are convincing for the use of either cephapririns or prostaglandins, but it perilous to compare these methods. In particular the aforementioned papers differed in their definition of endometritis (clinical versus subclinical), use of prostaglandin versus an analogue, use of ceftiofur versus cephapirin, the number of times dosed, and timing of treatment. Basic trends are apparent, but refinement is necessary to pinpoint which treatment is most effective for which condition at what time.

Discussion

Endometritis is the most detrimental postpartum uterine disease due to its high prevalence, negative impact on fertility, and diagnostic difficulty. It is unfortunate that many circumstances can lead to infertility and thus infertility is an insensitive measure of endometritis. Given the absent to subtle clinical signs of endometritis, additional research is necessary to better understand the pathogenesis and progression of the disease so as to better address it clinically. This leads to a fundamental challenge: is inflammation our ally or enemy and when does the immune response transition from helpful to hazardous?

Clinically, the diagnostic tool Metricheck is novel and appears to be effective in New Zealand's practice of herd wide endometritis metaphylaxis. However, in the fresh pens of the United States, Metricheck lacks the sensitivity to be used on individual cows. Since prostaglandin use is widespread in the United States, and it is as efficacious as Metricure, there is little reason to displace the use of prostaglandins for antibiotics. Likewise, the infrastructure of New Zealand supports identification of the at risk populations eligible for treatment with Metricure, whereas the use of prostaglandins falls under cumbersome regulatory scrutiny.

Endometritis is a universal disease of infertility; and despite a disappointing lack of translatability of techniques between husbandry systems, there is much opportunity for improvement and investigation. Hopefully in the future we can increase the sensitivity of diagnostics, refine selection criteria treatment, determine the true cure rates of each therapy, and establish an optimal treatment window so as to best reduce antibiotic and hormone usage.

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