Chapter

Equine Reproduction: Seasonality, Endometritis, and Twinning in the Mare

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Abstract

This chapter reviews the seasonality and effect of photoperiod in mares and how, as clinicians, we can shorten the vernal transition period and improve our efficiency in getting mares in foal. Different protocols have been utilized to shorten the vernal transition, and each will be discussed. We will also examine endometritis in the mare. The role of biofilms in causing endometritis in our equine patients, and potential treatment plans, in particular breeding the dirty mare, will be reviewed. Finally, we will examine the effect of twin pregnancies in the mare, the most common cause of noninfectious abortion, and offer two management therapies for dealing with twinning in the mare.

Keywords: mare, endometritis, photoperiod, twins, kisspeptin

1. Introduction

Our knowledge of reproduction in the mare has expanded considerably in the last few decades and continues at pace. The scope of this chapter is to try to answer some of the most common questions an attending veterinarian may be asked to deal with, namely, shortening the vernal transition, dealing with postmating endometritis, and dealing with twin pregnancy, all of which can frustrate even the seasoned clinician. The aim of this chapter is to give the reader the background knowledge of why certain therapies may or may not work and to give the clinician workable solutions to some of the more common aspects of clinical reproductive work in the mare.

2. Mare reproduction

2.1 Seasonality and photoperiod

The mare is a seasonal, long-day, polyestrous animal, meaning that her reproductive status is intrinsically linked to photoperiod. In the Northern Hemisphere, the normal physiologic breeding season starts in spring (April) and continues through to autumn (September). This corresponds to increasing photoperiod (increasing daylight length). The light signals are received by the retina, processed by melanopsin [1], which, as a pigment, is located in retinal ganglion cells, themselves being photosensitive. This information reaches the suprachiasmatic nucleus via the retino-hypothalamic tract [2]. Melatonin produced in the pineal

gland is suppressed during hours of darkness. This fall in melatonin as photoperiod increases during the spring stimulates the mare to, reproductively, enter the spring or vernal transition. The classical hypothalamic–pituitary-ovarian axis, known to many clinicians, is oversimplified.

In the last decade or so, numerous authors have examined the role of kisspeptin neurons in the relation of cyclicity in many animal models, including the mare. It appears that increasing photoperiod stimulates the main kisspeptin neuron population located in the arcuate nucleus of the hypothalamus [3]. Distinctly, the horse does not appear to have a secondary population of kisspeptin neurons in the preoptic area, unlike cattle and sheep [4]. A small population of these receptors are located within the ventromedial nucleus of the hypothalamus [5]. The kisspeptin neuron fibers are found throughout the septo-preoptic region, an area that the majority of gonadotrophin-releasing hormone (GnRH) neurons are located [6]. In 2007, Smith et al. were the first to note that kisspeptin neurons may be influenced by photoperiod [7]. They saw an increase in KISS1 mRNA in the arcuate nucleus in sheep in their physiologic breeding season. Our understanding of the role of kisspeptin in the mare and effects on her reproductive status is derived mainly from studies on sheep models; there have been limited studies in the mare.

In the sheep model, it appears that in artificially decreasing photoperiod, thereby eliciting a stimulatory effect in sheep (sheep are short-day breeders), there is a corresponding increase in the number of kisspeptin neurons [8]. Kisspeptin neurons appear to form numerous synapses with other neurons that produce dopamine [9], melanocyte-stimulating hormone [10], and GnRH [11], among others. The regulation of kisspeptin is still not fully understood, but it appears that it may consist of a combination of negative feedback via estrogen in the sheep model [12] and via dopamine. The dopamine neurons in the retrochiasmatic area of the hypothalamus exerts an inhibitory effect on GnRH secretion during anestrous but not during the physiologic breeding season [13]. There is an upregulation of dopamine receptors in the kisspeptin neurons during breeding season [13]. There appears to be a seasonal difference in the number of kisspeptin neurons in the population found in the arcuate nucleus of the hypothalamus, but no seasonal difference in the preoptic population. This has been confirmed in the mare [4]. This seasonality difference appears to be driven, or at least modulated, by photoperiod. However, from sheep models, we know kisspeptin does not express melatonin receptors [14], and it is proposed that any effect of melatonin on the functionality of kisspeptin may be indirect [3]. It has also been postulated there is an indirect effect of photoperiod that is modulated via the thyroid hormones [3]. Nearly all preoptic kisspeptin neurons express thyroid receptors [15]. It has been shown that the thyrotropes (the cells secreting these hormones) located in the pars tuberalis of the rostral adenohypophysis are melatonin responsive [16, 17]. It appears these cells display dramatic melatonin-dependent photoperiodic changes; under short photoperiod, there is low level expression, while under long photoperiod, there is high level expression [18, 19].

GnRH is a 10 amino acid peptide secreted by the hypothalamus. Its secretion, regulated by decreasing melatonin during increased photoperiod, is modulated via kisspeptin neurons mentioned above. Secretion of this peptide enters the hypothalamic-hypophyseal blood portal system, which bathes over the gonadotrophs located in the anterior pituitary, cells that synthesize and secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH). During the vernal transition, there are ever-increasing circulating concentrations of FSH and LH. This increase is gradual, with LH in particular remaining low prior to the first ovulation. It is thought that the low circulating LH is due to the low storage of LH within the gonadotrophs [20]. Under the regulation described above, the increased GnRH stimulates FSH secretion and thus drives the growth of ovarian follicles. However during the vernal transition,

mares undergo several follicular waves. Under these waves, follicles seemingly grow (although they rarely reach pre-ovulatory size), only to regress, and be replaced by another follicular wave. In a report by Watson et al. [21], only 31% of all FSH surges during this transition period lead to the production of a follicular wave. Only one a follicle under the influence of FSH, produces sufficient estrogen, will a follicle ovulate under the LH surge. Estrogen appears to be involved in numerous components of the mare reproductive cycle. It appears that estrogen has a negative and a positive feedback mechanism on kisspeptin within the arcuate nucleus [7, 22], while the fall in circulating estrogen levels leads to increased LH secretion at the level of the anterior pituitary. During the peri-ovulatory surge, declining FSH under the influence of increasing estrogen and inhibin production from the growing follicle, LH reaches maximum concentrations 1 day post ovulation. LH promotes the maturation and subsequent ovulation of the follicle.

Numerous issues can be seen related to this complex reproductive cycle. During the vernal transition, mares may be presented with multiple small follicles evident on the ovaries via transrectal ultrasonography. Follicles may grow and then regress. The unpredictable nature of the folliculogenesis and the exact duration of the vernal transition are not only frustrating for the owner but a headache for the attending clinician. It is imperative that the clinician has a thorough understanding of the mechanisms at play during this period. Having this knowledge will allow the veterinarian to potentially manipulate these mechanisms and the hormones involved and shorten this phase. Understanding the systems at play allows for proper management by the veterinarian and will increase the productivity of the mares under their control.

2.1.1 Taking control of the vernal transition

2.1.1.1 Light manipulation

In the Northern Hemisphere, it has become standard industry practice to place mares under light regimes starting December 1 [23] in the breeding of thoroughbred racehorses. Nonetheless many breeders of other types of horses also utilize light manipulation to hasten time to first ovulation. A 200 watt incandescent light bulb in a 12 × 12 foot stall is sufficient to begin stimulating follicular activity. Typically light is added to the evening; mares are brought into their stalls from pasture before dusk and then exposed to artificial light until 23.00 hours. It is now known that light in the short wave spectrum (465–485 nm) is most effective at inhibiting melatonin production [24]. It is important to allow the mare to receive some hours of darkness and that exposing them constantly to light stimulation actually extends the anestrous period. On larger horse farms, the use of indoor schools, housing numerous barren, and/or maiden mares can be effective. However, in those large building, it is important to check the light intensity at all areas. A loose rule of thumb is that the light should cast no shadows and that you should be able to read a newspaper anywhere in the building. For those who want to be more scientific in their approach, a photometer can be utilized. Exposure to this light regimen should continue for at least 70 days. Within 60 days, most mares will show some follicular activity, with the majority expressing their first ovulation with 70 days of onset of light exposure. While exposure to artificial light does not eliminate the vernal transition, it simply moves it forward. In the natural physiologic breeding season, the mare will not display follicular activity until April, and many will not experience their first ovulation of the season until May. Moving the vernal transition forward several months allows the clinician to start breeding these mares in February and March.

There are disadvantages to this regime. The mares must be housed either in stalls or a large barn, which intensifies their maintenance. Stalls need to be cleaned out regularly, adding to staffing responsibilities. In addition mares housed in groups in barns allow for opportune risk for injury especially if they are fed together—the lowly mare has nowhere to run from her aggressive barn mate.

2.1.1.2 Equilume™ face mask

To counter the problems of intensified housing of mares under light, researchers in Ireland have come up with a novel way to provide the mare with enough stimulatory light to advance the physiologic breeding season, while in their pasture. These masks provide blue light to one eye. It was concluded by Murphy et al. [23] that one light stimulation to one eye is sufficient to stimulate onset of follicular activity, is as effective as stall or barn light regimes, but also has added benefits of being more economic, especially to the small-scale breeder, while increasing horse welfare. Horses can remain out in their pastures, which reduces stress on these animals. However, these are currently one-time use items (as in for one season) and cost a few hundred dollars per mask, and occasionally inquisitive mares may pull off the face mask of another. On larger studs where the infrastructure for housing numerous animals under artificial lights, and with adequate staffing, it appears that the traditional light regimes remain the favor. Despite this, there is a place of the use for such masks. Mares seem to lose interest in the mask of other horses within a few days (the likelihood of a mask being pulled off is highest at the start). Providing there is nothing in the pasture on which the mare could hook the mask on (access to tree branches or fence posts above rails), and given that it is securely fastened, the mask should remain in place. Those that have only a handful of broodmares may prefer this method, as it reduces labor costs involved with stalling the mare.

2.1.1.3 Kisspeptin supplementation

As described above, kisspeptin appears to regulate GnRH secretion. As of yet, no commercial kisspeptin product is available. A recent report by Australian researchers found that although kisspeptin administered to mares as a constant rate infusion elevated circulating LH levels, it did not lead to an LH surge and therefore did not evoke ovulations within their group of mares during the vernal transition [4]. It remains to be seen whether the use of kisspeptin may shorten time to first ovulation, by potentially driving follicle maturation, under influence of LH, without necessarily causing ovulation.

2.1.1.4 Use of dopamine antagonists (domperidone, sulpiride)

As shown, dopamine plays an essential role in the stimulation of the reproductive axis in the mare. Dopamine has an inhibitory effect on GnRH release. For completeness it appears that dopamine antagonist acts via the stimulation of prolactin. For both domperidone and sulpiride, the dose is 1 mg/kg given PO and IM, respectively. Both are administered once daily for 25 days. The reports on the efficacy of the use of these preparations to shorten time to first ovulation in the mare are conflicting. A recent study by Mari et al. [25], comparing the two products, found that sulpiride significantly shortened time to pregnancy establishment (61 days) compared with domperidone-treated mares (83 days). That group concluded sulpiride is effective in advancing the vernal transition, whereas domperidone is only effective in some mares.

2.1.1.5 Use of progesterone

As mentioned the long transitional phase exhibited by mares is characterized by numerous follicular waves, unpredictable follicle growth, and follicle regression. Many protocols have examined the use of progesterone (P4) to dampen down these unpredictable features of the transitional phase and to drive a follicle to become dominant and one that will ovulate. The physiological effect of exogenous progesterone supplementation is relatively simple. P4 exerts an inhibitory mechanism with regard to LH but has minimal effect on FSH secretion. As described earlier, LH is required for maturation and final ovulation of the dominant follicle. While the mare is exposed to exogenous P4, LH is blocked at the level of the anterior pituitary, while FSH continues to be secreted. Therefore the follicles continue to grow under influence of FSH. Once the exogenous source of P4 is removed, this sudden fall in circulating P4 stimulates the LH surge, leading to final maturation and ovulation of a dominant follicle. The typical regimen is a dietary supplementation with altrenogest (Regumate®) at 0.044 mg/kg PO for 10 days. Injectable P4 products are becoming more routinely available. In the USA compounded products such as progesterone in oil can be utilized. Controlled release of P4 from these compounds last between 7 and 10 days. Daily application of oral altrenogest can be time-consuming. There also is a risk of noncompliance, should a mare be difficult to catch, not to mention potential side effect for the operator. The use of these long-acting P4 BioRelease products have been shown to be effective [26]. It appears that the use of exogenous P4 has maximal benefits when the mare exhibits a follicle of at least 20 mm in diameter and when administered in deep anestrous has little effect [27, 28]. An injectable altrenogest marketed via BOVA has recently become available in the UK for the first time, although no studies on its efficacy are currently available.

2.2 Endometritis

2.2.1 Endometritis in the mare

Endometritis is a leading cause of subfertility in the mare [29] and is the third most reported condition seen in our equine patients [30]. Endometritis, simply, the inflammation of the lining of the uterus, has historically been attributed to bacterial colonization and infection of the uterus. However there are a subgroup of mares that will exhibit persistent mating-induced endometritis (PMIE), in the absence of bacterial isolation. Furthermore we will also examine in the chapter the role of biofilm formation and bacterial endometritis.

Post breeding, a normal, physiologic endometritis will be observed in all mares [31]. This normal, transient event, which peaks around 8 hours post insemination, occurs to eliminate excessive spermatozoa, seminal plasma, and contaminants from the uterus [32]. This physiologic response should be over by 48 hours post insemination [33]. The subgroup of mares that experience PMIE appear to have an altered inflammatory response to the presence of spermatozoa and seminal plasma within the uterus. These mares tend to be aged, have increased parity, may exhibit chronic inflammatory changes within the endometrium [34], and exhibit failure to clear intrauterine bacterial challenges [35]. Susceptibility rates among thoroughbred broodmares is 15% [36], and crucially the early embryonic death rate is three times higher in this group of mares [37]. A persistent inflammatory uterine environment 5 days post fertilization is incompatible with embryo survival [38].

It has long been proposed that mares are classified as either susceptible or resistant to PMIE. It has been shown that susceptible mares do have altered protein

composition of their endometrial fluid [39] and these mares also exhibit higher levels of pro-inflammatory cytokines [40, 41]. It has also been shown that these mares with a delayed uterine clearance have contractile defects of the endometrium, possibly contributing to this delay in uterine fluid clearance [42]. It has been proposed that nitric oxide mediates smooth muscle relaxation [43]. It also important that mares that fall into this subgroup tend to have poor perineal conformation and a forward tilt to the uterus, such that it sits over the pelvic brim. It is therefore paramount to be able to identify these mares and initiate appropriate therapy.

Bacterial endometritis in the mare is primarily caused by four pathogenic species: *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [44–47]. By far the most commonly isolated are *Escherichia coli* and *Streptococcus equi* subspecies *zooepidemicus* (**Figure 1**). Diagnosis of bacterial endometritis is based on transrectal ultrasonography, uterine culture, and uterine cytology. In transrectal ultrasonography, these mares may show increased uterine edema and increased uterine luminal fluid, which may be echogenic in nature. Any mare that has uterine fluid with an accompanying corpus luteum (CL) should be highly suspected to have a uterine infection. There are now several reports of bacterial species becoming resistant to commonly used antimicrobials especially gram-negative species. It is therefore paramount that the attending veterinarian takes cultures to identify which bacterial species are present and then to select an appropriate antimicrobial based on antibiotic sensitivities.

A list of commonly used intrauterine antibiotics and dosages can be found in **Table 1** in the therapy section.

2.2.2 Fungal endometritis

Around 5 percent of infectious endometritis are attributed to fungal organisms [48], of which *Candida* spp., *Aspergillus* spp., and *Mucor* spp. are most frequently isolated. Again, mares that have anatomical defects are predisposed, and the use of previous intrauterine antimicrobial therapy is thought to increase the likelihood of fungal infections. Two schools of thought exist as to why this may be the case. Firstly, with repeated infusions, fungal organisms may be transplanted into the uterus (i.e., via contamination), and secondly, whether the antimicrobials may disrupt the normal bacterial flora of the caudal reproductive tract and subsequently

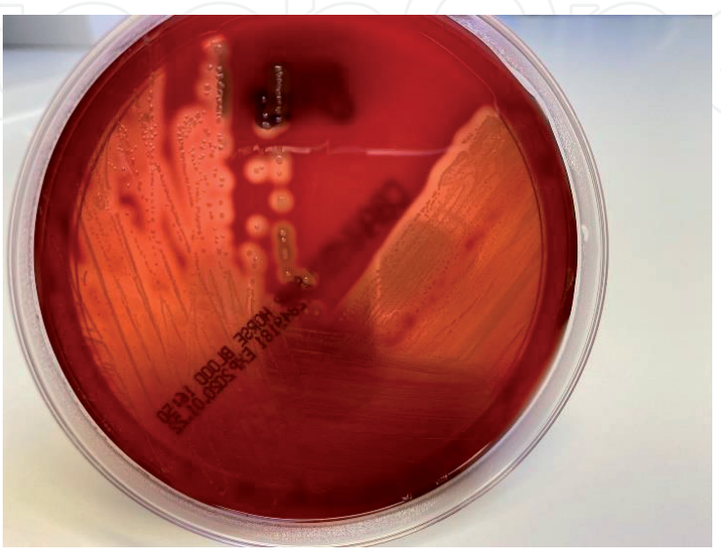

Figure 1.
Streptococcus equi *subspecies growing on blood agar showing distinct beta hemolysis. Image courtesy of BioTe veterinary laboratories, England.*

	Product	Dosage	Notes
Antifungals	Clotrimazole (100 mg/ tablet)	500 mg in 50 mls sterile saline	
	Fluconazole (200 mg/ tablet)	100–250 mg in 50 ml sterile water	Add 5 ml DMSO per 1 gram of fluconazole to aid in dissolving tablets
Antibacterials	Ampicillin (1 g vial)	1–2 g in 50 ml sterile saline	Mainly effective against gram-positive bacteria
	Ceftiofur (1 g vial)	1 g in 20 ml sterile saline	Broad-spectrum antibiotic
	Ciprofloxacin (10 mg/ml)	400–500 mg in 50 ml sterile saline	Mainly effective against gram-negative bacteria. Not first line; only utilize if strains are resistant to other antimicrobials
	Gentamicin (100 mg/ml)	1–2 g, buffer with 10 ml 8.4% sodium bicarbonate	Mainly effective against gram-negative bacteria
	Penicillin (procaine) 300,000 units per ml	15 ml, dilute to 50 ml in sterile saline	Mainly effective against gram-positive bacteria

Table 1.
Doses for commonly utilized antimicrobials for intrauterine administration (table reproduced courtesy of equine reproduction laboratory, Colorado State University).

allow colonization by the fungus. These sites (such as the clitoral fossa) can then serve as a nidus for uterine infection.

In any suspected fungal endometritis, it is imperative to send the sample swab to be tested for polymerase chain reaction (PCR). Fungal growth in routine laboratory cultures encompasses a long wait for results, whereas the turnaround for PCR is relatively quick.

2.2.3 Therapy

Therapy for endometritis in the mare will vary depending on whether the attending veterinarian is dealing with a bacterial endometritis, fungal endometritis, or indeed PMIE. Nonetheless there are some therapies that will be necessary in all cases, and they are dealt with first.

It is imperative to correct any caudal reproductive tract anatomical anomalies, such as poor perineal conformation. Surgical correction, such as a vulvoplasty (also known as a Caslick procedure), should be performed on these mares prior to breeding. A temporary Caslick can aid in treatment during the few days intrauterine access is required. A permanent Caslick can then be placed after treatment has ceased or after breeding (and resolution of any post-breeding fluid). An alternative is to place a permanent Caslick and to administer the treatment via a speculum, giving access to the cervix. Fixing anatomical defects in this area will prevent recontamination of the caudal reproductive tract and helps to "pull" the uterus into a more caudal position, aiding the natural mechanical cleansing mechanism of the mare.

All mares that have excess fluid should undergo uterine lavages. In cases of infectious endometritis, these uterine lavages reduce the organism load, aid in removal of biofilms (see below for further treatments), and reduce particulate matter that may interfere with the antimicrobials used.

2.2.4 Ecbolics

No attending veterinarian should underestimate the use of ecbolic when dealing with endometritis in the mare. The two commonly used preparations are oxytocin and prostaglandin F2α (PGF2α) in dealing with uterine fluid.

Oxytocin is by far the most commonly used of these two. Its ease of administration either given IM (intramuscular) or IV (intravenous) and its relatively short duration of approximately 30–45 minutes make it an essential product to have on standby when breeding mares. Side effects are minimal. However given its short duration of action, it does require multiple doses. Typically 1 ml either IV or IM of oxytocin given every 4 hours for 1 day, starting a minimum 4 hours post breeding, will be sufficient in treating most minor cases of uterine fluid retention.

The use of prostaglandins is not as straightforward as oxytocin. There are more side effects with the use of this preparation, and some are potentially quite serious. Prostaglandin is a known abortifacient. It is a good practice to always identify the mare in front of you for any reproductive treatment and, if in doubt, ultrasound the mare to confirm that she is indeed empty. Duration of action is approximately 4 hours. During this time, the mare may sweat, may act colicky, and may exhibit loose stools. It is recommended to monitor the mare during these 4 hours. Many clinicians are familiar with the use of PGF2α, as a luteolytic agent, and that is by far the most common use in equine reproduction. However, the veterinarian should not be afraid of its use when dealing with uterine fluid retention. Caution must be taken, however, when dosing PGF2α on the day of ovulation, as some studies have suggested that it can impact the formation of the corpus hemorrhagicum (which later becomes the CL, the source of progesterone required for maintenance of pregnancy). On the day of ovulation, it would steer the clinician away from use of prostaglandins, unless he or she is prepared to place that mare on an exogenous source of progesterone. The typical protocol initiated at my practice is that we would start with oxytocin for the first day and a half. If the mare has yet to respond satisfactorily to oxytocin therapy in that time, she is unlikely to respond. Throwing more oxytocin her way is futile. It is at this point we would consider the use of prostaglandin. In exceptional and severe cases, where there is significant fluid retention, it is not unknown to utilize both oxytocin and prostaglandin simultaneously on day 1.

2.2.5 Bacterial endometritis

Typically 3 days of intrauterine therapy is sufficient to see a positive outcome to therapy. It is bad practice to initiate intrauterine therapy for more than 3 days and predispose the bacterial inhabitants of the uterus to develop resistance to the antimicrobial utilized.

If the mare is presented with significant uterine fluid (in excess of 1 cm on ultrasonography), care must be taken to remove excessive uterine luminal fluid before commencement of the therapy. This is because we now know that certain antimicrobials may be affected by the fluid, but also there is a dilution factor to consider. Removal of fluid may include uterine lavages where 1–2 L of sterile fluid is distilled into the uterus and then allowed to flow back through the same giving set back into their original bags. Manual palpation of the uterus via the rectum at the same time the veterinarian is trying to remove the fluid may aid in evacuation of the uterine fluid. Ecbolics can be utilized concurrently, namely, oxytocin (see **Table 2**). For mares that present with minimal fluid, the use of ecbolics may be sufficient to remove the fluid. It is recommended to ultrasound the uterus prior to each intrauterine infusion.

Product	Dose and route of administration	Notes
Lutalyse® (dinoprost tromethamine)	5–10 mg IM once	Naturally occurring prostaglandin F2α
Estrumate® (cloprostenol)	250 μg IM once	Synthetic prostaglandin F2α
Oxytocin (20 units/ml)	20 units IV or IM	q 6 hours

Table 2.
Doses and routes of administration for the commonly utilized echolic agents (table reproduced courtesy of equine reproduction laboratory, Colorado State University).

2.2.6 Fungal endometritis

Further to the treatments below (**Table 1**), it is indicated to lavage the uterus with dilute acetic acid or dilute povidone-iodine.

2.2.7 Exercise

The use of exercise postmating, whether pasture turnout on the use of a horse walker, is widespread, yet the efficacy and examination in control studies are lacking. It is hypothesized that increases in intra-abdominal pressure from exercise transfer pressure to the uterus to aid in evacuating the contents and improve the lymphatic drainage [49]. Others have suggested that exercise can tone the hindquarters and leads to an improvement of perineal conformation [50]. Swift et al. [51] demonstrated that exercise was an effective management technique to aid in evacuation of uterine contents post breeding in mares. In their study, they note the lack of control studies on the efficacy of exercise alone as a treatment for uterine fluid retention post breeding in the mare.

2.2.8 Glucocorticoid treatment

The use of IV dexamethasone at a dose of 50 mg at time of treatment has become widespread following the classic studies by Bucca's group in Ireland [52]. It has been shown that there is a negative correlation between elevated endometrial score at time of breeding and pregnancy rates [53]. Dexamethasone has been shown to modulate the inflammatory process, possessing anti-inflammatory effects (decreasing IgG) while showing a stimulatory effect on α1-antitrypsin and transthyretin, which both enhance the defense mechanisms of the uterus.

2.2.9 Acupuncture

A recent and growing addition to the treatment of endometritis in the mare is acupuncture. It has been suggested that electroacupuncture stimulates afferent nerve fibers, leading to modulation of hormone release through ascending pathways to the hypothalamus as well as reflex activation of the autonomic efferent pathways to the uterus [54]. The first control study examining the use of electroacupuncture in the mare as a treatment modality for endometritis found mare resistance to treatment was a major limitation in the use of this treatment, and that given the multiple acupuncture points, as of yet, does not appear to be an effective mechanism when treating endometritis in the mare.

2.2.10 Breeding on a dirty cycle

In an ideal world, we would swab the uterus, and if found to have an infection, we would "clean" her up and wait for the mare's next cycle. However in the time-pressured breeding season, and in particular when dealing with valuable thoroughbred racehorses, time is seldom something the attending veterinarian has. This author has had great success breeding mares on dirty cycles, as long as there is at least 3 days prior to cover, to allow 3 days of intrauterine therapy. It is well established that the optimum time to swab the mare's uterus is when there is presence of uterine edema; swabbing when there is no uterine edema raises the risk of a false-negative result. An assumption is that the mare is infection-free only to be found negative on her pregnancy scan. Moreover it was inappropriate for the attending clinician to swab the uterus of a mare in diestrus (i.e., that she has a CL present). For one, the cervix will be tightly closed, and you may damage the cervix while trying to force the culture instrument through. Additionally, as the cervix is tightly closed, if you have accidently tracked bacterial isolates from the external vulva, or indeed the vaginal vault into the uterus, thereby inoculating the uterus with an infectious agent, the infection will take hold as the mare will be unable to "cleanse" herself with a closed cervix.

2.2.11 A frustrating scenario and the role of biofilm

We have all been there, as attending clinicians. We swab the mare, she cultures negative, there are no ultrasonic changes to make us think there may be an infection, and she returns negative on multiple cycles. There is a caveat here, that reproduction is a complex beast, and many, many things must fall into place for successful fertilization and subsequent embryonic development to take place. As the saying goes, it takes two to tango. However as this part of the chapter is dedicated to endometritis and often we do not have access to the stallion, it is fair for the clinician to start with the mare, and indeed her uterus, when beginning to evaluate why a mare may not become pregnant.

In the short breeding season, the author recommends that any mare that is negative on two cycles (i.e., she has been inseminated twice) should undergo a full reproductive examination that includes swabbing the uterus for culture. If there is any suspicion that the mare maybe dirty, but has a negative culture, then the clinician should explore other diagnostic routes. This would, namely, be low-volume lavage.

Nonetheless there are some mares that either routinely cultured negative but fail to conceive or conversely routinely cultured positive despite appropriate therapy based on sensitivities. In these cases, the attending clinician must consider the possibility of a biofilm. A biofilm as defined by Loncar et al. [55] is a community of bacteria that are attached to an interface or to one another, encased within an extrapolymeric matrix consisting of nucleic acids, lipids, proteins, and exopolysaccharides. These biofilm plaques are inherently resistance to both antimicrobial and innate immune defenses, which leads to a persistent, chronic infection, even in the face of prolonged antimicrobial therapy. The matrix reduces the penetration of antimicrobials. Gram-negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* are all capable of producing biofilms. The workout of Colorado State University has given us incredible information on treating biofilms. The work of Loncar et al. [55] showed that no single treatment was effective against all three bacterial species named above and suggests appropriate identification of bacterial species is paramount for successful treatment. The administration of dimethyl sulfoxide to the uterus shows promise in treating biofilms caused by *E. coli* and *K. pneumoniae*.

2.3 Twinning events in the mare

Twin or multiple pregnancies in the mare is the most common noninfectious cause of pregnancy loss. Twin pregnancies have been reported to account for up to 30% of abortions in the mare [56, 57]. When twin pregnancies are established, the pregnancy will continue as normal to approximately 6 months, when one or both fetuses will die due to insufficient placental supply. Typically mares carrying twins will abort around 5–9 months of gestation [58]. Only 14% of twin pregnancies resulted in foals surviving into their second week of postnatal life [56]. Mares producing live twins will inevitably require intense (and therefore expensive) neonatal care. The majority of twin pregnancies are dizygotic and arise from multiple ovulations which can be either synchronous or asynchronous in nature. Ginther [59] has proposed that there are familial lines higher than normal incidences of multiple ovulations and thereby there may be a genetic predisposition. Thoroughbreds show the highest incidence of multiple ovulations, while it is low in native breeds and yet to be reported in native Shetland ponies. Older mares also seem to have high incidences of multiple ovulations [60]; however lactating mares appear to have lower multiple ovulation rates, presumably due to the suckling effect on the hypothalamic–pituitary-ovary axis. Not only do mare normally abort twin pregnancies, they also show high incidence of dystocia, damage to the reproductive tract (including the cervix), retained placenta, and delayed uterine involution. These have ramifications on the future reproductive health of these mares. In one study only 38% of mares that had a twin pregnancy in the previous breeding season produced a viable foal the following year [61]. Given the significant risks associated with twin pregnancies, detection of these is paramount. The attending veterinarian should make detailed notes of the presence of large follicles on the ovary and, at ovulation detection, note all ovulations. However do not be fooled, if only one large follicle has ovulated and another large follicle remains. If this follicle should subsequently ovulate, there is a chance of the establishment of asynchronous twins. It is advised to examine the mare in stocks and have the mare adequately restrained. Checking for twins in the field, where a mare may be fractious and/or not restrained correctly, will lead the clinician to potentially rush through examination. There is a danger element to ultrasounding mares not in stocks. Occasionally owners will state that they do not wish to transport the mare to facilities that have the required setup. If this is the case, get the mare restrained as best as possible, and advise the owner that this is not optimal. No ultrasound examination is foolproof. Begin in a systematic manner. The author starts with the left horn, runs the ultrasound probe laterally until the left ovary is seen, and then returns to the bifurcation. This is repeated twice. The same is then done for the right horn. Finally the body of the uterus is examined twice. During the examination, it is paramount to retain the uterus within the center of the screen at all times. If you feel as though a section of the uterus has been missed, repeat. As can been seen, to do this in the field without stocks in a fractious mare can be difficult. Natural reduction of unilateral twins before day 40 is reported at 85% [62, 63].

Given the limitations of this chapter, only two techniques for dealing with twin pregnancies in the mare will be described. There are numerous other techniques described and the readers are encouraged to examine these. At approximately 16 days post ovulation, the embryo (in this case the embryos) become fixed. Up until this point, the embryos are highly mobile and move throughout the uterine lumen. Typically a twin check using transrectal ultrasound takes place before this day 16. Identification of the small embryo takes place. If the pregnancies are adjacent to one another, the probe is gently oscillated to move them apart. The smaller embryo is then moved to the tip of the uterine horn, while a downward pressure

from the ultrasound probe on the selected embryo is performed. While keeping the embryo in focus on the ultrasound screen, rupture of the embryonic wall will be observed, and leakage of the embryonic fluid into the uterine lumen will also be observed. A quick check on the remaining embryo should also be performed, following this procedure. Adjunct therapy typically includes a single dose of flunixin meglumine (1 mg/kg IV) given prior to the elimination procedure. Typically these mares are placed on oral Regumate® (dose of 0.088 mg/kg SID PO), until a P4 sample is taken around the heartbeat ultrasound check (approximately day 25 post ovulation). Success rates of continued survival of the singleton pregnancy after a twin reduction around this time is in excess of 90% [64].

If you are presented with twin pregnancies beyond this stage, the clinician has a few options to choose from. After day 40, 63% of these pregnancies result in loss of both fetuses [65]. One of the authors preferred mechanism of twin reduction after day 40, which is cranio-cervical dislocation. Here the clinician is dislocating the first cervical vertebrae from the cranium along with disruption of the ligamentous attachments and severing the spinal cord via transrectal manipulation. This technique can be utilized between 60 and 110 days' gestation. The mare is sedated and placed in stocks. Buscopan (2 cc IV) can facilitate manipulation of the fetuses. Flunixin meglumine (1 mg/kg IV) is administered prior. The small fetus is selected and identification of the head performed, via identification of the mandible. Stabilize the head between the thumb and the finger and move the head side to side. Place the thumb at the base of the cranium and apply pressure proximally and dorsally; this will result in dislocation, whereby a "pop" is felt. Adjunct therapy included altrenogest (Regumate® at dose 0.088 mg/kg SID PO). Fetal death should be confirmed in 1 week post procedure via transrectal ultrasonography. Viability of the remaining conceptus should be evaluated (viz., by continued growth and the presence of a fetal heartbeat). If both pregnancies continue to be viable, then further intervention will be necessary.

3. Conclusions

With a thorough understanding on the physiologic events in the spring/vernal transition, the clinician can aid in hastening time to first ovulation. Most mares, if not all, will show some transient uterine fluid accumulation post breeding. Having the skills to note which mares are likely candidates to have excessive fluid accumulation, or which mares have a uterine infection, will greatly improve pregnancy rates. Identification of mares that may develop twin pregnancies is a key skill of the equine theriogenologist, but transrectal ultrasonography has its limitations if the mare is examined in the field. Twin pregnancies are easily dealt with if identified prior to fixation.

Conflict of interest

The author declares no conflict of interest.

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