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Cardiology

Contents

Abstract Papers

Cardiac physiology and auscultation

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Cardiac physiology

The heart is made up of four chambers, the left atrium, left ventricle, right atrium and right ventricle. Four valves are present in the heart to keep the blood moving in a forward direction. These are the mitral valve on the left between the atria and ventricle, the tricuspid valve on the right between the atria and ventricle and the aortic valve exiting the left ventricle and the pulmonary valve exiting the right ventricle. The aorta is the main artery carrying oxygen-rich blood from the left ventricle to supply the organs of the body (brain, heart, skin, kidneys, liver, intestines, muscle). The aorta branches into smaller arteries and then to capillaries. Within the muscle cell, mitochondria are the small organelles that use oxygen to produce energy. Carbon dioxide is also produced as part of this process. Carbon dioxide then enters the bloodstream at the level of the capillaries and is transported by smaller veins into the major veins and then to the right atrium. This then is carried through the right heart to the lungs via the pulmonary artery where it enters the pulmonary capillaries. The pulmonary capillaries are located adjacent to the alveoli. These alveoli are small air-filled sacs at the very ends of the airway. There is an extremely thin membrane between the alveoli and the pulmonary capillary which allows oxygen to enter the capillary and carbon dioxide to pass across into the alveoli. The oxygen rich blood leaving the capillary enters into the pulmonary vein, then the left atrium and ventricle before exiting the aorta and the cycle starts again.

Oxygen and carbon dioxide are transported within the bloodstream by haemoglobin, which is a specialised protein within the red blood cell. The spleen of the horse has an important role in storing extra red blood cells. At the onset of exercise, adrenaline acts on the spleen and causes it to contract, releasing extra red blood cells into the system which increases the packed cell volume to 55-60% and causing a 50% increase in oxygen carrying capacity.

Rest to exercise differences

Racehorses have an enormous capacity for increasing oxygen delivery from rest to exercise. A table below summarises these changes:

Variable	Rest	Exercise	Exercise/Rest Ratio
Heart rate (beats/min)	30	210-250	7-8
Cardiac output (L/min)	30	240-450	8-13
Stroke volume (L)	1	1.7	1.7
Aortic pressure (mmHg)	120	180-230	1.7
Pulmonary artery pressure (mmHg)	20-30	90-140	3-5
Packed cell volume (%)	37-47	55-60	1.5
Oxygen uptake (L/min)	1-2	80-100	50-100

Table 1. Physiological changes from rest to exercise in racehorses

Autonomic control of heart rate

The sinus and atrioventricular nodes respond to changes in both sympathetic and parasympathetic tone. Increased sympathetic tone enhances automaticity (increases heart rate) whereas increased parasympathetic tone inhibits automaticity (reduces heart rate) and prolongs conduction time. Within the confines of the heart rate range, which for a horse is from approximately 20 beats per minute to 240 beats per minute, regulation of the heart rate is controlled by reciprocal activity of the sympathetic and parasympathetic nervous system. Sympathetic stimulation is moderated by noradrenaline and as actioned mostly by $\beta 1$ receptors. This increases heart rate, conduction velocity, force of contraction and active relaxation. The neurotransmitter of the parasympathetic nervous system is acetylcholine. This has a very short latency period and can influence heart rate over only a few cardiac cycles.

Cardiac adaptation to training

Racehorses have naturally large hearts, approximately 1-1.5% of bodyweight. In mammals, including humans and horses, exercise training will cause further adaptations to the heart which include an increase in heart size. In racehorses strenuous exercise training causes an increase in the blood volume which then causes increased stretch and pressure in the heart. This results in balanced enlargement of the heart chambers (atria and ventricles) and thickening of the heart muscle wall. This process is called athletic heart syndrome. As a result of the increase in heart size, the stroke volume (amount of blood pumped by each heartbeat) increases. The maximum heart rate for an individual horse is fixed and does not change with exercise training. The cardiac output is the amount of blood pumped by the heart each minute and is proportional to the amount of blood being delivered.

Cardiac output (L/min) = Heart rate (beats/min) x stroke volume (L)

Heart rate is controlled by the balance between the sympathetic (fight or flight) and parasympathetic (rest and digest) systems. The sympathetic system is modulated by adrenaline and increases heart rate. The parasympathetic system is modulated by acetylcholine and decreases heart rate. At peak exercise the system is entirely under the influence of the sympathetic system but the parasympathetic influence returns immediately after exercise and causes a rapid decrease in heart rate. Peak exercise and immediately post-exercise are the most vulnerable times for arrythmia. Pain and anxiety can also increase heart rate. Imbalance between sympathetic and parasympathetic influence can contribute to arrhythmia.

Auscultation

Notes for auscultation of cardiac arrhythmias are provided in the lecture on electrocardiographic recording.

Cardiac murmurs are very common in athletic horses and in most cases have no impact on performance or safety. However, some murmurs are indicative of important underlying structural heart disease that would preclude continued use of the horse. Definitive characterisation of a cardiac murmur requires echocardiography (heart ultrasound). Auscultation is helpful in determining the most likely lesion causing the murmur. Additionally, information gleaned from the signalment, fitness and current use of the horse provides important information as to the likely significance of the lesion.

In general, the intensity of the murmur reflects the severity of the lesion with louder murmurs usually being more significant.
Classification of cardiac murmurs is as follows:

1. Very soft murmur that requires extended auscultation to detect
2. Readily audible murmur that is softer than S1 or S2
3. Readily audible murmur that is moderately loud and similar in intensity to S1 and S2
4. Readily audible murmur that radiates widely and is louder than S1 or S2.
5. Very loud murmur with palpable thrill.
6. Very loud murmur with palpable thrill that is audible with the stethoscope held just off the chest.

A cardiac murmur is the sound generated by turbulent blood flow in the heart. It can be physiologic (benign flow murmur) or caused by shunt, regurgitation or stenosis. Physiologic murmurs and valvular regurgitation are common in horses and stenosis is very rare. Shunts are congenital and should be suspected in young, poorly grown animals with a murmur.

The auscultatory findings associated with potential cardiac lesions in horses with cardiac murmurs are as follows:

Left side thorax
- systolic murmur
  - physiologic (flow) murmur
  - mitral regurgitation
  - ventricular septal defect (ejection murmur relative pulmonic stenosis)
- diastolic murmur
  - physiologic (flow) murmur
  - aortic regurgitation
- systolic with diastolic component- aortopulmonary fistula (Friesians)

Right side thorax
- systolic murmur
  - tricuspid regurgitation
  - ventricular septal defect
- continuous
  - aorto-cardiac fistula

Echocardiography

A recent consensus statement from experts in the field suggested that echocardiography is warranted in association with the following clinical findings:[1]

1. A previously diagnosed murmur that is louder on serial examinations
2. A grade 3-6/6 left sided murmur compatible with mitral regurgitation or aortic regurgitation
3. A grade 4-6/6 right sided systolic murmur compatible with tricuspid regurgitation
4. Suspected ventricular septal defect
5. Continuous or combined systolic-diastolic murmurs
6. Clinically important arrhythmias, whether a murmur is present or not
7. Suspected myocardial injury
8. Suspicion of congestive heart failure

Physiologic (flow murmurs)

Physiologic murmurs are most commonly heard on the left side during systole and are caused by turbulent flow of blood through the normal pathways in the aorta and pulmonary artery. Occasionally a left sided diastolic murmur associated with ventricular filling may be heard. Such murmurs are usually less than grade 3/6 and are of no clinical significance. They are often heard in young foals and fit racehorses as these groups have a relative increase in blood volume.

Shunt

Ventricular septal defect is the most common congenital lesion in horses. This lesion is characterised by a left sided systolic murmur associated with relative stenosis of the pulmonary artery (blood is shunted from the left to ride side of the heart due to lower pressure in the right). A systolic murmur on the right side is also typically heard. A shunt carries a very poor prognosis if signs of cardiac failure are evident (poor growth rate, high resting heart rate). Horses with smaller defects may be suitable for riding but this must be assessed with echocardiography. Spontaneous closure of a VSD has been reported in the horse.

Atrioventricular valve (mitral and tricuspid) regurgitation in athletic horses

Cardiac enlargement and remodelling occur with exercise training in horses. A similar syndrome 'Athletic heart syndrome' is observed in human athletes, particularly those participating in strenuous aerobic events. Such adaptations include an increase in parasympathetic tone, expansion of plasma volume, cardiac hypertrophy, reduced cardiac contractility at rest and increased valvular regurgitation. Increased parasympathetic tone is manifest by a reduction in resting heart rate. Expansion of plasma volume with exercise training induces morphological changes in the heart and enhances cardiac output. Cardiac hypertrophy in horses is characterised by an increase in chamber size and a mild increase in relative wall thickness. Reduced cardiac contractility at rest is observed. Increased chamber size contributes to development of regurgitation in the atrioventricular valves. Mitral regurgitation is seen in 10-20% and tricuspid regurgitation in 20-40% of Thoroughbreds, depending on race type. The prevalence of regurgitation increases with fitness and generally does not affect performance. The clinical significance of murmurs in racehorses can be determined by thorough investigation including echocardiography, resting and exercising electrocardiography.

Mitral regurgitation

Mitral regurgitation also occurs in non-athletic horses and may be caused by mitral valve prolapse, mitral thickening or dysplasia, ruptured chordae tendineae or flail leaflet. Many horses can continue to be ridden if the regurgitation is mild. Atrial enlargement will occur progressively and may cause atrial fibrillation. Horses with an elevated resting heart rate, prominent jugular pulse, concurrent arrhythmias other than atrial fibrillation or severe haemodynamic changes should not be ridden. Mitral regurgitation is associated with ventricular arrhythmia in humans. It is not clear whether valvular regurgitation increases the risk for pathological arrhythmia other than atrial fibrillation in horses. Current guidelines recommend exercising ECG in horses with moderate to severe mitral regurgitation.

Tricuspid regurgitation

Tricuspid regurgitation is particularly common in racehorses and increases in prevalence and severity as part of athletic heart syndrome. Tricuspid regurgitation can also increase the risk of atrial fibrillation. In young horses, tricuspid dysplasia can be a cause of tricuspid insufficiency.

Aortic regurgitation

Aortic regurgitation is commonly observed in horses >15 years of age. It can also occur in young horses and might have a poorer prognosis and deteriorate more rapidly when presenting early. In many cases it is well tolerated and horses can continue to be ridden. However, it is appreciated that aortic regurgitation increases the risk for ventricular arrythmia therefore overnight holter monitoring and exercising ECG is recommended for these horses. The character of the arterial pulse can be helpful in determining the severity of aortic regurgitation as more severely affected horses have a bounding pulse.

References

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Unravelling the subfertile mare: Management options to improve fertility

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Introduction

Endometritis, or inflammation of the endometrium, is a condition that commonly affects the fertility of mares. Endometritis can be caused by the introduction of pathogenic organisms, semen, urine, or air into the uterus. Additional factors, such as age, perineal conformation, anatomic abnormalities, poor uterine contractility, defective lymphatic drainage and degenerative tissue changes can contribute to endometritis.

Interestingly, all mares develop endometritis because of breeding.[1] However, the response to endometrial inflammation differs between mares. Most healthy mares quickly eliminate inflammatory byproducts through intrinsic mechanisms such as uterine evacuation via muscular contractions and an open cervix. Additionally, influx of "healthy" inflammatory cells and lymphatic drainage contribute to a healthy uterine environment.[2] Aberrations in one, or all, of these mechanisms can lead to uncontrolled inflammation, infection and reduced pregnancy rates. Elucidating the root causes of endometritis can be challenging but is an essential first step toward improving fertility in subfertile mares.

Diagnosis of endometritis

Fundamental to the diagnosis of any problem is assessment of an animals' physical condition and review of medical/breeding history. Increasingly, medical professionals rely on diagnostic tests to provide answers to infertility conditions when the critical information may be as simple as body condition or previous breeding and foaling history. For example, factors that affect fertility of a middle-aged, nulliparous mare (i.e. poor cervical relaxation while in oestrus) are often quite different than factors that affect fertility of a middle-aged, multiparous mare (i.e. trauma to the genital tract, compromised uterine clearance). In addition to a general physical examination, a thorough examination of the reproductive tract is an essential first step for determining the cause of endometritis. Commonly used tools for evaluating a mares' reproductive function include visual evaluation of overall body condition and perineal area, transrectal palpation and ultrasonography of the reproductive tract, vaginal examination and sampling for uterine culture, endometrial cytology, and endometrial biopsy. While results from these tests can help with obvious, and often correctable, problems it is important to understand that one test, alone, is infrequently diagnostic.

General physical and perineal examination

Careful attention should be paid to body condition and perineal conformation of mares. Overall, good physical condition is important to reproductive health in mares. Mares with poor body condition frequently have compromised general conformation which affects perineal conformation. For instance, low body weight leads to pelvic tilt which results in a dependent uterus and sunken anal sphincter. These conditions increase risk for uterine contamination.

Vulvar anatomy is a critical first defence against uterine contamination. Poor perineal conformation predisposes the mare to "wind sucking," faecal contamination, and pooling of urine in the vagina. The long axis of the vulva should be vertical with no more than a 10°

angulation toward the rectum. Most of the vulva should be ventral to the pelvic brim. When two fingers are placed on the pelvic ischium, no more than 2.5 cm of the vulva should be dorsal to the pelvic floor.[3] The vulvar labia should have good apposition and be free of irregularities. When the vulvar labia are gently parted no obvious influx of air should be heard. The vestibule-vaginal seal should be intact when evaluated through the parted vulvar labia.

Transrectal palpation and ultrasound examination

Transrectal palpation and ultrasound examination are important and useful components of evaluating reproductive health in mares. Transrectal palpation, alone, provides little useful information about endometritis, specifically, other than size and position of the uterus in the pelvic cavity. However, in a recent study, gynaecological evaluation techniques, including transrectal palpation and ultrasound, culture swab, cytology brush, low volume uterine lavage and processing an endometrial fragment were compared for diagnosis of endometritis.[4] Uterine oedema and intraluminal fluid, detected using transrectal ultrasound, were highly predictive of (52% of mares) endometritis.[4] Transrectal ultrasonographic evaluation showed good sensitivity and specificity for detecting endometritis.[4] Furthermore, intraluminal uterine fluid around the time of breeding and ovulation has an established relationship to reduced pregnancy rates in cycling and postpartum mares.[5-7] Post-mating induced endometritis (PMIE) is the most common cause of uterine fluid accumulation in the periovulatory period of mares.[4] Mares with delayed uterine clearance often have defects in intrinsic myometrial contractility and drainage through the lymphatics resulting in lymphatic stasis.[5,6] These conditions frequently manifest themselves as intraluminal fluid accumulation and abnormal uterine oedema patterns during oestrus.[7] Specifically, these mares often have an unusually large amount of uterine oedema starting early in the oestrus period and persisting after ovulation. Interestingly, mares with bacterial endometritis do not consistently develop intraluminal fluid. Some studies reported that mares having uterine infections caused by *Escherichia coli* were less likely to have intrauterine fluid accumulation identified by ultrasound and less evidence of cytologic inflammation.[8;9] Mares with uterine infections caused by *β haemolytic streptococcus, Klebsiella pneumoniae, Enterobacter cloacae* or yeast had a higher incidence of ultrasonographically detectable uterine fluid. While a simple first step, transrectal examination of the uterus and reproductive tract are critical for identifying endometritis in mares.

Uterine sampling for culture and cytology

The natural next step when evaluating a mare for endometritis is the collection of samples for uterine culture and cytology. A variety of techniques can be used for uterine sampling including use of a double-guarded swab, cytology brush, endometrial biopsy, or low volume uterine lavage. Results from many studies have shown great inconsistency with regard to predictive value of these tests for detecting endometritis.[8] In general, most practitioners sample the uterus using a double-guarded swab for microbial assessment combined with a double-guarded cytology brush to detect inflammation. Double-guarded instruments have been shown to reduce the risk of contamination by over 30% compared to single or non-guarded instruments.[9,10] In some cases, instruments are passed through a vaginal speculum as an extra precaution for reducing sample contamination.[11] Most would agree that uterine culture and endometrial cytology samples should be performed together, as either test has a high prevalence of inaccurate results when used alone.[9,12] Given the proximity to faecal contaminants, and commensal organisms that inhabit the caudal reproductive tract, false positive microbial culture results are common. Conversely, aerobic culture may fail to diagnose fungal or yeast infections, which are more easily detected on cytology. In general, any bacterial

growth in conjunction with neutrophils on cytologic exam is considered diagnostic for uterine infection.

Organisms historically implicated in equine uterine infections are *β haemolytic streptococci* (*Streptococcus equi zooepidemicus),* coliform bacteria *(E. coli, Enterococcus spp), K. pneumoniae* and *Pseudomonas aeruginosa*. All of these organisms are aerobic, therefore, anaerobic culturing is rarely performed for equine uterine samples. Commensal organisms on skin (*Staphylococcus, Bacillus* and *Micrococcus* spp) are periodically obtained in significant quantities from uterine cultures and have been associated with low pregnancy rates.[13] It is difficult to predict whether the root cause of poor pregnancy rates is the commensal organisms or impairment of the natural defence mechanisms for managing endometritis. The challenge in determining the relationship of less common organisms and true infection is exacerbated by the fact that areas that were previously considered "sterile," such as the uterus, are known to have a microbiome of bacteria that are not identified using traditional culture tools.[14] Using sophisticated genomic tools, Heil and co-workers identified *Klebsiella, Mycoplasma and Aeromonas* bacteria in the uteri of mares that tested negative using traditional uterine sampling tools.[14,15] A better understanding of resident bacterial populations in the reproductive tract will aid in accurate diagnosis and treatment of conditions that are affecting fertility of mares.

Endometrial cytology adds an additional, important, tool for diagnosing endometritis in mares. Endometrial cytologic findings can reveal normal vs abnormal epithelial cells, inflammation, microbial organisms, mucus, fungal/yeast organisms, urine, spermatozoa, and red blood cells. Importantly, endometrial cytology provides an opportunity to diagnose uterine conditions beyond microbial infection. Specifically, post-mating induced endometritis (PMIE) and uterine irritation from air/urine can cause cytologic changes in the absence of infection. Given that most endometritis is caused by non-infectious inflammation, this tool is especially valuable for directing treatment of mares that do not have an infectious endometritis. When evaluating an endometrial cytologic sample obtained with a double-guarded brush system, the sample is evaluated for the presence of "rafts" or sheets of healthy endometrial cells and little else. (Fig 1).

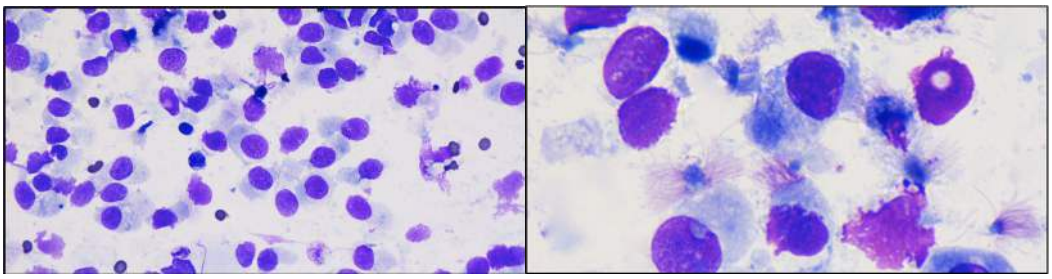

Figure 1: Normal epithelial cells (20x magnification and 100x magnification) obtained from the endometrium of a mare using a cytobrush (Photo courtesy of Dr. Patrick McCue, Colorado State University)

Mares with endometritis will have cytologic findings such as degrading epithelial cells (lack of epithelial sheets, foamy cytoplasm, pyknotic nuclei) presence of polymorphonuclear cells (PMNs), bacteria and/or fungal organisms (Fig 2). The number of PMNs are quantified as either

per high powered microscopic field (HPF; magnification 400x) or as a percentage of epithelial cells present.[12] On average, cytologies are scored as normal/mild (0 – 2 PMNs/HPF), moderate (3 – 5 PMNs/HPF) or severe (> 5 PMNs/HPF).[16,17] A second way to identify an "abnormal" cytology is if PMNs represent > 1% of the total cells.[8] Additional findings that can be identified using endometrial cytology include urine crystals (possible urine pooler), eosinophils (probable uterine irritant such as air or urine) and mucus. Exuberant uterine mucus production has also been associated with poor fertility in mares.[18] However, endometrial cytology obtained using a double-guarded brush is not always the best means to identify mucus as lubricant strands will be smeared on the slide along with cells. In some cases, diagnosis of uterine conditions is better served using low volume uterine lavage. In addition to exuberant mucus producers, mares having uterine infections with *E. coli* and *P. aeruginosa* may be detected more consistently using low volume uterine lavage.[19,20]

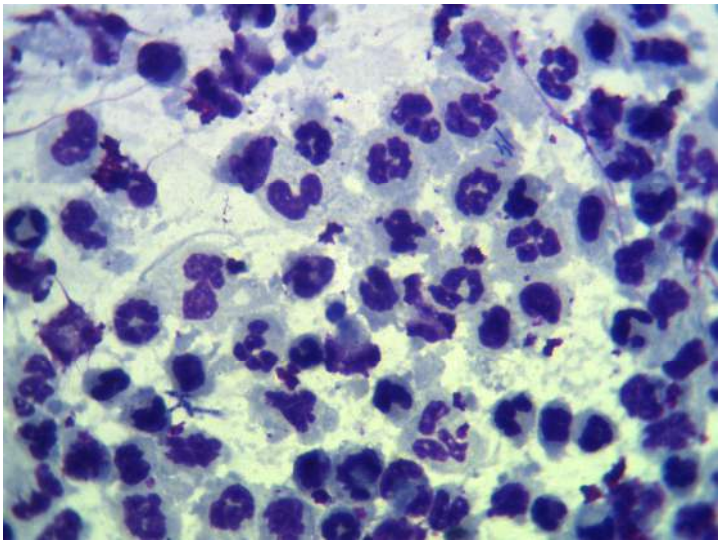

Figure 2: Degenerating epithelial cells and PMNs (20x magnification) obtained from the endometrium of a mare using a cytobrush (Minitube USA, Verona, WI, USA)

Low volume uterine lavage

Rationale for the use of low-volume uterine lavage (LVL) is to obtain a more representative sample of the uterine contents than occurs with focal samples obtained with a uterine swab or cytology brush.[16,21] Several studies have compared uterine swab/brush sampling methods with samples from low volume lavage, and results are conflicting.[4,9,20] Sensitivity and specificity of microbial culture results are reported to be lowest when using swab and brush techniques.[9,20] However, LVL is more time consuming and expensive to perform.

Low volume uterine lavage can be performed through a variety of techniques. To perform LVL, a sterile Foley-type catheter is attached to 150-250 ml of sterile saline (0.9% NaCl), lactated Ringer's solution (LRS) or phosphate-buffered saline (PBS) using a standard, large bore, single fluid line or a short, fluid connection system (Fig 3). The author prefers to use a high flow, fluid transfer set (https://www.milainternational.com/products/fluid-transfer.html product # 175490, Mila International, Inc., Florence, KY, USA) attached to the uterine catheter (Fig 4).

The fluid transfer set is 30 cm (12 in) long which reduces the dead space of the system and improves fluid recovery.

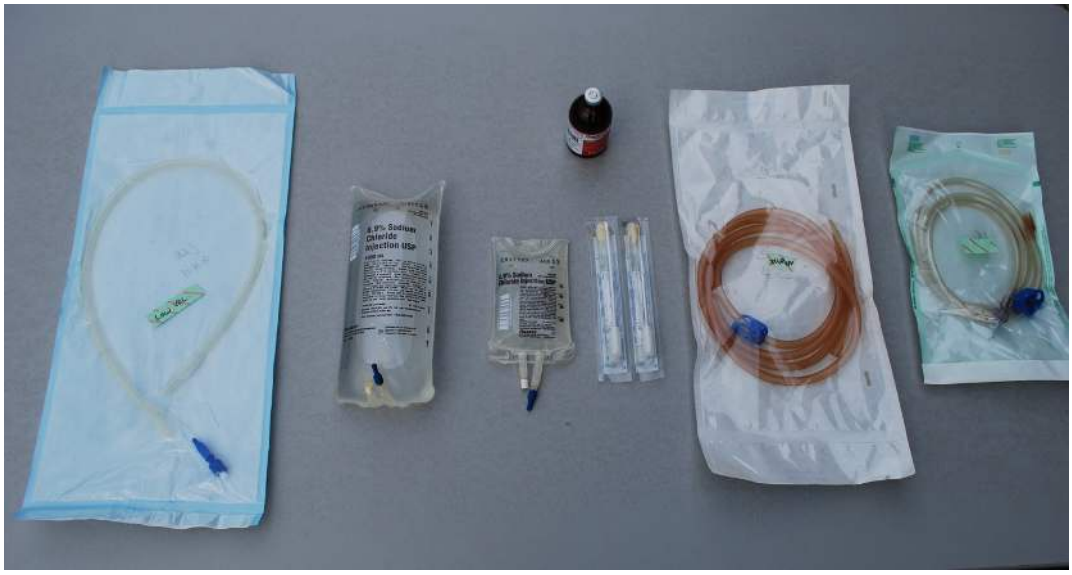

Figure 3: Supplies to perform low volume uterine lavage including (from left to right): uterine catheter, 1000 mL and 250 mL sterile saline bags, oxytocin, culturettes, 2 x single fluid line systems

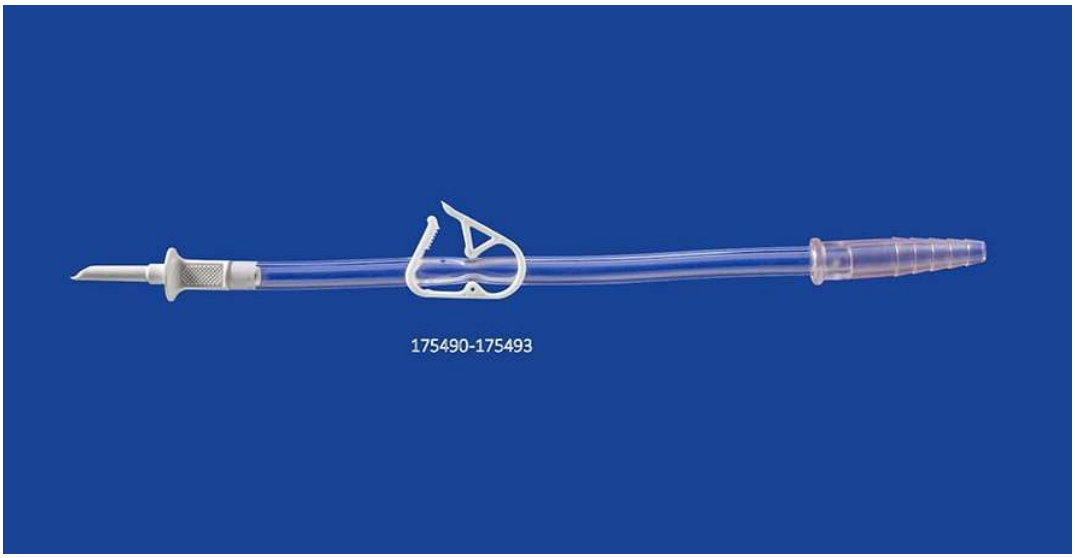

Figure 4: Mila International High Flow Fluid Transfer Set, product #175490 (30 cm) used to connect small volume fluid bag to uterine catheter for low volume uterine lavage (Mila International, Florence, KY, USA)

Prior to fluid infusion, the system is primed with fluid (to eliminate air introduction into the uterus). The mare's perineal region is cleansed and dried. Using a sterile sleeve and lubricant, the catheter is aseptically inserted through the cervix and into a uterine horn. With transrectal

guidance, the catheter is fed into the tip of one uterine horn. In the author's experience, the catheter balloon is left uninflated so that the catheter tip can be manipulated easily into fluid pockets which aids in fluid evacuation. The contents of the fluid bag are infused into the uterus with care to avoid introduction of air. Fluid is agitated throughout the uterus using rectal massage for at least one minute. Oxytocin (10-20 IU, IV) is administered to the mare to promote uterine contractility for optimal fluid collection. Using transrectal manipulation, fluid is moved to the tip of the uterine horn where the catheter tip is placed. With the operator's hand cradling the tip of the uterine horn around the catheter, the fluid is allowed to drain out through the catheter and back into the bag so that the system remains "closed." For full fluid recovery, it may be necessary to "lift" the uterus, transrectally, from one tip of the uterus, sequentially to the other uterine horn. Once most of the fluid is recovered into the fluid bag, the uterine tube is removed as cleanly as possible. Fluid within the tubing system is drained into the fluid bag taking care not to drain the final 10–20 mL in the line as that fluid has the highest probability of contamination from the caudal genital tract. Uterine fluid should be processed for evaluation within one hour of collection to avoid iatrogenic cellular changes.[16] Prior to processing, fluid is transferred into 50 ml conical tubes where it is assessed for clarity, presence of mucus strands and/or debris (Fig 5). This crucial step provides a unique opportunity to identify mucus in the uterine contents. Mucus can both aid and impair pathogen clearance in systems with a mucociliary apparatus, such as the uterus. It has been postulated that normal mucus production facilitates cilia-propelled removal of contaminants from the uterus after breeding.[22] Under persistently inflamed conditions, excess or reduced mucus production alters mucociliary function and impairs pathogen clearance. Excess mucus production has been identified in mares with chronic endometritis.[18] In one study, cloudy and mucoid effluxes were highly correlated with presence of microorganisms (*E. coli* and *β haemolytic streptococcus*).[20]

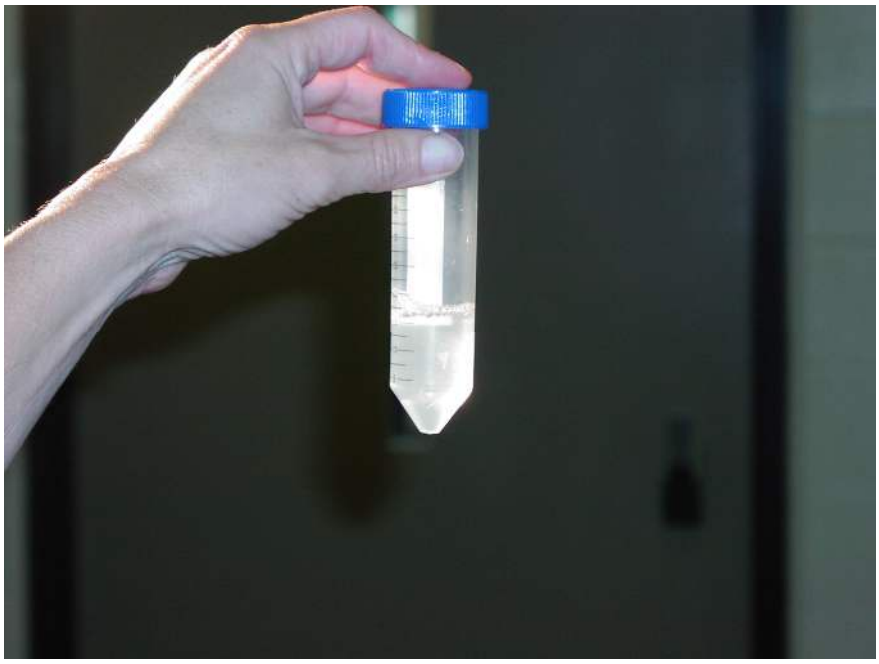

Fig 5: Cloudy uterine efflux obtained using low volume uterine lavage in a mare. (Photo courtesy of Dr. Michelle LeBlanc (deceased)

When possible, uterine fluid is processed using centrifugation for 10 min at 400 x g. Determining g force on a standard bench top centrifuge can be done with a nomograph and is well described by Vanderwall.[23] If centrifugation is not possible, the fluid contents should stand for at least one hour so that cellular material can settle in the bottom of the tubes or bag. After processing, fluid is decanted (if centrifuged) or aspirated (if allowed to settle) leaving 2-5 ml of supernatant and the pelleted cells. Two sterile cotton swabs are used to obtain samples from the pellet. One sample is placed in transport media for bacterial culture and the second sample is rolled directly on to slides for cytological evaluation.

An alternative method for performing uterine lavage to obtain uterine samples is to infuse 1 L of sterile fluid into the uterus (using the uterine catheter), agitate the uterus per rectum and recover the fluid back into the 1 L bag. This method also provides a closed system. The large volume of fluid makes centrifugation impractical, so fluid is allowed to stand upright in the bag for 1 hour and samples from the dependent portion aspirated off with a syringe and needle. Samples can then be processed directly or centrifuged for pellet formation. While working with a larger volume of fluid can be more unwieldy, this method offers the advantage of therapeutic uterine lavage combined with obtaining uterine samples.

Interpretation of LVL samples differs from those derived from uterine swabs/brushes. Following centrifugation, pelleted samples from LVL provide concentrated cells and debris that can aid in identification of subclinical infections. Interpretation of samples from low volume uterine lavage are best made in conjunction with additional evidence of endometritis such as abnormal intraluminal fluid accumulation (especially in dioestral or early oestral mares), exuberant uterine oedema, and repeated pregnancy losses after using good breeding management techniques. Positive indicators of subclinical uterine infection obtained from low volume uterine lavage include cloudy or mucoid fluid, isolation of known uterine pathogens and more than 0.5% PMNs identified per HPF of concentrated cells[16,17,20] (Fig 6).

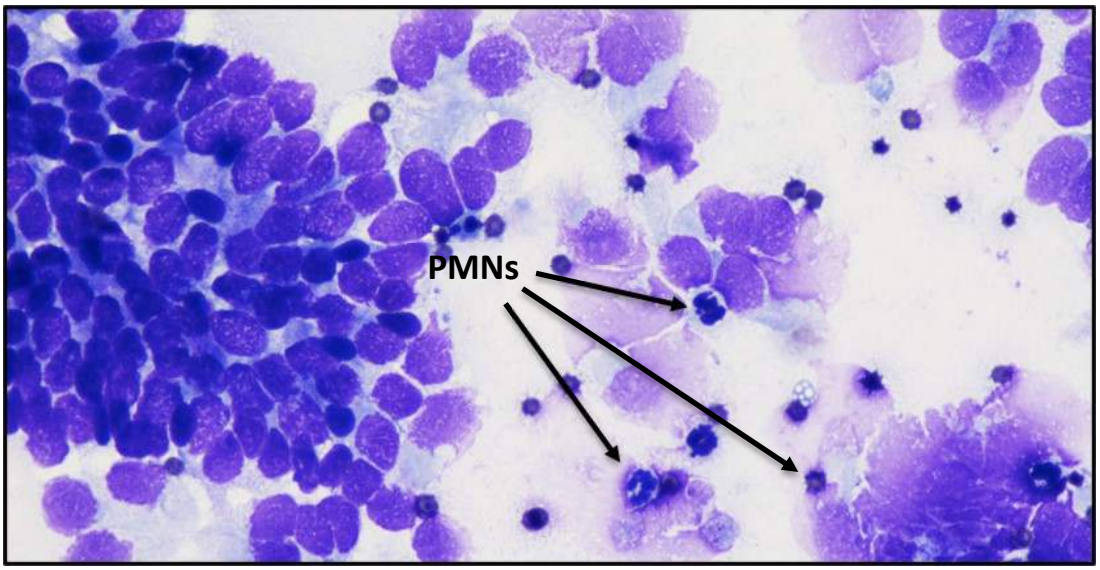

Figure 6: Mild endometritis detected by identification of PMNs and epithelial cells (20x) from uterine fluid obtained by low volume uterine lavage (Photo courtesy of Dr. Patrick McCue, Colorado State University)

Endometrial Biopsy

Uterine tissue obtained through endometrial biopsy is considered the 'gold standard' for detecting histologic reproductive pathologies in the mare's uterus, including endometritis.[24] Using a 60 cm, modified alligator forceps with a 4 mm x 28 mm jaw, a sample of endometrial tissue is taken from the uterine horn. Traditionally, the endometrial biopsy was taken at the base of one uterine horn, as this is the area that the embryo frequently fixes in early pregnancy. Based on work from Kenney, a single sample was shown to be representative of the entire uterus when considering inflammation or periglandular fibrosis.[25] More recent work showed that pathology could be different in each uterine horn, particularly in aged mares (> 17 years old).[26] It makes sense that pathologic changes would vary between uterine horns as mares age given that breeding, uterine insult and pregnancy could all cause endometrial changes. Therefore, single endometrial samples suffice when evaluating younger mares, but samples from each uterine horn should be considered when evaluating the aged mare. For histologic evaluation, the endometrial sample is submitted to a laboratory in an appropriate fixative (Bouins, Davidson's solution or 10% formalin), along with a detailed history of behavioural, ovarian, and uterine findings at the time of biopsy.

Endometrial biopsy samples can also be used for culture and cytology to specifically identify endometritis.[11] Results from several studies suggest that the incidence of false negative culture and/or cytology results are significantly reduced when sampled directly from a endometrial biopsy specimen.[11,27,28] In one study, endometrial tissue was obtained using aseptic technique through a specially designed vaginal speculum and sterile equine biopsy forceps.[11] Endometrial samples were obtained, blindly, in the uterine body. The tissue was used to inoculate a blood agar plate for culture and then smeared on a glass slide for cytologic evaluation. Samples were processed using traditional methods for microbial isolation and staining, as well as histologic evaluation after fixation. The sensitivity for bacterial growth using was almost 2.5 times greater when using an endometrial biopsy sample compared to a traditional double-guarded swab system.[11,27,28] However, subsequent studies did not demonstrate significant advantages of endometrial biopsy sample vs uterine swab/cytobrush samples for diagnosis of endometritis in mares.[4,29] Therefore, the method of choice for identifying uterine changes is best made based on the history of the mare, the suspected pathogenesis of disease and veterinarian comfort/time in performing specific techniques.

Additionally, microscopic examination of the endometrium provides the most accurate means of identifying inflammation and other pathologies in the mare's uterus. The components of the endometrial biopsy evaluated as part of the Kenney scoring system[24] include:

Inflammatory cells –types, location, and distribution of inflammatory cells in an endometrial sample are noted. Neutrophils are often indicative of acute inflammation while lymphocytes indicate chronic changes. The presence of a considerable number of plasma cells is a poor prognostic sign, as it indicates long-standing persistence of antigenic stimulus such as bacteria. The presence of eosinophils indicates uterine irritation such as urine pooling and/or pneumovagina. Hemosiderin-laden macrophages are often present for many months in postpartum mares or after other haemorrhagic events (i.e. uterine biopsy). While inflammation detected in endometrial tissue is pathologic, most inflammatory changes resolve with treatment. Resolution of endometrial inflammation can improve fertility.

Fibrosis – uterine fibrosis is connective tissue formation that is a normal degenerative part of aging. However, uterine fibrosis can also be incurred after insult to the uterus, and it can affect fertility. Importantly, uterine fibrosis affects glandular function, thus impacting support of the early embryo. Fibrosis can be widespread or focal and it is a permanent, untreatable change. Therefore, a mare with significant endometrial fibrosis has a much lower chance of carrying a foal to term thus affecting the endometrial biopsy score.

Dilated lymphatics – older, pluriparous mares tend to have large, pendulous uteri that do not provide adequate lymphatic drainage.[22] Lymphatic drainage is one mechanism contributing to uterine evacuation after breeding.[30] Dilated lymphatics in the endometrium suggest lymphatic dysfunction. Lymphatic dilation is often paired with clinical findings such as uterine cysts and/or poor uterine tone. Additionally, mares with lymphatic dysfunction have a propensity for pathologic uterine oedema, uterine fluid accumulation, and post-breeding endometritis.

Cystic glandular distention – cystic distention of uterine glands, when it is not associated with periglandular fibrosis, may indicate glandular stasis due to uterine dysfunction. The cause of cystic glandular distention is not known. Chronic glandular distention resulting in glandular epithelial atrophy renders the glands useless, thus affecting their ability to provide nutritional support to the developing embryo.

Vascular elastosis – degenerative lesions develop in vessel (arterial) walls in multiparous mares.[31,32] Arteries in both the myometrium and the endometrium undergo degeneration, and this condition has been statistically correlated to parity.[32] Specifically, elastic fibres within vessel walls undergo fragmentation and have reduced uterine perfusion.[31] It is hypothesised that compromised uterine blood flow impacts endometrial development, uterine clearance, endometritis, and general fertility.

When evaluating endometrial biopsy samples, the combined pathologies are used to categorise a mare's uterine health and provide prognostic information for the mare's ability to carry a pregnancy to term. Using the Kenney scoring system, the endometrium is categorised as Category I (> 80% chance of carrying a pregnancy to term), Category IIA (50 – 80% chance of carrying a pregnancy to term), Category IIB (10 – 50% chance of carrying a pregnancy to term) and Category III (< 10% chance of carrying a pregnancy to term).[24,25] Ideally, a trained theriogenologist evaluates an endometrial biopsy within clinical context so that treatment plans and prognostic information can be formulated. It is important to note that endometrial biopsy scores are not usually "fixed," and the Category score assigned is indicative of the uterine health on the day that the biopsy was obtained. Additionally endometrial architecture can be affected by season, transient insult, and treatment.

Treatment for the problem mare

Treatment strategies for problem mares are built on the premise of correcting inciting anatomic causes, cleaning the uterine environment and treating pathogens, when present. In many cases, anatomic abnormalities are the source of genital tract inflammation and/or contamination. Mares with poor perineal conformation, and/or compromise to the vulvar, vestibular, and cervical barriers often benefit from surgical correction of defects. A Caslick's suture is a simple remedy to most anatomic defects.[3] Less frequently, mares may require perineal reconstruction or cervical repair.

Antimicrobial treatment

Antimicrobial treatment can be a vital component for treating infectious endometritis. However, administration of antimicrobials to treat mares with endometritis should be based on supporting uterine culture and cytology information.[22] Given the changing landscape of microbial resistance to many medications, as well as differing regulations between countries for antimicrobial use, the therapies discussed here will focus on non-antimicrobial/non-traditional therapies. Many agents have mechanistic actions that can address functional deficiencies in uterine response to contamination, and some have a direct effect on microbes. Current therapies for uterine treatment focus on evacuation of uterine contaminants, altering the uterine environment through disruption of biofilm or mucus layers, managing inflammation and providing immunological support.

Ecbolic agents

Promoting uterine evacuation through the administration of ecbolic agents (oxytocin, prostaglandin) is important for treatment of the problem mare. Affected mares frequently experience poor evacuation of uterine contents after contamination. Oxytocin is a potent ecbolic agent that promotes strong, but short-lived, uterine contractions.[33,34] Oxytocin can be administered through an intravenous (IV) route or intramuscular (IM) route, and it is often combined with uterine lavage. Oxytocin therapy for uterine evacuation is not recipe-driven. Anecdotally, oxytocin is administered at 4-, 6- and 8-hour intervals depending on the time of breeding, presence/quantity of intrauterine fluid and access to the mare. Oxytocin may be administered to mares prior to, and after, breeding. However, one must be mindful of oxytocin's effect on the uterus, oviduct, and corpus luteum when determining a "safe" time to administer oxytocin in the peri-ovulatory period.

Mares with significant compromise to uterine contractility due to poor muscle tone or lymphatic drainage abnormalities require the prolonged contractile action that prostaglandin provides.[35-37] Both native prostaglandin (i.e. dinoprost tromethamine) and cloprostenol, a prostaglandin analogue, promote uterine contractility.[36] Cloprostenol is considered advantageous because it induces low-amplitude uterine contractions that last for 4-5 hours.[36] It is important to note that cloprostenol must be administered in the immediate peri-ovulatory period (either before or within 12 hours after ovulation) to avoid reductions in progesterone concentrations or effects on pregnancy rates.[38-40]

Uterine lavage

Uterine lavage is an important first-line tool for uterine treatment. Not only is lavage useful for "cleansing" the uterus of contaminants, but lavage also stimulates uterine contractions and influx of healthy PMNs. Uterine lavage is traditionally performed using crystalloid solutions (i.e. lactated Ringer's solution/LRS) or 0.9% saline (NaCl), both of which are isotonic solutions. Approaches to uterine lavage vary, with some veterinarians using one litre of solution, while others repeat one-litre lavages over several litres. Most veterinarians agree that the total volume of fluid used for uterine lavage is less important than the final effluent having little cellularity. Uterine lavage can be performed, safely, immediately prior to breeding when intraluminal uterine fluid is detected in the pre-breeding period.[41] Pre-breeding intrauterine fluid is most commonly identified prior to insemination of a second dose of frozen-thawed semen. In the study by Vanderwall, et al LRS was specifically chosen for pre-breeding uterine lavage of mares due to its similarity in pH and osmolarity with most semen extenders.[41] In the peri-breeding period, uterine lavage is more commonly used post-insemination. This tool can

be performed as early as 4 to 8 hours after breeding when the majority of sperm have entered the oviduct.[42] Uterine lavage early in the post-insemination period is especially important for mares with a history of post-mating induced endometritis or chronic subfertility because they lack the intrinsic mechanisms to clear the uterus of contaminants after mating.[43] However, uterine lavage 4 to 8 hours after breeding is not always convenient, and for most mares it is reasonable to wait until the following day(s) to perform uterine lavage. Administration of oxytocin is often combined at the end of uterine lavage (20 IU, IV or IM) to promote full evacuation of uterine contents.

Mucolytics/biofilm disruptors

Uterine therapy using mucolytic agents and solvents targets alterations in the uterine environments such as production of excessive mucus, biofilm, and/or exuberant exudate after breeding. Excess mucus production has been identified in mares with chronic endometritis and has been specifically associated with micro-organisms (*E. coli* and *β haemolytic streptococcus*).[16,18] While the uterine mucociliary apparatus is fundamental to evacuation of contaminants, excessive mucus impairs uterine function and sperm motility.[18] N-acetylcysteine (NAC) is a product that has been used as an intrauterine treatment to promote breakdown of mucus exudate in mares with chronic endometritis.[44] NAC, known as the "mucus dissolver", is widely used in human cough preparations due to its ability to break disulfide bonds in mucus.[45] Additional documented benefits of NAC include anti-inflammatory and antimicrobial properties.[45] As a uterine therapy, 30 mL of 20% NAC solution is added to 150 mL sterile saline to produce a 3.3% solution. The solution is infused into the mare's uterus. Uterine lavage is performed the following day. If mucus or excessive exudate is evident in the uterine lavage effluent, NAC is infused into the mare's uterus again followed by lavage 24h later. The effects of NAC were tested in a population of barren mares to evaluate both positive and negative properties of this product.[44] Evaluation of endometrial biopsies after treatment showed no negative effects on the endometrium of mares after uterine infusion with NAC. NAC treatment reduced endometrial mucus in some mares. A more recent study did not detect anti-inflammatory effects of NAC after intrauterine infusion and, instead, noted an increase of inflammatory cells in the endometrium.[46] Conflicting results were attributed to differences in study designs.[46] Important information examining the effect of NAC and/or dexamethasone at the time of breeding on fertility revealed interesting results.[47] In a population of 40 susceptible mares, treatment with NAC (infusion of 3.3% NAC solution) 6h before breeding combined with administration of dexamethasone (0.1 mg/kg IM) at the time of breeding resulted in lower endometrial oedema scores and higher pregnancy rates than either treatment, alone, or untreated control mares. These interesting data underscore the importance of controlling inflammation in mares with endometritis, however, the effect of NAC treatment, alone, requires further investigation.

Dimethyl sulfoxide (DMSO) has also been investigated for treating mares with endometritis. DMSO is a solvent that can control inflammation in some conditions. Uterine lavage using DMSO has been investigated for its anti-inflammatory effects, ability to reduce collagen formation in the equine endometrium and effect on biofilm.[48-52] Inflammation was reduced in some mares after uterine treatment with DMSO, while endometrial fibrosis was not changed.[48,50] Intrauterine administration of DMSO caused desquamation of the endometrial epithelium that regenerated within 21 days of treatment.[50] Pregnancy rates tended to improve in one population of barren mares.[48] Of interest, 30% vol/vol DMSO effectively disrupts biofilm produced by *E. Coli* and *K. pneumoniae* and inhibits bacterial growth, *in vitro.*[52,53] A similar

effect was not seen when *P. aeruginosa* were exposed to DMSO. The take-home message is that DMSO at a 30% vol/vol concentration may be anti-inflammatory and effective at reducing biofilm production and bacterial growth of some uterine pathogens.

Hydrogen peroxide (H_2O_2) has been advocated as a non-antimicrobial disinfectant agent for treating mares with endometritis.[52-54] H_2O_2 is an oxidizing compound that targets microorganisms through several mechanisms including release of high levels of reactive oxygen species (ROS), peroxidation, disruption of membrane layers, enzyme inhibition and many others.[55] An attractive property, H_2O_2 decomposes to hydrogen and oxygen by catalase resulting in inert compounds.[55] Importantly, H_2O_2 is effective as both an antimicrobial agent as well as a biofilm disruptor when infused into the uterus at a concentration between 1-3%. Ferris and co-workers demonstrated that biofilm biomass and/or bacterial colony forming units (CFUs) were significantly reduced when exposed to 1% H_2O_2.[53] Specifically, biofilm biomass and CFUs were reduced for *E. coli*, while only CFUs were reduced for *K. pneumoniae.* H_2O_2 had little effect on *P. aeruginosa* biofilm production or colony growth.[53] Two additional studies have further validated the use of H_2O_2 for antimicrobial effects in the uterus.[54,56] Using H_2O_2 at a concentration of 0.047%, *in vitro* bacteriostatic and bactericidal activities were noted against *E. coli*, *K. pneumoniae* and *P. aeruginosa* as well as other bacteria.[54] Similarly, Mazzuchini et al.[56], showed a 100% inhibitory effect of 3% H_2O_2 when exposed to *E. coli, P.aeruginosa, K. pneumoniae, S. aureus* and *Candida albicans*, *in vitro.* Therefore, H_2O_2 is a reasonable option for initiating a non-antimicrobial treatment while waiting for culture results in a mare with existing clinical signs of endometritis (intrauterine fluid, positive uterine cytology, vulvar exudate),

Buffered chelators

Buffered chelating agents have been advocated for targeting resistant bacteria, particularly those that produce biofilm. *P. aeruginosa, E. coli, Staphylococcus epidermis* and fungal and yeast species, have been identified as potent biofilm producers.[22,53,57,58] Buffered chelators have been examined for treating persistent uterine infections in both cattle and mares.[59-61] Early studies showed that first generation buffered chelators (ethylenediaminetetraacetic acid-2-amino-hydorxylmethyl-propane-1,3-diol; Tris-EDTA) used in the uterus of mares had no negative effects on the endometrium.[59,60] *In vitro*, Tris-EDTA reduced the MIC of a *P. aeruginosa* isolate recovered from a mare with endometritis. These studies showed that intrauterine application of Tris-EDTA was safe and effective against a problematic reproductive pathogen in mares. Additional studies in cattle showed that Tris-EDTA combined with an antibiotic was more effective at treating bacterial endometritis than antibiotics, alone.[61] Additionally, a third generation buffered chelator (disodium ethylenediameinetetraacetate dehydrate-2-amino-2-hydroxymethyl-1,3-propanediol; Tricide™, Molecular Therapeutics, LLC, Athens, GA) has shown to potentiate the effect of antifungal agents when applied to equine keratitis isolates, in vitro.[62] Ferris and co-workers examined the effect of Tris-EDTA (and other agents) on biofilm disruption as well as inhibition of bacterial growth in equine uterine isolates.[53] Tris EDTA had inconsistent results in biofilm disruption but showed good efficacy for inhibiting growth of *E. coli*. Based on this work, Tris EDTA, in combination with a known sensitive antimicrobial, is desirable for mares with known *E. coli* uterine infections.[53] Most often, 30 mL of Tris EDTA is mixed with an aminoglycoside or ceftiofur and used as an uterine infusion for at least 3 days.

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Ozone therapy

Ozone is a highly reactive gas comprised of three oxygen atoms that form a circular structure, often with exposure to ultraviolet light.[63] Ozone has gained popularity in equine medicine due to its proposed antimicrobial and anti-inflammatory effects.[63,64] Ozone has been investigated as an alternative, non-antimicrobial treatment for mares with endometritis.[64,65] When known reproductive pathogens were exposed to ozone (*in vitro*) as a gas, in distilled water or in oil, ozone gas and ozone in oil significantly reduced the bacterial load.[63] In contrast, ozonated distilled water had minimal effect on bacterial load. The same group evaluated the safety and efficacy of ozone foam infused into the uterus of mares with known subfertility. [64] Treated mares received an intrauterine infusion of commercially available ozone foam (Rigerâ Spray, Ozone Vet Services, United Kingdom) via sterile insemination pipette while control mares were administered an intrauterine infusion of lactated Ringer's solution (LRS). Uterine samples for cytology, bacterial culture and histologic evaluation were taken at varying time points (T0 = baseline; T1= 24 h after treatment, T2 = 1 week after treatment; T3 = 2 weeks after treatment; T4 = 27 days after treatment). Inflammation was initially greater in the ozone treated group but resolved by one week after treatment.[64] Uterine culture results and endometrial histology were not different between groups. Mares in the ozone-treated group tended (P = 0.07) to require fewer inseminations (1.69 ± 0.06) to achieve pregnancy than control treated mares (2.60 ± 0.89). Ozone, itself, appears to be effective in treating mares with endometritis, however, the vehicle for ozone delivery remains up for debate. Ozonated oil and gas consistently demonstrated good antimicrobial activity against reproductive pathogens while ozone in saline or distilled water has little effect against pathogens.[52,63,65,66]

Immunomodulators

A significant component of endometritis is inflammation. Influx of inflammatory cells into the uterus is necessary for removal of harmful byproducts of breeding. In some mares, inflammation persists and becomes pathologic. While clearance of uterine contents is a principal component of treating this "susceptible" population of mares, controlling aberrant inflammation is also important. In recent years, immunomodulatory agents such as steroids (dexamethasone/prednisolone), *Mycobacterium phlei* cell wall extracts (MCWE; Settle®, Bioniche Animal Health, Bogard, GA) and Propionibacterium acnes (EqStim®, Neogen Corp, Lexington, KY) have been tested for immunomodulatory effects in mares with persistent inflammation.[22,53,68-69] Studies have been highly variable in design, so the effect of these treatments is difficult to discern. Administration of dexamethasone at the time of breeding reduced intrauterine inflammation and improved pregnancy rates in a population of mares.[67] Additionally, a study examining the effect of both glucocorticoids and MCWE on endometrial gene expression of proinflammatory and anti-inflammatory cytokines in susceptible mares showed a positive effect of glucocorticoids for modulating the inflammatory response after induced infection.[68] Glucocorticoid administration decreased proinflammatory cytokines and increased anti-inflammatory cytokines after uterine infusion with *E. coli.* Both glucocorticoid and MCWE administration resulted in lower fluid retention and better clearance of pathogens after uterine infusion of *E. coli*. Evidence-based results from this study support a positive effect of immunomodulators for improving fertility in previously barren mares when administered at the time of breeding.

In addition to glucocorticoid administration to decrease post-breeding uterine inflammation, non-steroidal anti-inflammatory agents have been investigated. The conundrum with using non-steroidal anti-inflammatory agents for treating mares with endometritis is the potent inhibition

of prostaglandin production, via inhibition of cyclooxygenase, that ensues.[35] Prostaglandin is important for myometrial contractility which aids in uterine evacuation after mating.[70] When mares were administered phenylbutazone, a non-selective cyclooxygenase inhibitor (COX-1 and COX-2 isoenzymes), uterine contractility was impaired.[37] When flunixin meglumine, also a non-selective COX isoenzyme inhibitor, was administered to mares 2 hours after breeding, PMN counts in uterine fluid obtained at 8 hours increased, suggesting an impairment in uterine contractility.[71] Firocoxib, a COX-2 isoenzyme selective inhibitor, resulted in lower intrauterine PMNs when administered to susceptible mares in the peri-ovulatory period (from the time of ovulation induction through one day after artificial insemination) than untreated, control mares.[72] Ovulation was not inhibited after administration of firocoxib, but fertility data were not obtained in the trial. This limited information provides evidence that firocoxib can be used in the periovulatory period (e.g. for musculoskeletal reasons) without interfering with ovulation, and administration of the drug may reduce endometrial inflammation.[72]

Regenerative therapies

Regenerative therapies, including plasma products [whole plasma, platelet rich plasma (PRP), platelet poor plasma (PPP)], autologous conditioned serum (ACS), and mesenchymal stem cells (MSCs) have been investigated as treatments for endometritis in the mare. Early work by Asbury et al suggested that infusion of autologous plasma into the uterus of susceptible mares would enhance the uterine environment through the addition of complement, IgG and proteins.[73,74] While not examined as a sole treatment, a large-scale study showed that pregnancy rates were improved in mares administered intrauterine plasma combined with antibiotics vs mares administered intrauterine antibiotics, alone, or no treatment.[75] These data inspired the use of intrauterine plasma to treat mares with endometritis. However, the routine practice of intrauterine plasma infusion was not widely accepted due to the laborious methods for collection and storage of plasma. More recent attention has been paid to using PRP for treating mares with endometritis. PRP contributes a rich environment of growth factors and anti-inflammatory mediators having an immunomodulatory effect on the uterine environment.[76] Traditional blood processing to obtain PRP requires specialised equipment (RestigenPRPâ, Zoetis, Parsippany, NJ, USA). Interesting work by Segabinazzi and co-workers used readily available sodium citrate blood tubes to collect PRP for intrauterine infusion either 4 h prior to breeding or 24 h after breeding in known susceptible mares.[77,78] PRP-treated mares were compared to untreated control mares. PRP infusion either before, or after breeding, resulted in lower intrauterine PMN counts and lower endometrial COX-2 labelling. Importantly, pregnancy rates were higher in PRP-treated mares than untreated, control mares.[77] These results suggested an anti-inflammatory effect of PRP in the uterus independent of time of infusion. Subsequent work from the same group examined PRP and PPP intrauterine infusions compared to control treatment (LRS infusion) starting 2 and 1 days prior to breeding and 6 and 24 h after breeding.[78] Twelve susceptible mares were used in the study over three randomly assigned treatment cycles. A large volume (450 mL) of blood was collected into a blood transfusion bag (Jorgensen Labs, Loveland, CO, USA), to increase volumes of PPP and PRP. Treatment with both plasma products (PRP and PPP) resulted in reduced endometrial PMNs, intrauterine fluid and pro-inflammatory cytokines.[78] Further, bacterial inhibition was greater in PRP-treatment cycles, but bacterial inhibition in PPP-treatment cycles was not different from untreated, control cycles or PRP-treatment cycles. Embryo recovery rates were higher after PRP-treatments (83%) than control treatments (30%) but not significantly different after PPP-treatments (60%). These results showed a strong positive effect of PRP treatment after intrauterine infusion in susceptible mares. An interesting finding in the study was the positive

impact of plasma products (PRP and PPP) in general, suggesting that use of whole plasma may have utility for treating mares with endometritis when processing to produce PRP is not readily available. This point is further validated in a study by Mazzuchini et al where both PRP and PPP treatment significantly inhibited bacterial growth, *in vitro*.[79] An important factor to consider when using platelet products to enhance immunomodulation in the uterus is that all platelet processing methods do not deliver the same end products.[80] Careful consideration should be given to methods for processing plasma that have not been validated in horses and/or that do not deliver the same number of platelets, or other characteristics important to immune function.

Mesenchymal stem cells (MSCs) also provide valuable immunomodulatory and antimicrobial effects in various body systems.[76] (Reviewed) MSCs are derived from regenerative tissues such as bone marrow, foetal tissues and adipose tissues.[81] Problematic to the use of MSCs in equine practice is the need to harvest and culture the cells prior to application. Studies using allogenic MSCs, autologous conditioned serum (ACS), Wharton's jelly (from the umbilicus) conditioned medium and PRP for treating endometritis have been performed both in vivo and in vitro.[82,83] MSCs and ACS significantly reduced PMNs in the uterine lumen 6 h after spermatozoa challenge and MSCs stimulated production of anti-inflammatory cytokine IL-1Ra.[82] In a separate study, Wharton's jelly conditioned medium and ACS both demonstrated immunomodulatory properties, in vitro, while Wharton's jelly conditioned medium also showed antimicrobial properties.[83] The next step for regenerative therapies is formulating means of producing these useful substances, easily and efficiently, for use in private practice. To date, the use of allogenic stem cells vs autologous stem cells for intrauterine treatments had not been well established. While allogenic MSCs would provide a more efficient treatment option in mares with endometritis, it is not clear what the immune response of the treated animal will be to allogenic MSCs, particularly after repeated administrations.[84,85]

Conclusions

Endometritis is significantly more complex than originally thought, thus challenging dogma regarding causative factors, which in turn, affects treatment of the condition. While the "Big Four" equine uterine pathogens, *Streptococcus equi zooepidemicus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* are still responsible for infectious endometritis, we now know that there is a plethora of uterine inhabitants that comprise the uterine microbiome. A better understanding of the uterine environment will lead to more targeted use of antimicrobials, when needed. Additionally, decades of work have revealed that infection is not the sole cause of endometritis. Therefore, present, and future, approaches to managing mares with endometritis will rely heavily on accurate diagnosis of the cause of endometritis and effective treatments for the problem. The cellular and molecular tools available today will both improve diagnostic sensitivity but also cloud interpretation of test results. Now, more than ever, it will be important to incorporate obvious, clinical signs alongside sensitive technologies to arrive at an accurate diagnosis of endometritis so that appropriate treatment can be delivered.

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Oral and oroscopic examination for equine dental disease

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Thorough oral examination is fundamental to accurate diagnosis and effective treatment planning in equine dentistry. Oroscopic examination extends the clinician's ability to visualise structures that are otherwise inaccessible via direct examination, significantly improving detection of dental and periodontal pathology. The two techniques are best viewed as complementary, with oroscopy building on and enhancing findings made during the clinical oral exam. This summary describes a structured protocol for complete oral and oroscopic examination, based on clinical practice and supported by current literature.

Before commencing with oroscopy, a thorough history, general physical exam and systematic oral evaluation should be performed. The examination should be conducted in a suitable, enclosed location to avoid glare and poor contrast associated with outdoor lighting. Sedation is considered essential for a complete examination; an α2-agonist and opioid combination is typically used. Prior to sedation, it may be useful to observe the horse eating a range of feedstuffs, including biting a carrot—many horses with equine odontoclastic tooth resorption and hypercementosis (EOTRH) are unable to do so (although this test lacks formal validation, it may raise clinical suspicion of disease).
Oral examination begins with recording the signalment, history and any previous dental interventions using a standardised dental chart. A general physical examination is followed by an external examination of the head, including assessment for asymmetry, nasal discharge or odour, bony swellings or depressions, which may indicate dental apical pathology, sinus disease, previous sinus pathology or surgery. Manual palpation of the mandible, interdental space and lips is also performed.

The incisors must be examined prior to placement of the mouth speculum. In addition to assessing tooth number, eruption status and pathology, approximate age estimation may be made. The lateral excursion to molar contact (LMC) test is used to assess masticatory symmetry and identify potential pain responses.

Initial intraoral examination is performed using a bright head torch, full-mouth speculum, large-volume mouthwash, and manual tongue retraction. The initial assessment gives an overview of the dental and oral status, including soft tissue trauma or other abnormalities, any signs of impacted food, obvious pathology (shear mouth, smooth mouth, large overgrowths, fractures etc), asymmetries and any clues that there may be uneven mastication patterns. Any deviation from the expected or anticipated norm should be recorded and further evaluated to seek a cause. This initial examination will always be limited due to the configuration of the equine oral cavity—particularly the limited mouth opening and the rostral positioning of the lip commissures which prevents full visualisation from a rostral approach. Access to the buccal and lingual aspects of the cheek teeth, full assessment of occlusal surfaces in particular, is limited. To overcome this, the use of a dental mirror or oral endoscope (oroscope) is required.

The following structured protocol is recommended for oral examination:

- Record signalment, history, previous dental care
- General physical and brief clinical exam (cardiac auscultation, etc.)
- Observe mastication if applicable (e.g. referral for dysmastication)
- Extraoral and nasal examination (asymmetry, smell, discharges)

- Incisor and canine examination (including LMC test)
- Visualisation of cheek teeth via buccal retraction
- Sedation and placement of mouth speculum
- Large-volume oral rinse (3–4 syringes; catch fluid in a bucket)
- Manual palpation of soft tissues, lips, bars of mouth
- Direct intraoral inspection
- Oroscopic examination
- Closed mouth oroscopic assessment
- Recording of findings (e.g. video, photographs, dental charting)

Oroscopic examination generally requires a slightly deeper plane of sedation than basic oral examination, primarily to ensure tongue relaxation and reduce chewing or evasive behaviours. Equipment choices vary. For routine everyday use wireless high-definition digital oroscopes (e.g. WDE Pro) are increasingly preferred due to their portability, integrated LED light source, and image quality suitable for both routine and referral-level diagnostics. Other systems include USB-wired digital cameras connected to laptops, or optical telescopes (e.g. glass rod systems) fitted with digital cameras, although these are more fragile and less practical for ambulatory work. Modern portable systems negate the need for separate light sources, converters, and monitors. Images may be viewed live and recorded simultaneously. For ultimate image quality and close-focus possibility, clinic based full HD systems based on glass rod telescope systems with large chip camera attachments, imaging linked to wall monitors still provide superior imaging for fine control specialist work and should be considered for anyone considering establishing or working in a specialist or specialist-led referral clinic environment.

Oroscopic examination technique requires practice but should follow a consistent sequence for reliability and completeness. Operators should remember that oroscopes are fragile clinical instruments and should be handled with care, especially within the oral environment. As a general working rule, it is better to partially protect the lens digitally with one hand, also keeping the working tip well away from risk of direct dental or instrument contact.

The examination should begin with the maxillary arcade, typically at 106, and proceed caudally to 111. The same process is repeated for the contralateral side (206–211). For mandibular arcades, the camera or image is rotated 180° to maintain spatial orientation: 306–311 and 406–411. With digital systems, the software may include a ‘rotate’ or ‘flip’ function; for telescope–camera systems, the telescope may be rotated independently to achieve the same result.

Each tooth is examined methodically on its occlusal, buccal and palatal (or lingual) surfaces, with visual probing of any defects using an occlusal explorer. Periodontal pockets may be gently probed and flushed with a water pick for clearer visualisation. Each finding is recorded before progressing.

The oroscopic examination should further assess:
- **Oral soft tissues** – lips, palate, mucosa, interdental space
- **Incisors and canine teeth**
- **Wolf teeth** – erupted or unerupted
- **Cheek teeth** – occlusal, buccal, palatal/lingual surfaces

As well as recording all general pathologies, each tooth should be assessed individually, specifically recording:

Endodontic status

- Secondary dentine defects
- Pulp exposure or necrosis
- Gingival recession
- Discharging gingival tracts / parulis
- Dentine fissures, pulp stones or other reparative dentine changes
- Discolouration of dentine, often adjacent to infundibular caries

Infundibular caries

Using the modified Honma classification (Karma et al., 2025):

- Grade 1: Caries of cementum only (defect >2 mm)
    - 1.1: <50% cemental loss
    - 1.2: >50% cemental loss
- Grade 2: Cementum + enamel
    - 2.1: <50% cemental loss
    - 2.2: >50% cemental loss
- Grade 3: Cementum + enamel + dentine
- Grade 4: Coalescence of mesial and distal infundibula
- Grade 5: Tooth fracture, endodontic involvement, or apical infection

Periodontal status

- Classification of diastemata (buccal, lingual, full interproximal)
- Periodontal pocket depth assessment
- Periodontal grading:
    - Grade 1: Mild inflammation
    - Grade 2: Moderate attachment loss
    - Grade 3: Advanced pocketing and food trapping
    - Grade 4: Severe destruction, gingival recession
    - Grade 5: Involvement of apical pulp ("perio-endo" disease)
- Gingival pathology: recession, hyperplasia, draining tracts, proliferative lesions

Oroscopy not only facilitates the early detection of disease but also supports clinical decision-making, treatment planning, and monitoring of disease progression or resolution. The ability to digitally archive images or videos is particularly valuable for longitudinal case management and client communication.

Electrocardiographic recording (including smartphone and wearable devices)

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Introduction

Cardiac arrhythmias may be seen in all breeds and disciplines but are particularly relevant to racehorses and performance horses. The clinician is tasked with diagnosing the arrhythmia and determining the likely cause, impact on horse and rider safety and long-term impact on life expectancy. Atrial fibrillation is the most common performance limiting arrhythmia in horses and frequently occurs in the absence of detectable structural cardiac abnormalities. The ECG in the horse gives important information about heart rhythm and P, QRS, and T complex morphology but is unreliable for prediction of cardiac chamber size. Many arrhythmias in horses are paroxysmal, meaning they are short-lived. Such arrhythmias are unlikely to be observed at rest but could cause poor performance or collapse during exercise. Recent technological advances have allowed for uninterrupted recording of an exercising ECG and long term holter monitoring for enhanced capture of paroxysmal arrhythmias. In addition, new devices such as the Kardia ECG, and digital stethoscopes with ECG capability (Eko) are suitable for use with a smartphone and have allowed for rapid stall side diagnosis of arrhythmias without the use of cumbersome equipment. Other technological advances include smart textiles and implantable loop recorders and a renewed interest in the 12 lead ECG and vectorcardiography. Such new developments have led to greater understanding of the frequency and importance of cardiac arrhythmias in horses.

Indications for an ECG

Resting electrocardiography

1. When an abnormal heart rhythm is heard at rest
2. In horses and foals with bradycardia or tachycardia, even when the rhythm sounds regular
3. During monitoring of surgical procedures, including general anaesthesia and standing surgery
4. During and following cardioversion of horses with atrial fibrillation with pharmacological treatment or electrocardioversion
5. In horses with a history of collapse at rest
6. Pre-purchase examination
7. Determination of heart rate variability

Exercising electrocardiography

1. In horses with a previously diagnosed exercising or post-exercise arrhythmia
2. In horses with a history of poor performance, staggering or collapse during exercise
3. In horses with persistent atrial fibrillation that wish to continue low level exercise
4. In horses with arrhythmias at rest such as second degree atrioventricular block, that are suspected to be vagally mediated
5. In horses with clinically important structural heart disease such as valvular regurgitation
6. In horses with high grade exercise induced pulmonary haemorrhage (EIPH)
7. In a horse returning to exercise following an episode of myocardial injury/ myocarditis

What are we measuring with an ECG?

The surface ECG records the magnitude and direction of electrical activity between two electrodes. A bipolar lead consists of a positive and negative electrode. Electrical activity which is overall towards the positive electrode will result in a positive deflection in the ECG. Activity towards the negative electrode results in a negative deflection. The amplitude of the deflection will be greatest when the electrodes are positioned parallel to the mean electrical axis (MEA) of the heart. The mean electrical axis of ventricular depolarisation in the horse is tilted in ventrodorsal-caudocranial direction, and from right to left in the frontal plane. The direction of atrial depolarisation is dorsoventral and craniocaudal. This typically results in a positive p wave (atrial depolarisation) deflection and a negative QRS (ventricular depolarisation) deflection in lead 2 of a surface ECG (Fig 1).

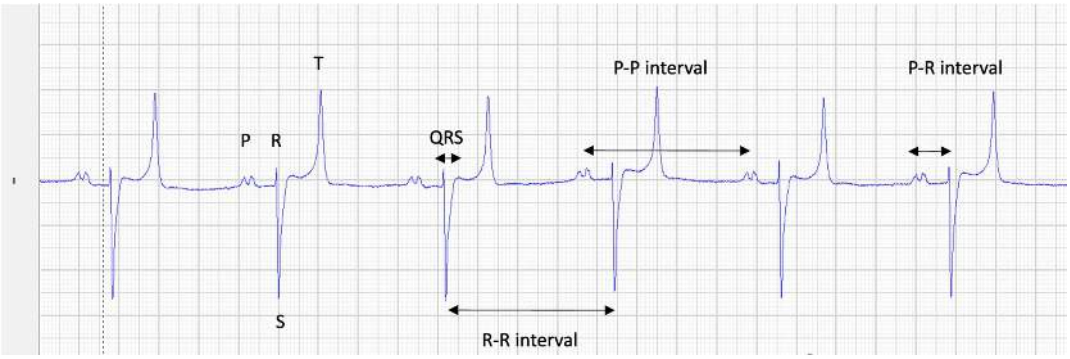

Figure 1. Resting ECG acquired in lead II with the Televet. Paper speed 25mm/sec, gain 10mm/mV. There is a normal bifid p wave with a positive deflection, a normal QRS with negative deflection and a positive T wave deflection associated with ventricular repolarisation. Normal sinus rhythm is present, based on regular R-R interval and regular P-R interval. There is a P wave for every QRS and vice versa.

Electrode positioning

Electrodes should be positioned to align as near as possible to the MEA. Table 1 details the colour of each electrode depending on whether they ascribe to the USA or European conventions. In Australia, manufacturers might use either of these so it is helpful to be familiar with both. Figure 2 demonstrates the orientation of each lead. The easiest way to remember this is the number of L's. ie. Lead I is RA to LA (one L), Lead II is RA to LL (two L's), lead III is LA to LL (three L's). The electrode names indicate the correct placement of leads for a surface ECG in a human. In humans, the MEA is from the right arm to the left leg. Lead II is the default lead, which is parallel to the MEA.

Orientation of the electrodes parallel to the MEA in horses is readily achievable at rest (Fig 3). It is important to remember that the MEA of the heart of the horse is very different to humans. Historically, electrodes have been placed on the elbows and stifles to mimic the armpit and upper thigh of humans. However, the MEA of the horse is mostly ventrodorsal. Therefore, electrodes should be placed dorsally and ventrally to optimise the ECG. The orientation of the electrodes used at rest can be modified for exercising ECG (Fig 4). Having the leads oriented under the girth allows them to be secured. Whilst P wave amplitude is not optimised by this orientation, it still provides an ECG of diagnostic quality.

Electrode	USA	Europe
Right Arm (RA)	White	Red
Left Arm (LA)	Black	Yellow
Left Leg (LL)	Red	Green
Right Leg/ Earth (RL)	Green	Black

Table 1. Colours of electrodes according to conventions in USA and Europe

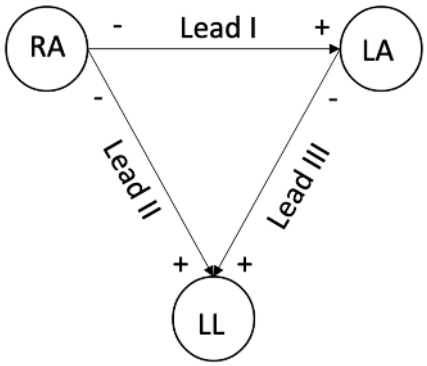

Figure 2. Diagram of alignment of ECG leads and electrodes

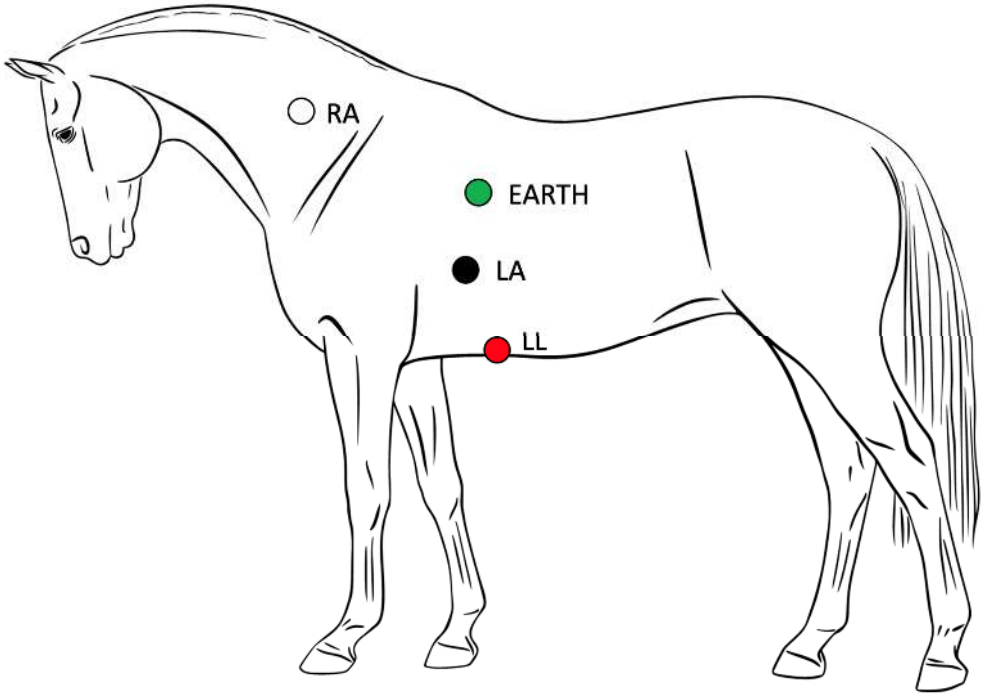

Figure 3. Placement of ECG electrodes for recording at rest. The RA electrode can be placed on the left or right side of the neck

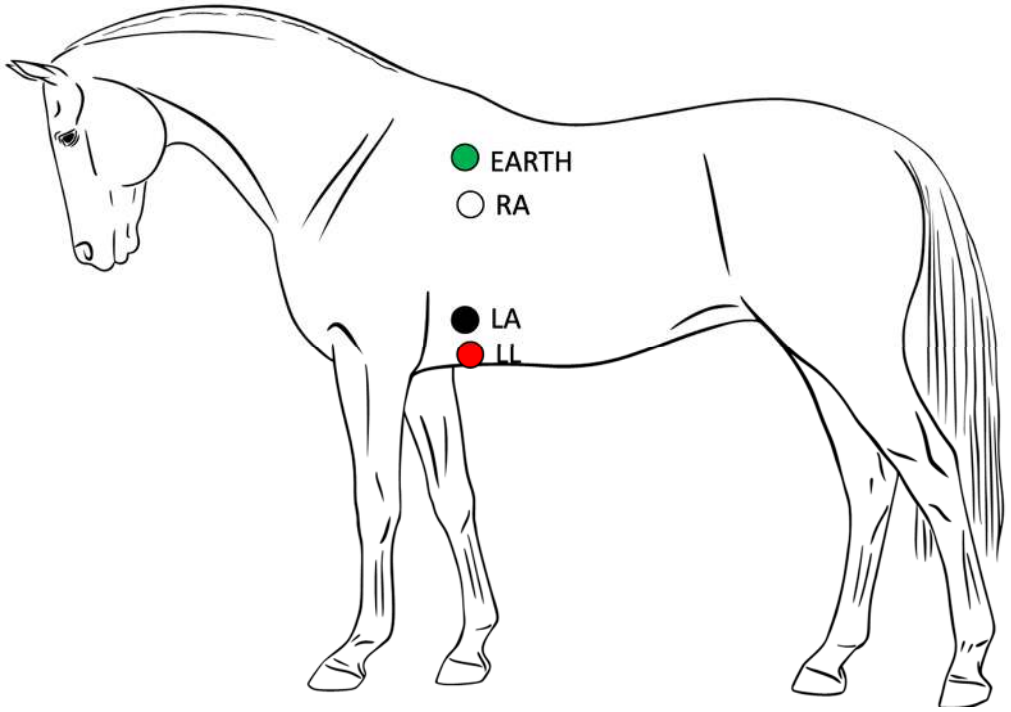

Figure 4. Placement of ECG electrodes for exercising electrocardiography. This placement is less optimal for visualisation of the P wave. The earth and RA electrode can be placed on the left or right sides of the thorax. In this orientation, leads I and II are very similar.

Recording devices

It is important that any device used has the capacity for longer term storage of the recording. Electrocardiography devices can be paper based or digital, with the major benefit of digital devices being that they can easily be stored attached to a patient's electronic file and can be readily shared.

Multiple lead devices for resting electrocardiography

Most commercial electrocardiography units designed for use in humans can be used for horses. Some units will allow for changes to the paper speed and amplitude (wave height) to optimise the appearance of the ECG. Devices with leads are most comfortable for the horse when combined with adhesive electrodes rather than using alligator clips on the skin. A better quality ECG is produced when the horse is comfortable because the cutaneous muscles are not stimulated and twitching which causes artefacts. Some adhesive electrodes are better quality than others. The author typically uses KRUUSE electrodes which are designed for veterinary use. It is not usually necessary, but contact can be improved by clipping or application of methylated spirits or ultrasound gel.

Televet https://www.televet.de (Fig 5)

The Televet ECG system was developed for use in small and large animals. It is commonly used in equine practice and confers some advantages. The televet unit can be used for telemetric monitoring. This means that the device is connected to the horse and the ECG can be stored onto an SD card within the device and streamed via Bluetooth for recording or display on a mobile phone, tablet or computer. If recording to the SD card, this card can then be removed from the device and inserted into a computer to download the ECG recording. This allows the unit to be used for long term holter monitoring, up to 24 hours,

and can also be used during exercise. The device offers 3 standard leads, and 3 augmented leads. The device has its own software which allows for visualisation of long recordings, automated detection of arrhythmias, and displays the heart rate. A simpler app is available for visualisation of the ECG on a smartphone or tablet device. ECGs are recorded in a standard format so that they can be viewed and shared outside the in differing software formats. Screenshots can be used to capture and share the ECG outside the commercial software. The device can also be used for foetal heart rate monitoring. When used with the app, the device can GPS track the speed and altitude of the workout if connected to a mobile phone service. The device can also stream remotely to allow the workout to be viewed in real time, without the need for Bluetooth connection. However, this capability can be challenging to achieve in practical terms. Exercise tests can be performed on the lunge, on the treadmill or under saddle depending on the indication for the test. When used for exercise the leads need to be carefully secured to prevent movement and dislodgement to optimise the quality of the recording. This is relatively easily achieved at speeds up to a slow gallop. In racing Thoroughbreds at speeds exceeding 15 m/s artefacts can interfere with interpretation of the ECG. The author uses a breastplate, in addition to custom made neoprene surcingle and elastic bandage (https://www.horseworks.com/collections/the-saratoga-bandage) to secure electrodes for holter monitoring and exercising ECG (Fif 5). This is preferable to super glue or stapling of electrodes.

Figure 5. Breastplate, and neoprene surcingle used to secure Televet device and electrodes for exercising and longer term resting ECG recording (Left). A saddle can be placed directly over the surcingle. In the right image, the Televet box is attached to the breastplate with the LA and LL electrodes under the girth and the RA and earth electrodes under the saddle just below the wither. Loose electrodes are more subject to artefact and detachment

Smart textiles

Smart textiles, which allow for implanted ECG electrodes in wearable garments have been validated for ECG recording in horses. They provide ECGs of equivalent quality to standard silver/ silver chloride patches. These garments are not yet commercially available. However, in future they might offer a more affordable, semi disposable option for holter monitoring at rest and during exercise.

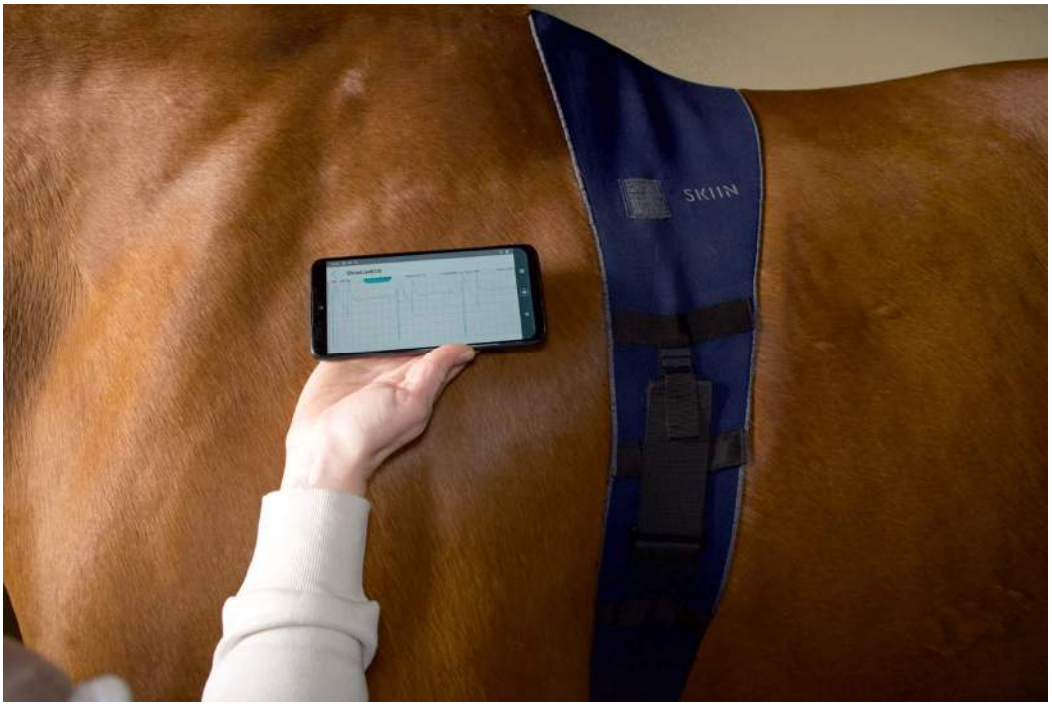

Figure 6. Example of skin device for recording of resting and exercising ECGs

Single lead devices for resting electrocardiography

Alivecor/ Kardia https://www.alivetec.com (Fig 7a)

The Alivecor veterinary device and app were originally modified for veterinary use. This app is no longer providing technical support. The Kardia device and app have been developed for humans but can be used in horses. The device allows for rapid acquisition of a single bipolar lead when applied directly to the precordium in the left or right parasternal region. The author typically applies methylated spirits and gel to the electrodes to improve contact. The device is suitable for identification and recording of resting or post-exercise arrhythmias. The diagnostic quality is adequate in trained personnel. The device has the capability through the app to generate a PDF of the recording (Fig 7b). The duration of the recording can be selected from 30 seconds to 5 minutes. The device also has an algorithm for automated detection of arrhythmias. Using this algorithm, the recording can be classified as atrial fibrillation, bradycardia, tachycardia, normal sinus rhythm, sinus rhythm with premature ventricular contractions, sinus rhythm with supraventricular ectopy and sinus rhythm with wide QRS (https://www.kardia.com/blog/advanced-determinations). These determinations have been validated for use in humans but might not be appropriate for detecting arrhythmias in horses. One study found the device was able to distinguish sinus rhythm from atrial fibrillation in horses post-exercise. The major source of error for the Kardia algorithm is oversensing, resulting in misidentification of T waves as R waves. It is important that an experienced practitioner evaluates any ECG recording to identify the cardiac rhythm. A major limitation of the device is that the electrodes are close together. This makes it more challenging to align the device with the MEA and obtain an ECG of diagnostic quality. Furthermore, because there is only a single lead, the alignment of the device to optimise the QRS visibility results in a less clear P wave. The KardiaMobile 6-lead has electrodes slightly further apart than the KardiaMobile single lead. Therefore the 6-lead device is recommended for use in horses even though it is generally used only to record a single lead. Some practitioners have attached longer leads to the device to allow it to be used with standard electrodes and the 3 lead function might then be applied. ECG 3-D

printers have also been used to create sleeves for the device which allow attachment of longer ECG leads. This could improve the diagnostic capability of this device for veterinary practitioners. An additional possible utility of the Kardia device compared to other ECG devices is that it is inexpensive ($300 AUD). It can be purchased by horse owners and trainers who are shown how to use it, meaning that a recording can be made rapidly in horses demonstrating unusual activity such as a high heart rate, ataxia, staggering, exercise intolerance. This recording can be electronically shared and reviewed by a veterinarian to determine if a cardiac arrhythmia is involved. It can also be used by horse owners to monitor horses with a history of recurrent atrial fibrillation.

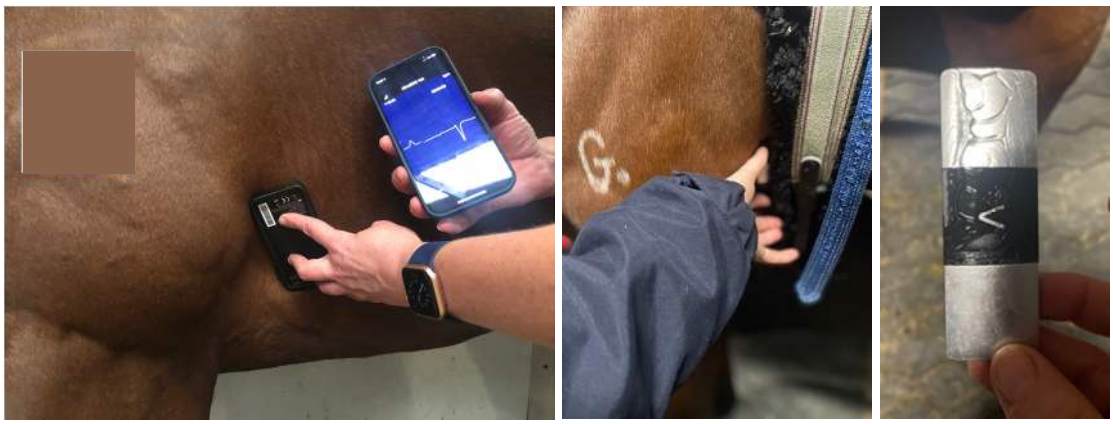

Figure 7a. Alivecor Veterinary Monitor (left) and Kardia ECG (right) applied to the left precordium in a horse

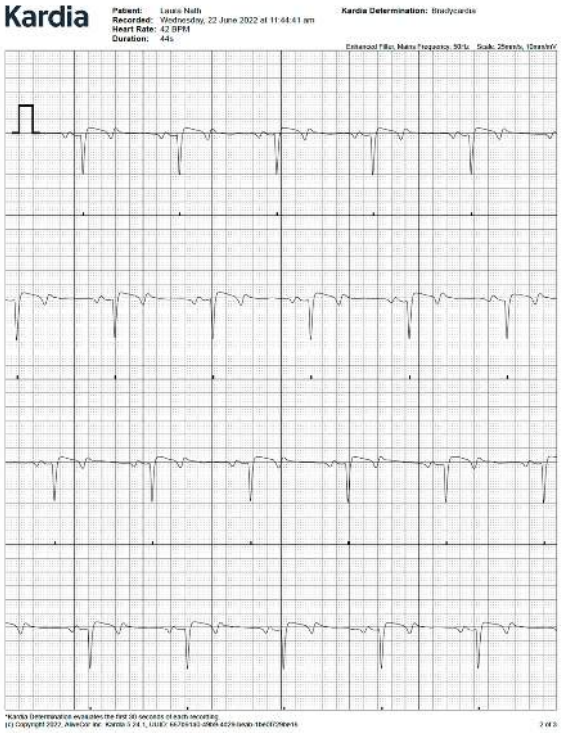

Figure 7b. Example of a PDF produced by the Kardia App demonstrating sinus rhythm in a resting horse

Eko Duo

The Eko Duo is a digital stethoscope which can be combined with a digital ECG. This allows recording of both the heart sounds (phonocardiogram) and the ECG. Similar to the Kardia, the device connects to a smartphone for real-time viewing of the ECG. The recording also uploads to the Eko dashboard. It does not produce a PDF of the entire recording in the same way as the Kardia so is less easy to share.

Figure 8. Eko Duo stethoscope with electrodes on back of device.

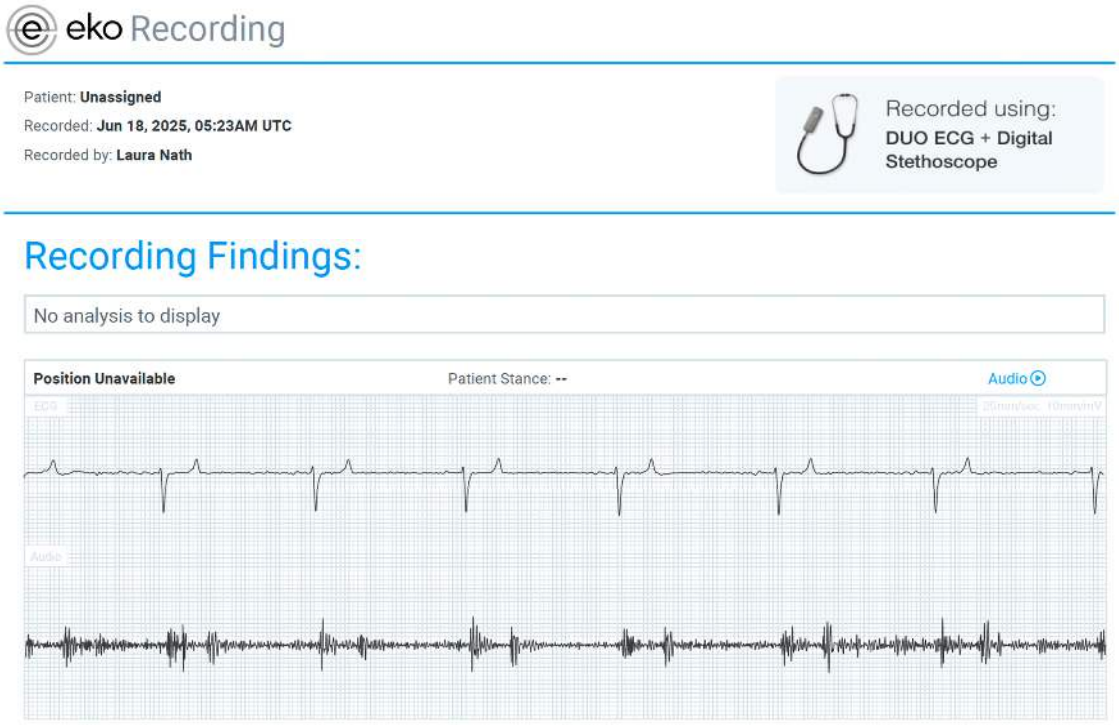

Figure 9. Example of reporting dashboard for Eko Duo

Implantable loop recorders

Implantable loop recorders have been validated for ECG recording in resting horses. [They are very small devices which sit under the skin in the precordial area (Fig 10). They can be used for long term (>12 months) monitoring of rhythm in horses with paroxysmal arrhythmias. They are comfortable and can be placed in front of the girth such that the horse can continue to be used for riding. The device autodetects an abnormal heart rhythm which it captures and stores in the memory of the device. Information on the device can be downloaded through an app and reviewed approximately every 2 weeks. It is currently used as a research tool. ECG quality is inadequate for recording during exercise. This device has future utility in monitoring paroxysmal arrhythmias and investigation of episodic collapse as it allows for long term capture of heart rhythm abnormalities. One limitation of the device is that new devices are expensive (>$5000) and require specialised equipment for transfer of information through the app.

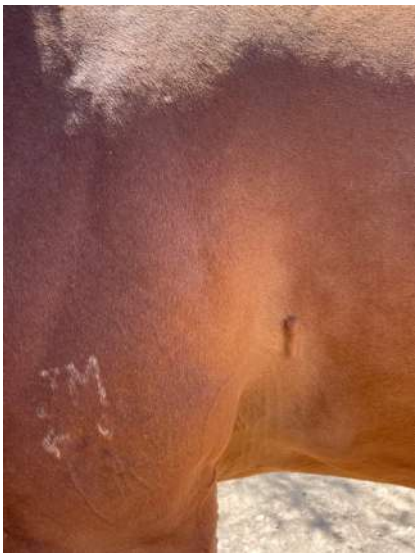

Figure 10. Reveal Linq device sitting under the skin in the left precordium of a Thoroughbred horse

Figure 11. Example of ECG and report produced by the Medtronic Reveal Linq device

Adhesive patches
Adhesive patches designed for use in humans, offer medium-term (1 week) recording of the ECG and have been validated for use in horses. These patches obtain excellent quality recording at rest but are unsuitable for recording during exercise. The patches are disposable, with each patch being used on a single horse for a single investigation. These patches are more economical than the implantable loop recorders and therefore might be more suitable for medium term recording of an ECG in horses with episodic collapse or paroxysmal arrhythmias.

12-lead ECG
In humans, a 12-lead ECG is used to detect changes in cardiac conduction associated with cardiac disease. For this procedure, electrodes are placed in a standard manner and the shape of complexes in each lead is evaluated. Abnormalities in the appearance of ECG segments in different leads can be used to identify the region and likely source of any change in conduction even when the rhythm is regular. Examples include myocardial hypertrophy, myocardial infarction and cardiac dysplasia. Recently, there has been renewed interest in developing a standard 12-lead ECG placement for horses and a proposed protocol has been published. Experimentally, abnormalities in ventricular repolarisation were identified in a horse with induced myocardial infarction. The 12-lead ECG was recently used to identify the region of origin of experimentally induced atrial premature complexes using vectorcardiography. With further research, the adoption of the 12-lead ECG technique holds promise in allowing for more precise identification of cardiac conduction abnormalities in horses.

Devices for exercising electrocardiography
The previously described Televet is one of the most commonly used devices for recording an exercising ECG and the only device to the authors knowledge that is capable of recording multiple leads during exercise.

Equimetre (https://vet.arioneo.com/en/equimetre-vet-ecg/)
The Arioneo developed Equimetre is a device that has recently been adopted as a training tool by many Thoroughbred trainers in Australia. The device is very user friendly and consists of an elastic surcingle which sits under the saddle and can be attached to the girth with Velcro so that it does not slip during exercise (Fig 12). This device has an in-built capability for recording a single lead ECG during rest and exercise (Fig 13). In addition to this, the device obtains data relating to heart rate, speed and stride length and stride frequency (Figure 14). This information appeals to trainers in that heart rate is an indicator of fitness and training load. Heart rate elevations can be an early indicator of injury, illness or cardiac arrhythmia. Changes in speed and stride can also indicate a predisposition towards musculoskeletal injury. Whilst many devices are capable of monitoring heart rate during exercise, the accuracy of the recorded heart rate can only be determined by evaluating the simultaneously recorded ECG. In many instances in which a suspiciously high or low heart rate was detected by the device, review of the ECG indicates a period of poor recording quality with artefacts. Apart from the Televet, the Equimetre is the only other commercially available device to the author's knowledge which has been validated for exercising ECG recording in horses. The device is simple to use, and owners and trainers can be taught to apply the device independent of a veterinarian. The electrodes need to be maintained and kept clean and water needs to be applied to the electrodes before placing on the horse to optimise the ECG quality. A major limitation of the device is that it does not have its own software for viewing the ECG aside from live viewing within the app. The app does not have a recording capability other than through using the in-built screen recording

of the mobile device. For viewing the entire recording, the captured ECG can be downloaded through the equimetre server as a .csv file, which can then be imported into other ECG viewing software such as Kubios (https://www.kubios.com).

Figure 12. Example of the Equimetre placed under the saddle and attached to the girth. A commercial saddle blanket is available to store the box which records the digital information obtained through the recording

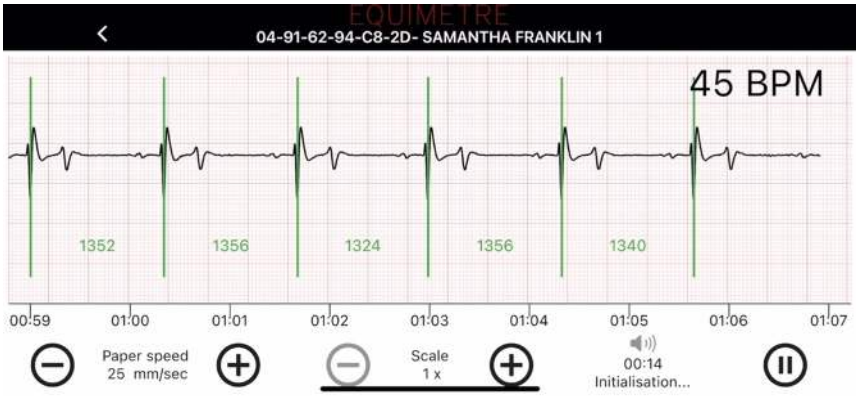

Figure 13. Example of Equimetre ECG obtained at rest through the device app

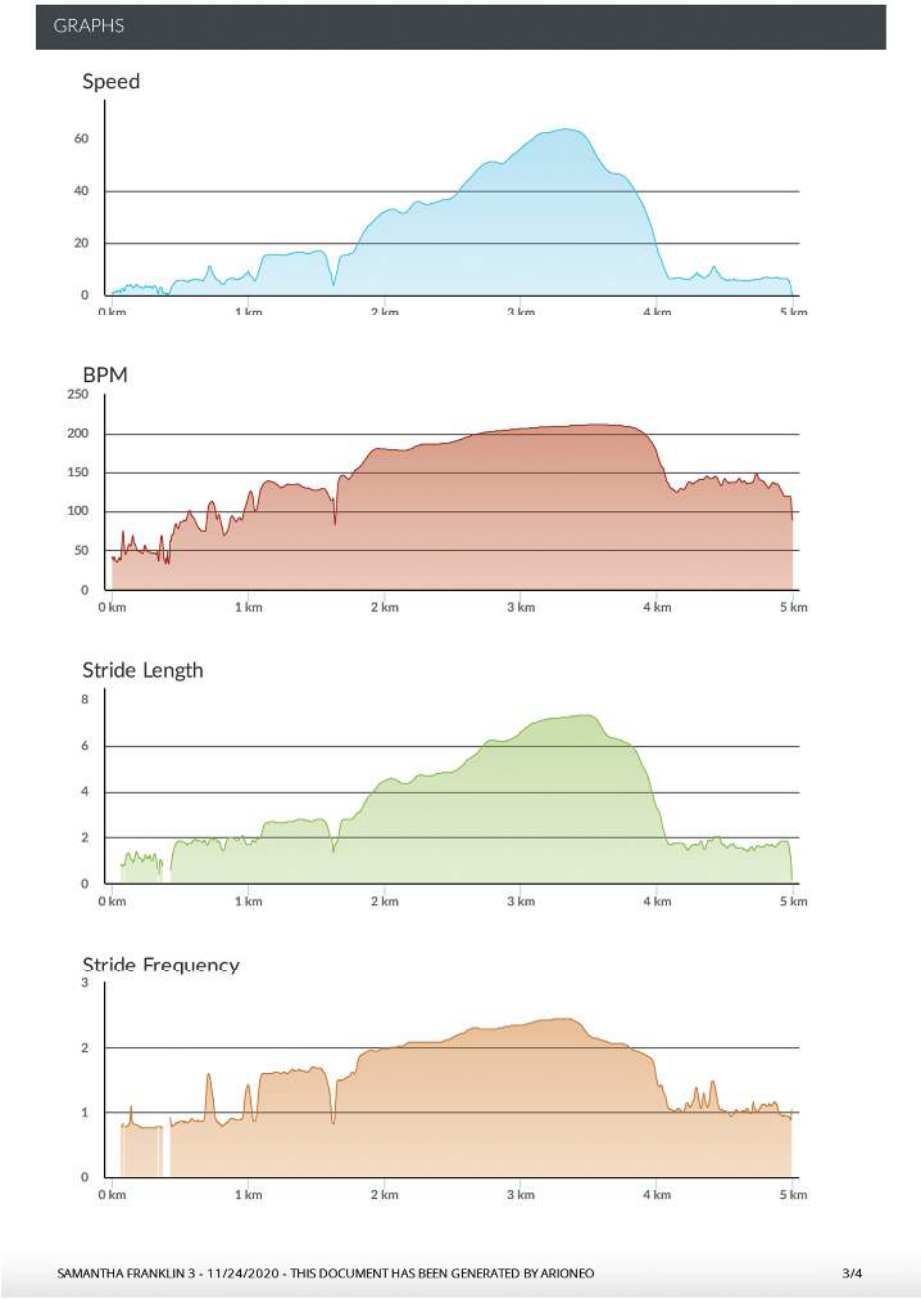

Figure 14. Example of graphs reported by the Equimetre device with information regarding speed, heart rate, stride length and stride frequency

Designing an exercising ECG test

When recording an exercising ECG, it is important that the level of effort performed by the horse is similar to or slightly above what would normally occur. This means that Thoroughbred and Standardbred horses need to exercise maximally, which is interpreted as a heart rate exceeding 200 beats per minute for at least 30-60 seconds. When the heart rate is beyond 200 beats per minute, vagal tone is abolished. The return of vagal tone in the immediate post exercise period is an important vulnerable time for arrhythmias and it is important that this part of the exercise test is also recorded. The author usually terminates recording of the exercising ECG when the heart rate is below 100 beats per minute. For

horses with a history of staggering or collapse, it is appropriate to first investigate the ECG on the lunge or on a treadmill before performing any testing with a rider. In cases of poor performance, staggering or collapse, it might be appropriate to simultaneously record dynamic endoscopy to evaluate the interaction of heart rhythm and upper airway function.

ECG interpretation

A detailed explanation of ECG interpretation is beyond the scope of this article and readers are directed to several excellent articles outlining this skill. The normal impulse originates in the sinoatrial node, located in the right atrium and passes across the right and left atria to the atrioventricular node. This produces the P wave. There is a delay as the impulse passes through the AV node, followed by conduction along the deep Purkinje fibres into the ventricular myocardium. This produces the QRS complex associated with ventricular depolarisation. Ventricular repolarisation produces the T wave. Therefore, in sinus rhythm, this co-ordinated impulse conduction results in a regular P, QRS and T wave. There will be regular R-R intervals, regular P-P intervals and a P wave for every QRS and a QRS for every P. The morphology of P waves can vary from single positive to bifid (two peaks). These two peaks reflect conduction through the right and left atria respectively and are a normal finding. The QRS complex is typically long and narrow and in a negative polarity. Changes in P wave morphology can sometimes be seen in atrial ectopy when the impulse originates within the atria rather than the sinoatrial node. Similarly, changes in QRS complex morphology can indicate ventricular ectopy but can also reflect aberrant conduction of a supraventricular impulse. Changes in QRS morphology are due to a change in the conduction pathway within the ventricle and can be wide and of inverse polarity. It is important not to be too prescriptive in ascribing the origin of premature complexes as supraventricular or ventricular. As sometimes ventricular ectopy can be narrow and sometimes supraventricular ectopy is wide. T wave morphology varies widely and the significance of these changes, if any, are not understood.

Importance of arrhythmias

The importance of cardiac abnormalities in athletic horses has been reviewed and a consensus statement on managing these athletes is published and free to access. This publication is enormously helpful and gives specific recommendations for the suite of disorders commonly seen in horses. Readers are encouraged to consult this publication when evaluating horses with cardiac disease.

Physiological arrhythmia

Horses have high vagal tone at rest which promotes vagally mediated arrhythmias. Such arrhythmias are abolished with exercise as the heart rate increases and vagal tone subsides. The most common of these arrhythmias are second-degree atrioventricular block (Fig 15) and sinus arrhythmia. Provided they do not persist during exercise they have no impact on horse safety or performance.

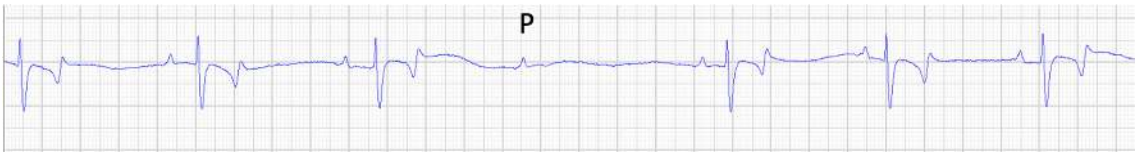

Figure 15. An example of second-degree atrioventricular block. There is a P wave without a corresponding QRS complex. The T wave in this recording is biphasic (positive and negative deflections). This is sometimes described as being "regularly irregular" as the dropped QRS does not interfere with the underlying rhythm of the sinoatrial node. Paper speed 25mm/sec, 10mm/mV

Atrial fibrillation

Atrial fibrillation (AF) is the most common clinically important arrhythmia in horses. In racehorses and performance horses it typically occurs in the absence of gross structural abnormalities. In athletic horses, AF is usually paroxysmal, converting to sinus rhythm within 72 hours without specific treatment. Persistent AF lasts beyond 7 days and rarely spontaneously converts to sinus rhythm. In the general horse population, recurrence occurs at a rate of 15-65%. The high rate of recurrence supports the contribution of individual factors, such as atrial size and microstructural changes affecting mechanical function and atrial fibrillation cycle length in the development of AF in horses. Age is a risk factor for arrhythmia in Standardbreds, and Thoroughbreds likely due to training associated cardiac remodelling. Persistent atrial fibrillation requires conversion to sinus rhythm if the horse is to continue with strenuous athletic activity. Conversion can be achieved with either nasogastric intubation with quinidine sulphate or electrocardioversion, which involves placing intracardiac catheters and discharging an impulse through the myocardium. The prognosis for conversion depends on underlying structural disease and the duration of atrial fibrillation prior to treatment as atrial fibrillation itself will cause microstructural and electrical remodelling of the myocardium that further promotes AF. Horses with permanent atrial fibrillation (owners declined treatment or treatment unsuccessful) might still be suitable for riding at lower intensity. It has been recognised that at high heart rates horses with AF are at risk of ventricular conduction abnormalities and ventricular ectopy. Therefore, screening the horse for such abnormalities with exercising ECG at the intended level of exercise is recommended.

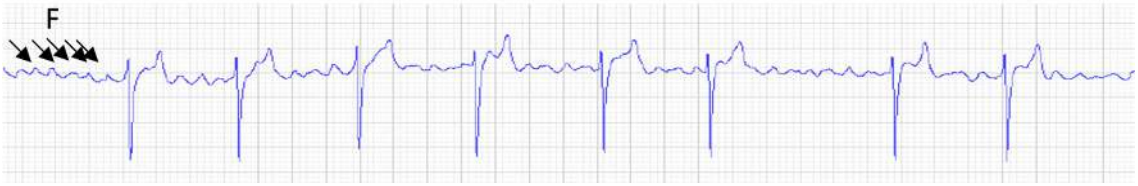

Figure 16. An example of atrial fibrillation. There are irregular R-R intervals and undulation of the baseline with F (flutter waves). This rhythm is sometimes described as being "irregularly irregular" as the underlying rhythm is chaotic. Paper speed 25 mm/sec, 10 mm/mV

Exercising rhythm abnormalities

An exercising ECG is required to determine the significance of exercise-induced arrhythmias. However, the use of only 3 leads for the exercising ECG limits precision in characterising arrhythmias in horses. Most studies report only on changes to cardiac rhythm and the significance of morphological alterations in the T wave and variations in timing of electrical events are not well understood in horses. In strenuously exercising horses, cardiac arrhythmias (other than AV block and sinus arrhythmia) occur in approximately 50% of examinations with complex, potentially pathological ventricular arrhythmias occurring in 3-16% of examinations. Supraventricular tachyarrhythmias are generally not considered life threatening in horses. Supraventricular premature complexes are indicated by a beat which comes earlier than expected and most have a pause before the subsequent complex (Fig 17). A P wave with abnormal morphology might precede the premature QRS complex which usually has normal morphology. Ventricular premature complexes most often have a change in QRS morphology (Fig 18). Potentially pathological ventricular arrhythmias occur with equal frequency during peak exercise and in the immediate post exercise-period which are also common times for sudden death to occur. With return of vagal tone, heart rate drops precipitously in the immediate post exercise

period, from more than 200 beats per minute to approximately 120 beats per minute over 90 seconds. Arterial hypoxaemia occurs in strenuously exercising horses due to a combination of hypoventilation and diffusion limitation. Hypoxaemia is exacerbated by dynamic upper respiratory tract obstruction or lower airway disease, which may promote arrhythmia. Autonomic instability, myocardial hypoxia and electrolyte disturbances including lactic acidosis, hyperkalaemia and ionised hypocalcaemia explain the vulnerability to arrhythmia during exercise and in the immediate post exercise period. Electrolyte abnormalities promote arrhythmia by changing the threshold potential. Abnormalities of potassium, calcium, magnesium and pH are particularly important. These changes can occur in strenuously exercising horses particularly in hot and humid conditions. Horses with other diseases such as enterocolitis, anterior enteritis and ruptured bladder may also develop arrhythmia due to metabolic derangements associated with the underlying disease.

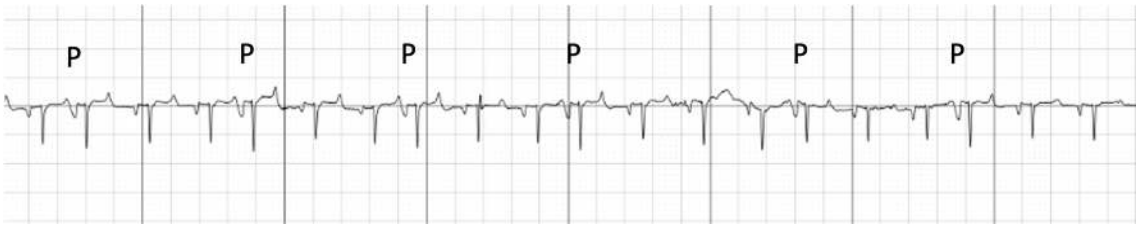

Figure 17. An example of supraventricular premature complexes. Each early complex is preceded by an abnormally shaped P wave and followed by a pause. Frequent atrial premature complexes are thought to predispose to atrial fibrillation

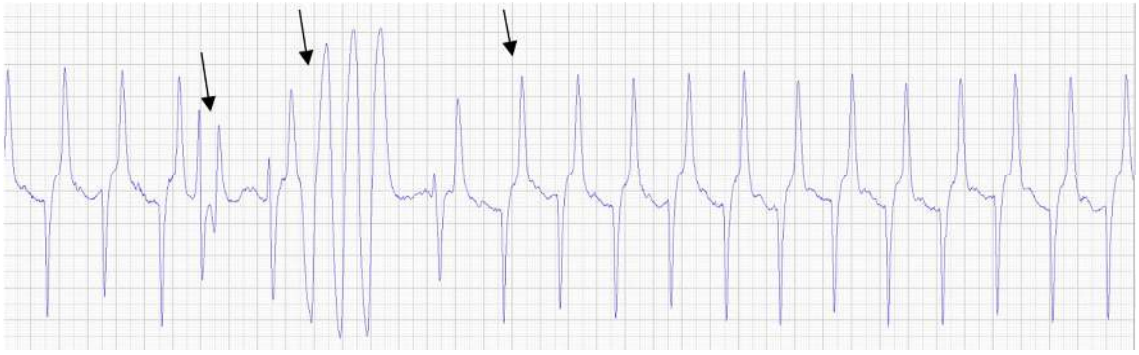

Figure 18. A post-exercise ECG demonstrating suspected ventricular ectopy. In this recording, the morphology of QRS complexes is variable with some being wide and some inverted. The first black arrow indicates an inverted QRS complex. The second arrow indicates a run of ventricular premature complexes with R on T. Complexes with multiple different morphologies likely indicate multiple ectopic regions within the myocardium. Complexes which occur in runs of couplets, triplets or more are also of concern and considered to be more likely to deteriorate to cardiac arrest. The third arrow indicates return to sinus rhythm. Paper speed 50mm/sec, 10mm/mV

Summary

There have been rapid advances in technology over the past 10-20 years which have facilitated new techniques in identifying and diagnosing cardiac rhythm abnormalities in horses. Further developments will allow for not only more advanced diagnostic capabilities but also translate to improved treatment options for horses with cardiac arrhythmias.

Further reading

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4. Kee P, Anderson N, Gargiulo GD, Velie BD. 2023. A synopsis of wearable commercially available biometric-monitoring devices and their potential applications during gallop racing. *Equine Vet Educ* 2023;*35*(10):551-560.

Assessment and treatment options of fractured incisors and cheek teeth

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Careful assessment of all fractured teeth is important to determine if the fracture involves sensitive dentine or pulp tissue which may compromise the viability of the tooth. Various classification systems exist that help to clarify which dental structures/ tissues are involved and help to formulate a treatment plan. Many of the classifications systems used in brachydont species are not applicable to equines as they have no clear crown/root distinction and the presence of an unerupted reserve crown is not accounted for. The AVDC classification system is based on the dental tissues involved. On a very basic level, complicated crown fracture indicates pulp involvement, and uncomplicated crown fracture indicates no pulp involvement.

Enamel Infraction (T/FX/EI)	A crack in the enamel without any loss of tooth substance.
Enamel Fracture (T/FX/EF)	A fracture that removes a piece of enamel, exposing the dentin beneath.
Uncomplicated Crown Fracture (T/FX/UCF):	A fracture that extends into the dentin but does not expose the pulp (the soft tissue inside the tooth).
Complicated Crown Fracture (T/FX/CCF):	A fracture that exposes the pulp.
Crown-Root Fracture:	A fracture involving both the crown and the root. This can be further classified as uncomplicated or complicated based on whether the pulp is exposed.

Table 1: AVCD dental fracture classification system. Note that the last classification (Crown-Root) is not applicable in equines

Traumatic fractures to incisors are relatively common, especially in younger horses. In contrast cheek teeth fractures secondary to trauma are very rare and more likely secondary to occlusal fissures or due to chronic dental disease (infundibular caries or apical disease with pulp death). Traumatic incisor fractures may involve multiple incisors or just one, and in some cases, avulsion fractures may also involve alveolar bone. Assessment of the fractured surface includes visualisation, probing and in some case, careful burring of the fractured surfaces with a high- speed water cooled diamond endodontic burr to determine if there is pulp or sensitive dentine involvement. Radiographs are essential to determine the extent of the fracture and longitudinal fractures may be present that will affect the prognosis. Acute fractures may show no or minimal apical changes on radiographs even if there is pulp horn involvement. Chronic fractures may have marked apical or peri-apical changes such as root blunting and peri-apical bone sclerosis. Some cases may even show the presence of a reparative dentine bridge which would indicate good viability of the tooth. Treatment options include endodontic treatment or extraction, and this is determined by assessment of the macroscopic and radiographic findings. In some cases, the fracture is purely cosmetic, and no further treatment is required. Equine endodontic treatment of

incisors are relatively easier compared to cheek teeth and have been shown to have a good success rate.

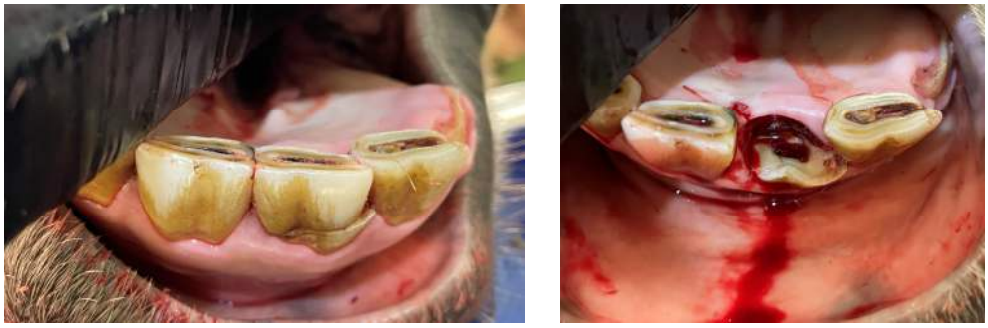

Figure 1: Careful assessment of a transverse fracture of a 301 incisor in a young horse revealed an open pulp horn

Cheek teeth fracture patterns have been well defined in previous studies and have been shown to be mostly secondary to other dental disease. Severe infundibular caries will weaken the tooth centrally and may result in a mid-sagittal fracture with displacement of either the buccal or palatal fragment. Extraction is almost always indicated in these cases as they have severe apical disease with compromise of the endodontic system. In cases of chronic apical disease, pulp death and necrosis with secondary caries they will weaken the endodontic system with eventual fracture. These fracture patterns can involve any of the affected pulp horns and may result in buccal, palatal, mesial or distal clinical crown fractures of the teeth. In cases where the pulpitis and fracture are limited to only part of the tooth, endodontic and restorative treatment may be an option. In cases where the entire endodontic system is diseased, the fracture is more likely to be extensive, and extraction is most commonly indicated.

The most difficult fractures to assess are the fractures involving the secondary dentine of the buccal or palatal pulp horns with no obvious other pulp horn involvement. These may be secondary to occlusal fissures that gradually weaken and suddenly fracture. A recent study has shown that up to 73% of these fractures have apical involvement, indicating the importance of assessing every fractured cheek tooth very carefully. If the fractured surface is at or below gingival level, it can be difficult to determine if pulp horns are open/ diseased. In addition, some cases show no or very mild apical changes on radiographs. Ideally, these cases should be assessed on CT scan as this will show pulp horn viability or non-viability as well as any subtle/ early-stage apical changes. Where CT examination is not an option, careful burring and probing of the fractured margin to determine if there are any open pulp horns is indicated. If there appears to be one or two pulp horns diseased with minimal apical disease, endodontic treatment may well be a viable option. If still not confident about tooth viability, repeat examination and radiographs in 2-3 months is recommended.

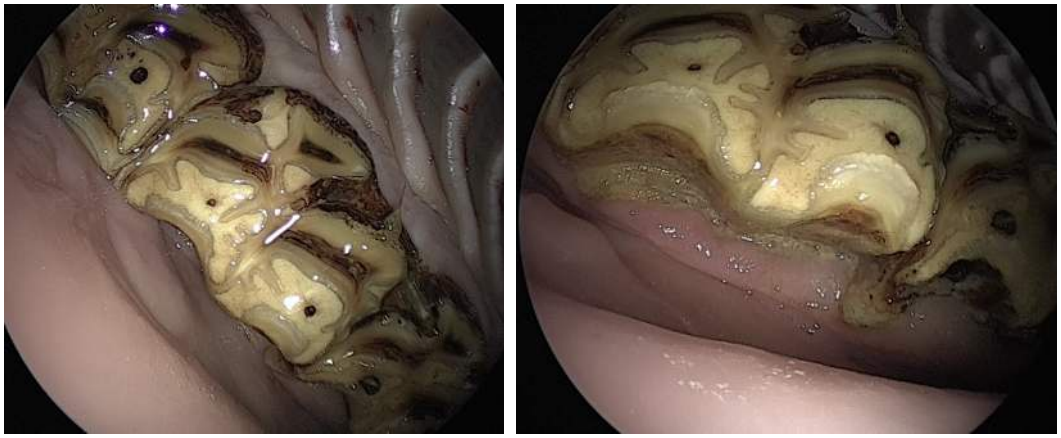

Figure 2: Oroscope images of the 109 with a buccal slab fracture though pulp horns 1 and 2 secondary dentine. Image on the left shows the occlusal surface, image on the right shows a close up of the fractured buccal margin with no obvious pulp horn involvement

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Imaging of the head (radiography and computed tomography)

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Due to the anatomy of hypsodont teeth in equids, we are only able to visualise 10-20% of the entire tooth length during oral examination, and radiography or computed tomography (CT) examination is essential once oral pathology has been identified. The equine skull is a complex three-dimensional structure with areas of air and very dense mineralised tissues (dental structures) allowing for good radiographic contrast. However, due to the overlapping of the structures in the equine skull, radiography has its limitations. Numerous studies looking at equine cheek teeth dental disease have shown that radiography is very specific (approximately 95%) but not very sensitive (approximately 50%).

Digital radiography is a very easy practical tool that can be performed in equine practice, although appropriate training and experience is required to take good diagnostic images. Good sedation and supporting the head with a head stand or dental halter is essential to ensure there is minimal movement. This is a common oversight made in clinical practice and result in views having to be repeated and increased radiation exposure to operators. Additional training and attendance of relevant professional courses are strongly encouraged to ensure that accurate techniques are used in getting the correct views.

The radiographic findings most consistent with cheek tooth apical disease include apical lucent halo, peri-apical bone sclerosis, root blunting/ clubbing and severity of the clubbing. Other changes such as, loss of *lamina dura denta*, root fragmentation, hypercementosis and widening of the periodontal ligament, may also be seen in cases of apical disease. Due to the complex endodontic system of cheek teeth with multiple pulp horns, no specific evaluation of the endodontic system can be performed on cheek teeth. When using intra-oral techniques to evaluate incisors, there is minimal superimposition of structures, and radiography of incisors is thus very useful. Assessment of individual incisor pulp horns can be performed with relative confidence. Apical changes such as apical lucent halo, peri-apical bone sclerosis, root blunting and hypercementosis can be evaluated. The pulp horns can be assessed for presence of reparative dentine/ mineralised bridges or comparative widening of the pulp horns indicative of pulp death i.e. cessation of secondary dentine production. Recent studies, have shown that early cases of Equine Odontoclastic Tooth Resorption and Hypercementosis (EOTRH) will show changes on CT examination not yet evident on radiographic images, although it could be argued that early stage EOTRH is not clinically relevant and continued monitoring for clinical signs or increased radiographic changes is the best way to manage these cases.

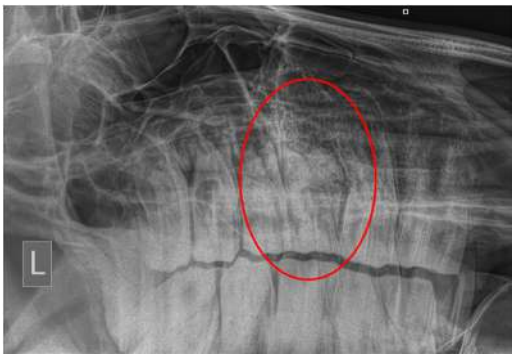

Figure 1: A left lateral dorso-ventral oblique view showing root blunting, apical hypercementosis, reserve crown lucency, periodontal ligament widening and peri-apical bone sclerosis of the 208, indicative of severe, chronic apical disease

Helical CT examination is where the x-ray beam rotates around the patient while the patient moves through the beam. This creates a spiral/ helical path of data acquisition with the creation of computerised 3D images. For the purposes of the equine head, this allows for evaluation of the pulp horns, pulp chambers, root canals, periodontal ligament and alveolar bone of individual teeth. CT allows for differentiation between structures of different densities, and this is very useful to identify gas within pulp horns indicative of pulp disease/ death in acute cases. The different sinus compartments, nasal conchal bulla and nasal passages can be individually evaluated for presence of fluid, soft tissue or other mineralised structures. In cases of young horses with apical disease and secondary sinusitis, the CT is invaluable to confidently identify the infected teeth and allows for more accurate treatment planning of the sinus disease.

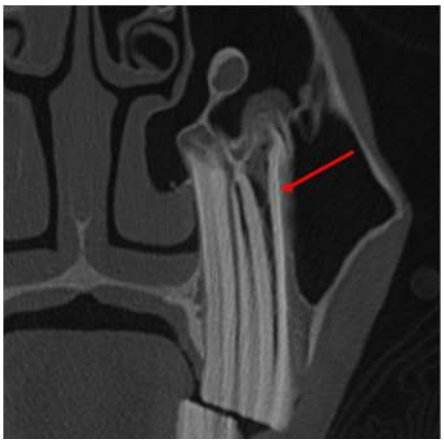

Figure 2: A transverse CT image of the 208 showing soft tissue attenuation over the apices, loss of alveolar bone and gas attenuation within pulp horn 2 (red arrow)

Although CT is superior to radiography when evaluating the equine head, radiography is still a very good practical, rapid, diagnostic tool that can give you a lot of information. Where there is marked pathology, radiography can be definitive. But in cases of more subtle pathology or where endodontic treatment is being considered CT examination is essential. Similarly, radiography can give you an indication of sinus disease, but CT examination gives better diagnostic definition that allows for better surgery planning, treatment and prognostication.

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Options for twin management after day 18: how did we get here?

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Introduction

The introduction of transrectal ultrasound for pregnancy evaluation has revolutionised the identification and management of twin pregnancies in mares. The rate of abortion due to twin pregnancies has dropped from an average of 20% to 6%.[1,2] While improvements in management of twins have dramatically reduced pregnancy losses, missed twin pregnancies can be financially and emotionally devastating. Additional methods for managing twins in the post-fixation period are available. Advantages and shortcomings of twin management techniques vary by procedure, and considering all points when managing post-fixation twins is important for decision making.

Twin management using manual reduction during mobility phase

Twin pregnancies are optimally detected between Days 13 and 15 of gestation. During this time period, the embryonic vesicles are mobile within the uterus.[3] Twin pregnancies that are detected during the mobility phase (up to Day 16 or 17) are best managed by manual reduction of one embryonic vesicle.[4] Survival rates after manual pregnancy reduction exceed 90% in experienced hands.[5] Approaches for manual reduction of one embryonic vesicle are well described but highly variable between clinicians.[6] In addition to high live foal rates, manual reduction of one embryonic vesicle is minimally invasive, highly reliable and inexpensive, therefore it is the optimal choice for managing equine twins.

Transvaginal ultrasound-guided twin reduction

Selective reduction of pregnancy using transvaginal ultrasonography has been advocated for mares with twins between 25 and 50 days of gestation.[7 - 9] Advantages of using a transvaginal ultrasound-guided approach for twin reduction in mares include twin reduction prior to placentation, minimal trauma to the mare and standing, outpatient procedure. Disadvantages of this approach include expense, need for specialty equipment and success variability between operators.

The technique involves a 5 or 7.5 MHz microconvex, transvaginal ultrasound transducer and casing designed for use in large animals. More recently, the use of a 5 MHz, linear transducer, (commonly used in oocyte aspiration) has been applied to transvaginal ultrasound-guided pregnancy reduction. Prior to the procedure, mares are often administered prophylactic antibiotics, anti-inflammatory agents and progestins. An operator carries the transducer into the anterior vagina, replaces the hand in the rectum and secures the pregnancy. The transducer is manipulated (transvaginally) until the pregnancy is imaged on the ultrasound screen. A 16 to 18-gauge, 60 cm needle is advanced through a needle channel in the transducer casing. The needle is passed through the vaginal wall, uterine lumen and into the yolk or allantoic space using a sharp jab. Fluid is aspirated and/or foetal trauma is induced with the needle.

The success rate of transvaginal ultrasound-guided twin reduction has been highly variable.[8,10-13] Operator experience, parity, day of gestation and bilateral vs unilateral position of pregnancies are all factors impacting success. Data from a prospective study described live foal delivery rates after transvaginal twin aspiration was performed in mares between 25 – 62 days gestation.[13] One skilled operator performed all twin reduction procedures. Nearly half (20/41; 49%) of treated mares delivered live foals. The live foal rate was highest in mares who underwent the procedure during Days 31 -35 gestation (9/14; 64%) and was lowest (0/5) in mares > Day 42 gestation. A large, multi-centre study provided additional supportive evidence for transvaginal ultrasound-guided twin reduction in the mare.[14] Examining records from 464 cases of transvaginal ultrasound-guided twin aspirations, 40% (127/317) of mares delivered a live, singleton foal at term. The procedure was significantly more successful during earlier in gestation (Days 25 – 35; 74/150 mares; 49.3%). Furthermore, pregnancy outcome was inversely correlated with gestational age.[14] Additional factors that influenced pregnancy outcome in this study included parity (multiparous mares suffered greater pregnancy loss after the procedure than uniparous mares) and number of punctures.[14] When gestational age was normalised at Day 30-34, mares who underwent a single puncture had higher pregnancy rates (37/53; 69.8%) than those undergoing two punctures (15/32; 46.9%) or three (6/18; 33.3%). Aspiration volume also had a negative effect on pregnancy rates, and a volume aspirated < 20 mL reported to be most successful.[14] In a small population of mares where embryo/foetal puncture was performed, fewer live foals were born (8/38; 21.1%) than in cases where no embryo/foetal puncture was performed (94/230; 40.9%). Overall, the data in this study demonstrated that transvaginal ultrasound-guided twin reduction was a viable option for twin management for most mares before 35 days gestation.[14] Factors to consider when selecting transvaginal ultrasound guided pregnancy reduction for twin management include the time of gestation, need for specialty equipment and operator skill.

Cranio-cervical dislocation

Cranio-cervical dislocation (CCD) is described as the dislocation of the first cervical vertebrae from the cranium, disrupting the ligamentous attachments and severing the spinal cord. This procedure can be performed to resolve twin pregnancy using transrectal or transabdominal techniques between 60 and 110 days of gestation.[15] The basis for this procedure is to eliminate one twin before placental formation is complete, allowing the remaining foetus to utilise the entire endometrial surface for nutrient and oxygen exchange.

Foetal termination using cranio-cervical dislocation is best performed using a surgical, flank approach.[15] This procedure has been used for twins between gestational ages of 58 and 150 days. Mares are administered propantheline bromide (30mg, IV, for uterine relaxation), flunixin meglumine (1 mg/kg, IV), broad spectrum antibiotics and exogenous progestins (altrenogest, 0.044 mg/kg, PO) prior to the procedure. Foetal size and location are determined using transabdominal ultrasound. Foetal access is considered more important than foetal size, in most cases, when determining which foetus to terminate. A standing flank laparotomy is performed ipsilateral to the horn containing the selected foetus. Identification of the preferred uterine horn is not always possible because of foetal movement and/or image quality. In cases with poor image quality, the right flank is selected for uterine access because fewer abdominal structures impede access to the uterus. Using one arm, the operator locates the uterine horn and identifies the foetus for termination. Cranio-cervical dislocation is performed by manipulating the foetus through the uterus, without incising the uterine lumen. The foetal head

is identified by its dome shape. The mandible and cervical vertebrae are identified and grasped. Cranio-cervical dislocation is performed by stabilising the head between the thumb and forefinger and bending the head from side to side. The side-to-side motion damages the ligaments attaching the head and neck. Dislocation is completed by placing the thumb at the base of the cranium and applying pressure proximally and dorsally. A distinctive pop is felt if dislocation is achieved, and the thumb and forefinger can be placed in the space created between the head and neck. The flank incision is closed using routine procedures. Mares are administered antibiotics and anti-inflammatory agents for 5-7 days and exogenous progestins for at least 30 days after the procedure. Foetal viability should be determined using transabdominal ultrasound at least once weekly, In one mare, foetal death did not occur after cranio-cervical dislocation. To protect the fertility and life of the mare, the pregnancy was terminated at 7 months gestation. Cranio-cervical dislocation in a population of healthy mares produced one live (normal) foal in 24/38 (63%) mares (KE Wolfsdorf, unpublished data).

Using cranio-cervical dislocation for twin management offers the advantages of reducing twin pregnancies to a singleton prior to complete placental formation and no need for specialised ultrasound equipment. However, the cranio-cervical dislocation procedure is an invasive procedure (similar to transvaginal or transcutaneous ultrasound-guided twin reduction), requires abdominal surgery and can be technically challenging. Identifying a foetus, and maintaining a grip on the selected foetus during the procedure requires patience and diligence. Further, prolonged time to foetal death can be disconcerting to the mare owner.

Ultrasound-guided foetal thorax compression

Foetal damage, manually or with the ultrasound transducer, has been advocated as a means of reducing twin pregnancies to a singleton using a variety of techniques. Of interest, is foetal thorax compression, which was originally described without the aid of ultrasound.[16] Arnold and co-workers modified the procedure by introducing transrectal ultrasound as a means of identifying, and monitoring, thoracic compression to cause death of one foetus in equine twins between 51 and 79 days gestation.[17] The authors reported application of thorax compression to 16 mares with 14 mares having unilaterally-fixed twin pregnancies and 2 mares having bilaterally-fixed twins. Ten of the 16 mares were pregnant with dizygotic twins while 6 mares carried monozygotic twins (derived from ART procedures). Mares were administered detomidine (0.01 mg/kg) and butorphanol (0.01 mg/kg) prior to the procedure. In some cases, rectal relaxation was achieved by administering mares N-butylscopalamine (0.2–0.4 mg/kg, IV) and/or 20 mL 20% lidocaine solution infused, per rectum. In the cases of unilateral twins, the foetus located closest to the tip of the uterine horn was selected for compression. For bilateral twins (n = 2), the foetus with easiest access was selected for compression. The ultrasound transducer was applied for lateral and downward pressure while stabilising the selected foetus against the pelvic brim or lateral abdominal wall. The foetal heart was identified and used as a target during the compression procedure. Steady, consistent pressure with the transducer was applied over the foetal heart until the heart rate slowed to less than one beat/second or stopped all together. Thoracic compression was applied between 1 and 5 minutes, and some mares required more than one attempt to cause foetal death (up to 5 attempts). Some mares were administered altrenogest (0.044 mg/kg, PO, q24h) through 100 days gestation or until both foetuses were confirmed dead. Live foals were delivered from 7/10 (70%) mares having dizygotic twins (6 unilaterally fixed twins and 1 bilaterally fixed twin pregnancy).[17] No live foals (0/6; 0%) were delivered after the procedure in mares with monozygotic twins, and the procedure was deemed unsuccessful (both foetuses dead the day following the procedure) in

all cases.[17] These data provide an alternative means of managing post-fixation twins that is minimally invasive, inexpensive and requires no special equipment. Furthermore, foetal location/fixation had little influence on procedure success with the majority of live foals born from mares having unilaterally-fixed, dizygotic twins. It was reported that more than one attempt was made in the majority of mares (8/10) and that operator experience with rectal procedures was a factor in success. Finally, the authors reported that turgidity of the foetal structure prior to 55 days gestation made the procedure challenging as did size and access after Day 80 gestation.[17]

Transcutaneous ultrasound-guided twin reduction

Transcutaneous ultrasound-guided twin reduction in the mare was pioneered by Rantanen and Kincaid.[18] The technique has been broadly used and offers the advantage of consistent live foal rates (≥ 60%) when performed by experienced individuals.[18,19] This technique is often used when the window for early twin reduction, by either manual crush or transvaginal ultrasound-guided procedures, has passed. Performing twin reduction at a later date may prove advantageous for unilateral twins that have undergone placental development and have greater stability. Conversely, twins with significant shared placental interface will not regain endometrial contact after the procedure is performed, thus placental insufficiency may result in premature termination of pregnancy or delivery of small, unthrifty foals.[20]

The original suggested time to perform this procedure was between 115 and 130 days gestation, however, a range of days has been described.[20,21] As with other described procedures, mares are often treated prophylactically with broad-spectrum antibiotics, anti-inflammatory agents, and exogenous progestins. The procedure is performed in the standing, heavily sedated mare. Sedation promotes movement of the foetuses into the cranial abdomen allowing for easier accessibility and to minimize foetal movement during the procedure. The foetuses are identified with a 2.5-3.5 MHz curvilinear transducer to determine foetal positioning and size. The most accessible foetus is targeted for reduction. The mare's abdominal area adjacent to the foetuses is clipped and surgically prepared prior to the procedure. Typically, a 6 inch, 18-gauge spinal needle is used to perform the procedure. The needle is passed through the skin and abdomen in one motion. Once the needle is advanced into the peritoneal space, the needle tip is located on the ultrasound image. Using a quick, thrusting motion, the needed is advanced through the uterine wall and into the uterine lumen. A foetal heart is identified and the needle is advanced, rapidly, into the foetal thorax/heart. Penetration of the foetal thorax and heart can be challenging as the foetus frequently moves away from the needle. Free flow of blood from the needle after removal of the stylet indicates needle placement within the foetal heart. Potassium chloride (KCl, 2 mEq/ml, up to 32 mEq KCL) or procaine penicillin (10-20 ml) are injected into the foetal heart, thorax or abdomen.[22] Potassium chloride generally results in rapid foetal death, particularly with intracardiac needle placement. Cardiac activity of the treated foetus is monitored immediately after the procedure. The foetus does not always die immediately in which case the mare is monitored over subsequent days to assess the status of both the treated and untreated foetuses. Generally, mares are administered flunixin meglumine at the time of the procedure and for up to 4 additional days (twice daily). Progestin therapy and prophylactic antibiotics are also prescribed for a period of 5 days.

Success rate of foetal cardiac puncture for twin reduction exceeds 50% in most cases. Factors that contribute to success of the procedure include proximity of the foetus to the adjacent twin

(and placentation involved), operator experience and conditions for performing the procedure. The terminated foetus is mummified and delivered in a small placental sac along with the live foetus.

Termination of pregnancy

While termination of pregnancy is not generally a chosen method for management of twins, it might be the most economical choice for the mare owner faced with twins that cannot be resolved using manual reduction. Further, some mare owners prefer not to subject the mare to the risks of more advanced twin reduction procedures. It is incumbent upon the veterinarian to educate an owner about the inherent risks of carrying equine twins into advanced gestation. Late term twins can result in abortion, dystocia, premature delivery, and death of the mare and/or foals. For these reasons, mare owners should be advised to terminate twin pregnancies that cannot be resolved using other means. Termination of twin pregnancy before 40 days is best achieved using a single dose of prostaglandin $F_2\alpha$ (10 mg, IM). Pregnancies from 40 to 100 days gestation are supported by multiple corpora lutea which necessitates multiple doses of prostaglandin over sequential days to terminate pregnancies. Advanced pregnancies (> 100 days gestation) require induced abortion under direct supervision, ideally in a hospital. In all cases, pregnancy status should be monitored using transrectal ultrasound examination of the reproductive tract during the termination procedure. Uterine lavage, in combination with ecbolic agents such as oxytocin, are useful for removing foetal tissues after the termination procedure.

Conclusions

Manual reduction of twin embryonic vesicles to a singleton is the most viable option for managing twin pregnancies in mares. When twins are identified in the post-fixation period, other management options must be considered. The pros and cons of all procedures, including success rates and stage of gestation, should be considered prior to selecting a method of management for late embryonic or foetal twins.

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Atrial fibrillation

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Atrial fibrillation (AF) is the most important performance limiting arrhythmia in racehorses. It should be suspected in horses with poor or erratic performance, a history of exercise induced pulmonary haemorrhage (EIPH), horses with heart murmurs, horses with elevated heart rates detected using heart rate monitors and in horses with a history of weakness or collapse.

Figure 1. Normal heart rhythm which is regular

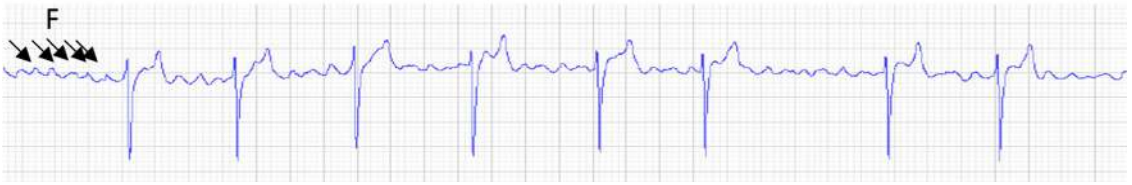

Figure 2. An example of atrial fibrillation. There are irregular R-R intervals and undulation of the baseline with F (flutter waves)

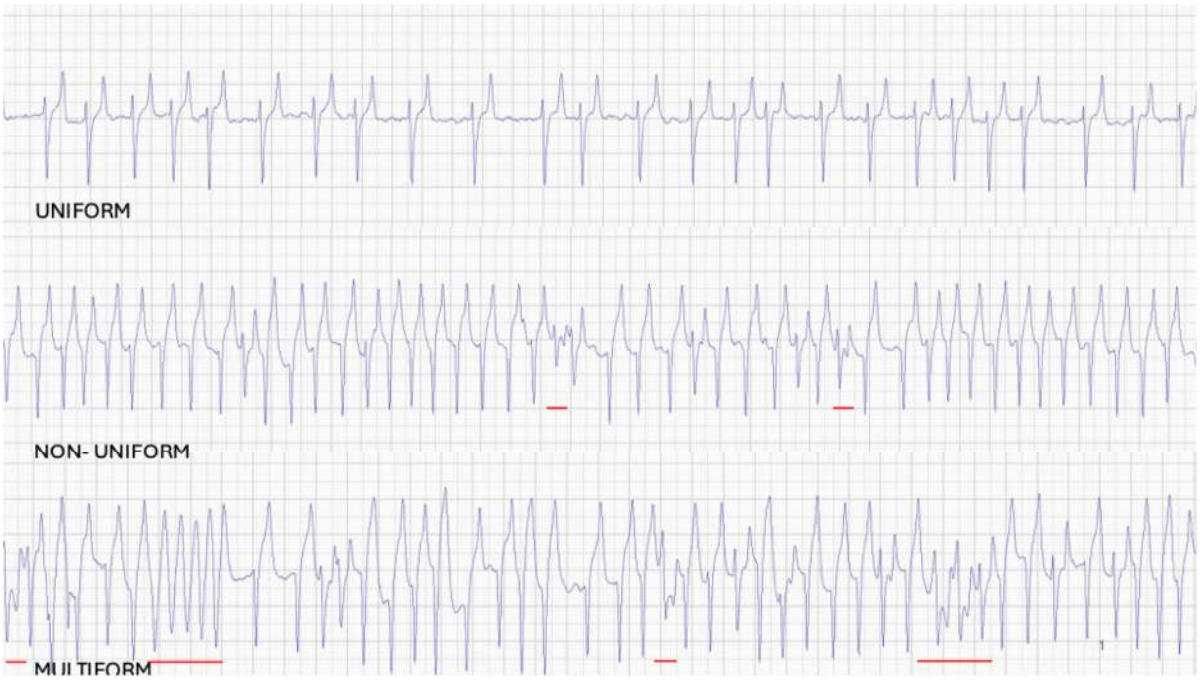

Figure 3. Example of atrial fibrillation at high heart rate. Uniform AF has the same morphology throughout. Non-uniform AF has a single morphological change, and Multiform AF has two or more morphological changes. Changes in QRS morphology are common and likely reflect ventricular ectopy or aberrant conduction

Consequences of atrial fibrillation

AF typically presents whilst horses are young and in active competition. Structural heart disease, other than cardiac enlargement and valvular regurgitation is generally absent in these young horses. Older horses with AF, including former racehorses that have transitioned to equestrian disciplines, are more likely to have identifiable structural cardiac pathology. AF in racehorses is usually paroxysmal, converting to sinus rhythm within 72 hours without specific treatment in approximately 90% of identified cases. Persistent AF lasts beyond 7 days and rarely spontaneously converts to sinus rhythm.

Although the majority of AF in racehorses occurs in the absence of detectable structural cardiac disease, atrioventricular regurgitation is commonly found in horses with AF that are presented for cardioversion. Increased left atrial size is associated with reduced cardioversion success and increased risk of recurrence. Structural remodelling in AF includes atrial enlargement and development of myocardial fibrosis. Atrial dilatation supports re-entry and myocardial fibrosis impairs conduction and can have pro-arrhythmic effects.

There is a strong association between AF and poor athletic performance in racehorses. The highly irregular heart rate in AF impairs ventricular filling and cardiac output. Ventricular filling is limited further by atrial mechanical dysfunction.

In addition to poor performance, there is a concern that AF in horses might increase the risk for collapse or SCD. Horses with AF that are exercised strenuously can have very high heart rates, commonly exceeding 300 BPM. Several studies have reported wide-complex tachycardia and R on T phenomenon in horses with AF, particularly at higher heart rates. The wide complex tachycardia might reflect aberrant conduction of a supraventricular impulse, or ventricular ectopy. Either of these conditions are likely to make the arrhythmia less stable and increase the risk for deterioration into ventricular fibrillation and death. Ventricular pre-excitation might also increase the risk of fatality in horses with concurrent AF.

A small number of studies have identified an association between atrial fibrillation and exercise induced pulmonary haemorrhage. Atrial fibrillation causes increased left atrial pressure and consequently increased pulmonary pressure which could exacerbate capillary failure. The impact of upper and lower airway disease on risk for atrial fibrillation has not been clearly established but it is expected that through exacerbation of exercising hypoxaemia airway disease could potentiate AF.

Atrial fibrillation mechanisms

Atrial premature depolarisations appear to be an important potential trigger for AF and atrial burst pacing can induce AF in horses. Atrial premature depolarisations are commonly seen during exercise in horses. A single study has evaluated cardiac arrhythmias before and after racing in Standardbred horses and this suggested that the onset of AF is most likely during racing. High pulmonary arterial, pulmonary artery wedge and pulmonary capillary transmural pressures are observed in strenuously exercising horses. Right and left atrial pressures also increase markedly. Left atrial pressure is a principal determinant of

pulmonary capillary pressure and AF is both a cause and consequence of raised left atrial pressure. Atrial fibrillation impairs atrial distensibility and acutely induces further elevations in left atrial pressure. These changes in atrial pressure, alongside fluctuations in autonomic tone, arterial hypoxaemia and electrolyte imbalances could explain the increase in incident AF during exercise in horses. Electrolyte imbalances include metabolic acidosis, resulting from excessive lactate production from exercising muscle and losses of chloride, sodium and potassium through sweat. Hypocalcaemia is also common. Due to their important role in membrane depolarisation, transient potassium and calcium depletion in particular, are suspected to affect AF development in horses. Atrial wall stress associated with atrial stretch incurred during exercise might cause myocardial inflammation and fibrosis. Racehorses commonly have atrioventricular regurgitation and this could further promote atrial structural remodelling and increase AF risk.

Similar to humans, myocardial sleeves in the pulmonary veins of horses have been identified and might be involved in the initiation and maintenance of AF. Recent research suggests that the myocardial sleeves of the caudal vena cava might be equally important as the pulmonary veins in inducing arrhythmias in horses. One study has demonstrated spontaneous ectopic firing from the pulmonary vein triggering a short period of non-sustained AF during electro-anatomical mapping in a horse. Research in Standardbred racehorses has supported a genetic basis for AF based on epidemiology and pedigree analysis. A recent study on Australian Thoroughbreds found the heritability of AF in this population was low.

Atrial fibrillation treatment

In horses, persistent AF is treated by either administration of quinidine sulphate via nasogastric tube, which is performed in standing horses. The dose is 22 mg/kg via nasogastric tube. It can be obtained from medical compounding pharmacies. Bioavailability is variable and the therapeutic window is narrow. Adverse effects include tachycardia, widening of QRS interval, arrhythmogenesis, hypotension, diarrhoea, colic, upper airway oedema. Dosing intervals are commonly reported as 2 hours for 5 doses, however longer intervals of 3-4 hours are commonly applied due to concerns for safety and toxicity. If quinidine sulphate is not effective as a sole treatment, it can be combined with digoxin (11 micrograms/kg PO q12).

An alternative is transvenous electrocardioversion (TVEC) which is performed under general anaesthesia. This procedure involves placement of specialised cardioversion catheters (https://www.digitimer.com/product/veterinary/transvenous-electrical-cardioversion/equine-atrial-fibrillation-catheters/) into the right atrium and left pulmonary artery. Defbrillation is delivered by a Lifepak defibrillator.

In the absence of structural heart disease, the prognosis for conversion with either procedure is good, likely exceeding 80%. Transvenous electrocardioversion might be successful in cases that do not respond to quinidine or demonstrate toxic effects of this drug. In particular, TVEC is appropriate in cases that have more long-standing AF or atrial enlargement. One of the major limitations of cardioversion is that recurrence is common and the risk of recurrence is likely to be similar with either procedure.

In the general horse population, approximately 15-65% of horses have recurrence of AF following cardioversion. The recurrence of AF in racehorses is approximately 20%. In racehorses that are treated for persistent atrial fibrillation this is increased to 40-60%. The high rates of recurrence of AF indicate that vulnerable individuals have alterations in both

electrical triggers and susceptible substrate. Atrial premature depolarisations are suspected to be important in AF onset in horses, and greater than 25 atrial premature depolarisations per 24 hours in the 5 days following cardioversion also increased the risk of recurrence.

In humans, isolation of the pulmonary veins with three-dimensional electro-anatomical mapping and guided ablation has been adopted as an effective method to reduce AF burden in suitable patients. In horses, it has recently been shown that similar myocardial sleeves are present in the caudal (inferior) vena cava and are responsible for focal ectopic discharges causing atrial tachycardia. Importantly, this site lends itself to ablation procedures as it is located adjacent to the right atrium and therefore is more readily accessible than the pulmonary veins which require puncture of the atrial septum.

Atrial tachycardia promotes atrial electrical remodelling and can induce AF. In some horses, atrial tachycardia is observed during treatment of AF with pharmacological or electrical cardioversion. In such cases, ablation procedures previously described for atrial tachycardia might be appropriate follow up treatments for management of AF.

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Grading of infundibular caries and introduction to restoration techniques

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Infundibular caries (IC) of maxillary cheek teeth are among the most prevalent and clinically significant dental conditions in horses. Their development is closely linked to pre-existing developmental defects of infundibular cementogenesis, especially hypocementosis, which predisposes to food impaction, bacterial colonisation, and progressive carious breakdown of dental tissues. These lesions, if left untreated, may result in sagittal fracture, pulpar necrosis, and apical infection.

Accurate diagnosis and risk stratification of infundibular lesions depend on robust classification systems. The Honma classification, originally developed for human dental caries, has been modified to reflect equine dental anatomy and clinical relevance. The Modified Honma Classification System (MHCS) grades lesions by tissue involvement, as visualised occlusally:

- Grade 1: Caries of cementum only
- Grade 2: Cementum and enamel
- Grade 3: Cementum, enamel, and dentine
- Grade 4: Coalescence of mesial and distal infundibula
- Grade 5: Structural failure or apical/pulpar disease

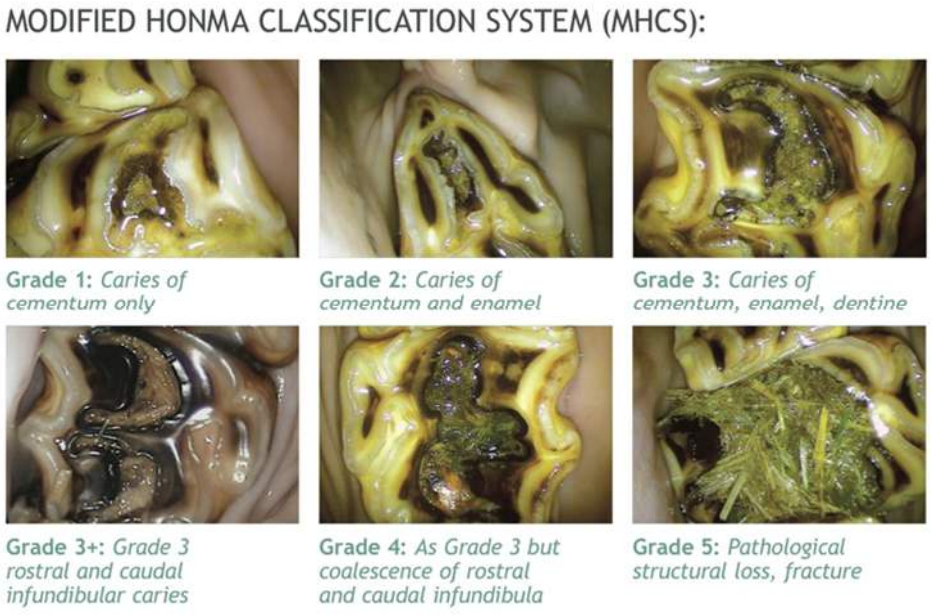

Figure 1: A 5-grade scale for occlusal assessment of infundibular caries

Further refinement has been proposed by Karma et al., dividing Grades 1 and 2 into subgrades (1.1, 1.2, 2.1, 2.2) based on the proportion of healthy cementum remaining. However, as these lesions are inherently three-dimensional, the Windley classification based on CT imaging has also been adopted. It categorises lesions into Group A (no visible occlusal lesion) and Group B (visible occlusal lesion), with subdivisions A0–A3 and B1–B3, respectively, based on lesion depth and

morphology. B3-type lesions with deep structural defects are most strongly associated with advanced disease and often require intervention.

Case selection for restoration is based on lesion severity, tooth location, age, and absence of endodontic or apical pathology. Horses aged 10–18 years with deep developmental infundibular defects and early signs of caries—especially maxillary 09 teeth—are the most suitable candidates. Restoration is typically indicated for:

- Occlusal Grade 3 lesions with ≥ 10 mm cavity depth
- Occlusal Grade 2.2 lesions (especially contralateral to a tooth with Grade 5 disease)
- B2 or B3 type lesions on CT without pulpar involvement
- No evidence of apical pathology or sinusitis

Diagnostic tools include oroscopy, endodontic probing, diagnostic burring, and imaging (radiography or CT). A cavity depth of 10 mm or greater, as assessed with a probe or endodontic file, is generally regarded as clinically significant when concurrent caries is present.

Restorative techniques require full debridement of food material and carious tissue using low- and high-speed burs, endodontic files, and irrigation. Cavity disinfection (e.g. NaOCl, CHX) has been shown to be unrelated to long term success of the procedure and is therefore optional and should not be considered an alternative to complete cavity debridement. Bonding systems and restorative materials are applied as per the manufacturers' instructions with a wide choice of options available. It is likely that the technique is more critical than the make or type of system used. Dual-cure resin composites are most commonly used by the speaker due to their favourable handling, polymerisation characteristics, and wear compatibility. A flowable microhybrid composite is placed incrementally from apical to occlusal aspect, with delayed or minimal light curing to reduce polymerisation shrinkage. Recent studies support the use of directed shrinkage protocols, delayed curing, and apical-to-occlusal filling to enhance marginal adaptation and longevity. Obturation is performed utilising the narrow sections of the cavity to aid material retention (capillary action).

Cavo-surface margins are optimised by preserving the natural crescent shape of the infundibulum. Bonding systems (either total-etch or single-step self-etch) must be selected based on substrate composition, with a preference for enamel or dentine bonding. Bonding to cementum, while sometimes necessary, is less predictable and associated with higher failure rates.

Long-term follow-up of over 200 restorations performed by the author demonstrated a success rate of 87% over a 6–11 year period. Success was defined by retention of restorative material, absence of caries progression, fracture, or apical disease. Failures were more commonly associated with Grade 2 lesions (vs. Grade 3), absence of bonding adhesive, or bonding to cementum. Restoration of contralateral 09 teeth prevented sagittal fracture in multiple cases. Infundibular restorations, when carefully selected and correctly performed, offer significant benefits in preserving cheek tooth function and preventing major dental complications. They are now established as a valid and effective component of advanced equine dental care, and their use is likely to expand further as techniques and materials improve.

Restoration Techniques

Restorative procedures in equine dentistry are increasingly recognised as valuable therapeutic tools for preserving structurally compromised yet functional teeth, reducing fracture risk, and managing or preventing progression of caries and endodontic disease. Indications extend beyond infundibular caries to include:

- Restoration of deep infundibular lesions (Grades 2.2–3+) to prevent sagittal fracture or food-related disease progression
- Restorative sealing following vital pulp therapies, including partial pulpotomy or apexification procedures
- Occlusal caries restoration of peripheral or central carious lesions, especially when food-trapping or progression is evident
- Protective occlusal sealing in younger horses with open developmental defects or in cases with mild occlusal fissures at risk of progression

In all cases, careful case selection and imaging are essential to exclude apical or endodontic pathology unless treated concurrently. The technique generally involves:

- Complete visual and mechanical debridement of the defect using low-speed tungsten-carbide burs, endodontic files, and lavage with sodium hypochlorite or chlorhexidine
- Disinfection and conditioning of the cavity walls as appropriate for substrate (enamel/dentine preferred over cementum)
- Application of a bonding system (total-etch or self-etch), followed by
- Incremental placement of a dual-cure resin composite, from apical to occlusal surface using flexible cannulae or extended rigid bendable mixing tips

For pulp restorations and apexification cases, internal cavity shaping must accommodate a dentinal bridge or apical barrier, and additional intermediate materials (e.g. MTA, CaOH, or bioceramics) may be placed before definitive composite restoration. Where occlusal sealants are placed in less cavitated defects, a conservative approach with minimal cavity shaping and flowable material placement is used.

Restoration is now considered an essential component of advanced equine dental practice, and its application continues to expand as techniques and materials evolve.

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Assisted reproduction in equine practice: A 30,000 foot view

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In the past decades, options for assisted reproductive technologies (ART) have exploded for the mare. Starting simply with embryo transfer, technologies now include:

- Embryo transfer (ET)
- Embryo cryopreservation/vitrification
- Oocyte recovery (follicular aspiration)
- Oocyte recovery from post-mortem ovaries
- Oocyte transfer/gamete intrafallopian transfer
- Intracytoplasmic sperm injection (ICSI)
- In vitro fertilisation (IVF)
- Nuclear transfer (NT; cloning)

While many of these technologies have been used to successfully achieve live foals, some are more practical than others. Most commonly, today, ART focuses on traditional embryo transfer, follicular aspiration to retrieve oocytes, oocytes recovered from deceased animals, embryos produced via ICSI and cryopreservation of oocytes/embryos. While live foals have been achieved through GIFT and cloning, these practices have not been broadly adopted due to expense and lower pregnancy rates. More recent success of IVF-derived foals has created great excitement in the industry, but consistent production of IVF foals remains limited to select practices.

In all cases, ART adds a layer of expense to the traditional management of mares for breeding. It is important to have a thorough discussion with mare owners about the pros and cons of each procedure so that expectations are in line with success rates, time and cost. Factors such as mare availability (performance mares), mare age, reproductive pathologies (ie, ovulation failure, oviductal disease, severe degenerative changes in the uterus, persistent uterine infections, cervical damage), and stallion availability (performance animals, stallion subfertility, deceased stallions) and estimated expenses should be discussed, candidly, before enrolment into any ART program. Below the pros and cons of the most commonly used ART procedures in equine practice will be reviewed.

Embryo transfer

Embryo recovery and transfer are procedures that have been widely adopted in equine reproductive practices. Factoring in mare breeding management, semen quality, recipient quality and synchrony and transfer success, the expected live foal rate lands between 65 – 75%.

Pros:

- Readily applied in equine practice
- Requires only a moderate amount of (new) equipment
- Utilizes skills already in place
- Pregnancy rates reliably good

Cons:

- Limited number of embryos produced per cycle
    - 1.2 embryos
- Can impact performance schedule while breeding mare

Oocyte recovery

When collecting oocytes from the mare, oocytes can either be collected from dominant preovulatory follicles, or from immature follicles. The advantage of using the dominant follicle is that the oocyte is mature and can easily be collected from the follicle yielding 1 or 2 oocytes. The immature follicles are significantly more numerous and yield a higher number of oocytes. However, recovery of oocytes from immature follicles is more technically challenging and the oocytes must be matured in vitro before they are fertilisable.

Oocytes are recovered from mares using transvaginal ultrasound-guided aspiration (ovum pick up; OPU). In most cases, a curvilinear ultrasound transducer is placed in a specialized casing containing a needle guide. The apparatus is inserted into the vagina, lateral to the cervix and ipsilateral to the ovary of interest. The ovary is palpated transrectally and positioned next to the probe. A needle is then placed through the vaginal wall to the ovary, and the follicular fluid and oocyte are removed by suction and lavage.

Pros:

- More embryos produced per cycle than ET
    - 2.2 embryos per session reported by Claes and Stout (2022)
- Many oocytes can be recovered in one session (immature oocytes)
- Maximises use of valuable semen via ICSI
    - Partial straws of frozen semen used
- Decreased impact on training/performance
    - Can be scheduled every 14 days
    - Return to training in as little as 2 days
- Can be performed at anytime (i.e. specific days, year-round)

Cons:

- Requires skilled personnel
- Requires specialised equipment
- Competence of cryopreserved embryos?
- Short and long term effects on the mare?
- Expense!!!

<u>Aspiration of a dominant, preovulatory follicle</u>

The goal with oocyte collection using the dominant preovulatory follicle is generally to harvest the oocyte as close to ovulation as possible. This ensures that both nuclear and cytoplasmic maturation have occurred and that the oocyte is viable.

The donor mare is examined daily using transrectal palpation and ultrasonography similar to a mare that is managed for breeding. Follicular growth is monitored carefully and measurements recorded. Cycling mares should also demonstrate signs of oestrus, including uterine oedema, relaxed uterine and cervical tone, and oestrous behaviour. An ovulatory agent is administered to the mare when she develops a follicle that is >35 mm in diameter. Human chorionic gonadotropic (hCG; 1500 IU, IV) and histrelin (1.5 mg,IM) or deslorelin acetate (1.8 mg, IM) administered together, approximately 24h prior to aspiration, is a protocol routinely applied prior to OPU mares at Colorado State University. This combination of agents is thought to help mature the follicle while also "loosening" the granulosa cells in the ovary to facilitate recovery of the oocyte.

Aspiration of immature follicles

Numerous oocytes can be retrieved from a single ovary when recovering immature oocytes (average 10 per mare); however in vitro maturation is required prior to ICSI. Additionally, oocyte aspirations can be performed multiple times on the same mare throughout the breeding season. Aspiration sessions are recommended no less than 14 days apart for ideal recovery of the mare. Most commonly, immature oocytes are shipped to a laboratory proficient in ICSI for further management.

After follicular aspiration, collected fluid is examined using a stereomicroscope similar to identifying an equine embryo. Oocytes are significantly smaller than embryos, therefore meticulous examination of recovered fluid is essential. The cumulus complex surrounding the oocyte is translucent and can aid in oocyte identification. Once located, the oocyte-cumulus complex is transferred to a holding dish containing clean embryo holding medium. Oocytes can be held or transported in embryo holding medium at 20°C for up to 24 hours before maturation without detrimental effects. Oocytes and medium should be packaged in an Equitainer® at room temperature and shipped overnight by courier to arrive at the lab the next day.

Ovary harvest and transport

If a mare dies suddenly, or is euthanised, her ovaries can be harvested and transported to an approved laboratory for oocyte recovery. Alternatively, oocytes can be harvested from the ovaries and transported to a laboratory for further processing. Ovaries should be harvested as soon as possible post-mortem. Once the ovaries are removed, they need to be shipped to an approved laboratory facility for processing as quickly as possible. Research has shown that ovary processing times greater than 6 hours negatively impact the pregnancy rates achieved with harvested ovary oocytes.

Current recommendations for ovary transport:

- For transport times less than 2 hours, ovaries should be kept at body temperature (37° C).
- For longer transport times it is recommended to transport the ovaries at room temperature.
- For transport by car, place the ovaries in a bag containing either normal saline or if not available, a closed plastic bag. Place the bag with ovaries in a Styrofoam container with bottles or bags of water at room temperature around them. An Equitainer® may also be used and may be a preferred method due to constant temperature control.

- For transport by air, place the ovaries in an Equitainer® either in a bag containing normal saline or by themselves. Do not use the frozen coolant cans. These cans should be at room temperature for 24h before shipment. If they are unavailable, a rectal sleeve filled with water can be placed in the receptacle for the coolant cans.
- Ovaries should be shipped by the fastest method possible. For best results ovaries should be received within 6 hours of the mare's death. If ovaries are received >12 hours from death, oocyte viability is compromised significantly.

Postmortem oocyte retrieval and shipment

A more desirable method for managing oocytes in the post-mortem period is to collect oocytes from follicles and ship to a laboratory for ICSI procedures. It is recommended to use a small volume of embryo holding medium that is warmed to body temperature. Sterile technique is essential to avoid contamination. Visible follicles should be opened first using the scalpel blade so that the follicle is completely opened and the interiors of both halves entirely visible. Medium is aspirated from the cup into the syringe with a 20-gauge needle. A bone curette is used to scrape the follicular wall and the tissue is rinsed into a Petri dish with a stream of medium from the syringe. Using this method, the entire follicular wall is scraped until no more tissue comes off the curette. All visible follicles are opened and scraped and then the ovaries are sliced at 3-mm slices to open follicles within the parenchyma. If a follicle is big enough to see, it is also scraped.

Identification of oocytes after scraping is similar to identification after aspiration except that more tissue bits will be in the rinsed material. After identification, oocytes are placed in embryo holding medium for maturation procedures or transport to a facility for ICSI.

Intracytoplasmic sperm injection (ICSI)

Intracytoplasmic sperm injection, or ICSI, is a technique where an immobilised sperm cell is injected directly into an oocyte. While laborious, this technique has been broadly applied to produce pregnancies from oocytes collected from mares. Frequently, oocytes are injected with cryopreserved sperm which significantly simplifies the management of oocytes. Additionally, ICSI using cryopreserved sperm greatly extends the use of frozen semen straws as a portion of a straw can be cut off for ICSI, and the remaining straw portion is stored at -196°C for future use.

ICSI is performed using a mature oocyte in metaphase II that has been collected, directly, from a donor mare or from oocytes that have been matured, in vitro. A micropipette is used to select a single spermatozoon, which is immobilized by breaking its plasma membrane. A larger micropipette holds the oocyte in place by applying gentle suction. A Piezo drill is used to break the fragile oolema. The sperm is then injected directly into the cytoplasm of the oocyte. Embryos are cultured in vitro until the blastocyst stage when they are transferred to the recipient mare for cryopreserved for future use.

Embryo cryopreservation

With the onset of OPU/ICSI produced embryos, there has been an increased demand for cryopreserved embryos. Cryopreservation of embryos reduces the need for immediate transfer. However, there are significant limitations to cryopreservation of equine embryos. First, viability of embryos is higher with smaller embryos (< 300 μm). In order to obtain embryos less than

300 μm, embryo flush must be performed on days 6-6.5 of pregnancy. Recovery of embryos at this phase is reduced because the embryo has not entered the uterus from the oviduct or because the embryo is not located due to its small size. Second, pregnancy rates are lower (approximately 55%) when cryopreserved, thawed embryos are transferred vs fresh embryos (approximately 75-80%). While there are limitations to cryopreservation of equine embryos, the procedure has certainly increased options for obtaining pregnancies.

There are two methods for cryopreserving embryos: slow freezing and vitrification. With slow freezing, embryos are exposed to a cryoprotectant (i.e. glycerol) in a controlled, stepwise cooling process that involves increasing concentrations of cryoprotectant. Aberrations in timing can lead to ice crystal formation which impacts the viability of the embryo after thawing.

Alternatively, embryos are frozen using an ultrafast method called vitrification. Vitrification involves a rapid transition of intra- and extra-cellular fluids from liquid to solid state without forming ice crystals. Vitrification requires the use of high concentrations of cryoprotectant (i.e. 4-5 x greater than the concentration used for slow freezing) and rapid drops in temperature. While the overall procedure requires significantly less time, and expense, there is a small margin for error. This is especially true in the final step where the concentration of cryoprotectant is highest and the time exposure cannot exceed one minute before freezing. In spite of limitations, vitrification of equine embryos has gained popularity because it can be done, easily, in a practical setting and it is less expensive than the slow freezing method.

In vitro Fertilisation

In vitro fertilisation (IVF) is the incubation of oocytes with sperm for fertilisation and is commonly performed in many domestic species, including humans. Success rates for IVF in horses has been limited, largely due to effective, repeatable methods for sperm capacitation necessary for fertilisation. Recent advances by Felix and co-workers have led to more repeatable results for embryo production after IVF using both fresh and cyroperserved semen. With advances in this technique, a completely different arm of ART in horses will develop.

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Inflammatory conditions of the heart: endocarditis, myocarditis, pericarditis

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Inflammatory conditions of the heart occur sporadically. These conditions share the common clinical features of fever, poor appetite, lethargy and inflammatory leukogram.

Endocarditis

Endocarditis typically affects young horses, mean 2.12 ± 3.32 years, although it has also been reported in broodmares. Horses usually have a high, unrelenting fever and marked elevation in white cell count, in addition to a cardiac murmur. Endocarditis develops following haematogenous spread of bacteria to infect the cardiac valves, with *Actinobacillus, Streptococcus* and *Staphylococcus* spp. being most commonly involved. The aortic and mitral valves are affected more frequently than the tricuspid and pulmonary valves. The condition often affects more than one valve concurrently and leads to severe distortion of the valve, with thickening of the leaflets, fenestration or ruptured chordae tendineae. The diagnosis is confirmed with echocardiography, which can identify these features. Further confirmation and identification of the bacterial aetiology can be achieved with blood culture. Treatment comprises of anti-inflammatories, anti-microbials and ACE inhibitors. The prognosis is generally poor, but survival has been reported and the prognosis appears to be better for tricuspid endocarditis than mitral and aortic valve endocarditis.

Myocarditis

Myocarditis involves inflammation of the heart muscle (myocardium). Horses may or may not have a fever at the time of presentation. The inciting cause can be toxic (ionophore toxicosis, Hypoglycin A = atypical neuropathy) or inflammatory/immune mediated (especially following viruses or infection with *Streptococcus* sp.). Occasionally white muscle disease is reported in association with selenium deficiency and selenium deficient soils are encountered throughout Australia. Horses often have tachycardia and cardiac arrhythmias. The diagnosis is confirmed by measurement of cardiac troponin which is typically markedly elevated. Supportive echocardiographic findings include impaired cardiac contractility and myocardial wall thickening, in addition to subjective myocardial dyssynchrony. Advanced echocardiographic imaging with measurement of myocardial strain using doppler or speckle tracking will provide additional supportive information. Treatment consists of anti-inflammatories including corticosteroids and supportive care. The prognosis for survival is fair, some cases are likely to develop myocardial fibrosis which could impact return to exercise following recovery. Colchicine administration might assist in preventing this.

Pericarditis

Pericarditis causes pericardial effusion and thickening of the pericardium. Clinically, it is characterised by muffled heart sounds. The underlying cause can be idiopathic (non-septic) or septic, involving either bacterial (*Actinobacillus, Streptococcus* or *Staphylococcus* spp.) or viral infection. In the USA, an outbreak of pericarditis in broodmares was seen in association with the mare reproductive loss syndrome caused by processionary caterpillars. Signs are due to pericardial effusion and or restriction of the heart by the thickened pericardium, both of which can cause cardiac tamponade. Diagnosis is confirmed with echocardiography which shows pericardial effusion and thickening of the pericardium of varying severity. Treatment consists of drainage of the pericardial fluid. Anti-inflammatories,

including corticosteroids in non-septic cases are indicated. Analgesics are also necessary. Colchicine might also improve recovery and lessen restrictive pericardial disease. The prognosis is fair.

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When is a pregnancy "at risk" and what can be done about it?

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Introduction

Several conditions can jeopardise the pregnant mare. Conditions contributing to pregnancy loss in the late pregnant mare include infectious agents (viral, bacterial, fungal), twin pregnancies, foetal or placental abnormalities, structural abnormalities (hydropic conditions, ruptured prepubic tendon or abdominal wall herniation) and systemic illness. Managing the equine high risk pregnancy relies on many of the same premises as those employed for women. Determining the root cause of a condition affecting the late pregnant mare is central to proper management. Serial monitoring of the mare is critical to assess health and effects of treatment. Often, an acute insult begins the disease process but the symptoms become chronic. Management approaches must be modified to meet the needs of the mare and developing foetus. In all cases, the end goal is to facilitate adequate gestational length for foetal maturation and delivery of a healthy foal.

Examination and serial monitoring of the high risk mare

Physical examination findings are often the first signals of an abnormal pregnancy. Serial monitoring of the mare and foetus is useful to determine the progression of disease as well as response to treatment. Daily physical examinations can reveal aberrations in heart and respiratory rates, body temperature, mucus membranes, gastrointestinal sounds, digital pulses and ambulation. Appetite and alertness provide information about the overall status of the mare. Premature mammary development and production of secretions can indicate stimulation of the hypothalamic-pituitary-adrenal axis and initiation of parturition/foetal expulsion.[1] Vulvar discharge can reveal ascending bacterial placentitis or vaginitis. Musculoskeletal changes, such as lameness, obesity or abdominal enlargements provide important information regarding the care of the pregnant mare. As a part of the overall assessment of the pregnant mare, laboratory data can add additional information in pregnancy. Laboratory information that can provide additional information includes complete blood count, serum chemistry, blood lactate, hormone profiles (progesterone, oestrogens) and acute phase proteins such as serum amyloid A.[2] Mammary secretion electrolyte values provide information about foetal readiness for birth, impending parturition, and sometimes, response to treatment. [1,3,4]

Ultrasonographic tools to monitor the high risk mare

Serial transrectal and transabdominal ultrasound examinations provide real-time information regarding placental integrity, foetal fluid character and foetal health. A thorough ultrasonographic examination (transrectal and transabdominal) at admission provides baseline information that can be used for comparison in subsequent examinations. When possible, transrectal ultrasound evaluations should be performed twice weekly, or weekly, to assess subtle changes in the placental unit and fluids that can indicate progression or resolution of disease. The frequency of transabdominal evaluations for foetal well-being should be as often as possible. Ideally, every other day to once daily transabdominal ultrasonographic

assessments are performed in high-risk mares for real-time assessment of foetal health and viability.

Transrectal ultrasonography of the reproductive tract in late gestation is a useful tool for evaluating placental integrity (at the cervical star), foetal activity and foetal fluid character.[5] Gestational age can also be estimated from a transrectal ultrasound examination.[6] The caudal aspect of the allantochorion is the most frequently affected area in mares with ascending bacterial placentitis, therefore careful examination of this area is essential. Using transrectal ultrasonography, the combined uterine and placental (chorioallantoic) unit is measured. Values of the combined thickness of the uterus and placenta (CTUP) for mares with normal pregnancies have been established (Table 1).[5] Mares with placental infection or inflammation will have increased CTUP measures or separation of the membranes with presence of purulent material.[7] Additional information obtained with transrectal ultrasound evaluation of the caudal reproductive tract include foetal activity and fluid character. Allantoic fluid is generally anechoic (black) with occasional particulate "floaters" noted after foetal activity.[8] Amniotic fluid has a higher degree of cellularity which gives the fluid a light grey appearance.[8] The amniotic membrane, seen as a thin, undulating strand, separates the two compartments. Increases in echogenicity of either fluid compartment, and/or thickening of the amniotic membrane are abnormal changes that are often indicative of infectious conditions.

Gestational Day	CTUP (mm)
Day 271-300	< 8 mm
Day 301-330	< 10 mm
> Day 330	< 12 mm

Table 1. Normal values for ultrasound measurement of the combined thickness of the uterus and placenta (CTUP)[5]

Foetal health is best assessed through transabdominal ultrasonographic measures of foetal heart rate, tone, activity and size.[9,10] The simplest indicator of foetal well-being is foetal heart rate (FHR). The average heart rate in a foetus greater than 300 days gestation has been cited as 75 ± 7 bpm, with foetal heart rate slowing by approximately 10 bpm at greater than 330 days gestation.[9] Renaudin provided additional parameters for foetal heart rates throughout gestation: Days 100 – 250, FHR > 100 bpm; Days 250 – 300, FHR > 80 bpm; Day 300 on, FHR > 60 bpm.[8] Activity level can affect foetal heart rate throughout late gestation. Furthermore, significant individual variation in foetal heart rate occurs (Macpherson, unpublished observation). Consistently low (less than 55 bpm) or high foetal heart rates (> 120 bpm) are associated with foetal stress warranting re-examination and possible intervention.[9]

Foetal activity level and tone are easily determined while examining a foetus for heart rate.[9,10] Foetal activity can vary during the examination period as foetuses have periods of sleep and wakefulness. Foetuses are also very active after transport or prolonged exposure to the ultrasound beam. Foetal "tone" is a subjective term describing the viable foetus. A live foetus has excellent "tone" in that it actively flexes and extends the torso, neck and limbs.[9] An atonic foetus is flaccid and lies passively within the uterus. A non-viable foetus often folds in upon itself and traditional landmarks (ie, thorax, beating heart) may be obscured by flaccid, folded

limbs. As expected, definitively determining that a foetus is dead is substantially more difficult that identifying a live foetus.

Foetal fluid character can also be evaluated from a transabdominal approach. Ideally, ultrasonographic examination of pregnancy, including foetal fluids, is performed in the quiet mare and not immediately after transport or exercise. Echogenic particles (snowfall appearance) can be noted in allantoic fluid after maternal or foetal activity, but will resolve over time. Similar to transrectal ultrasound findings of foetal fluids, allantoic fluid is anechoic (black) while amniotic fluid is mildly to moderately echogenic (light grey) in a normal pregnancy. Fluids that are highly echogenic or consistently have flocculent material present often indicate infection. In mares with ascending bacterial placentitis, amniotic fluid echogenicity increases long before echogenic changes occur in the allantoic compartment. Thickening of the amniotic membrane also occurs more frequently in mares with ascending bacterial placentitis. In contrast, allantoic fluid character changes profoundly (more echogenic) in mares with nocardia-form placentitis, while amniotic fluid character is unchanged.

Hormonal Assays and Biomarkers

Physical examination and ultrasound findings, while somewhat insensitive, are the most reliable means to diagnosing conditions in pregnant mares. Hormonal assays and blood biomarkers have been investigated to provide additional information that can suggest aberrations in pregnancy and/or systemic health.

Progesterone assays

Several progestagens are synthesised by the foeto-placental unit to support pregnancy in the latter two thirds of gestation.[8] Progestagens are metabolites of progesterone (P4) and pregnenolone (P5). Ousey and co-workers used gas chromatography-mass spectrometry to measure serial concentrations of progestagens throughout pregnancy in mares with normal and abnormal pregnancies.[11] All progestagens, except progesterone (P4), rose gradually in mare serum as pregnancy advanced. Mares with placentitis had higher levels of P4, P5 and metabolites when compared to normal, pregnant mares. Mares experiencing stress other than placentitis (colic, laminitis) showed normal, or slightly lower, levels of P4 and P5 metabolites. Unfortunately, accurate measurement of progestagens requires sophisticated mass spectrometry equipment that is unavailable to practitioners. However, many P4 immunoassays (radioimmunoassay and ELISA) cross react with progestagens produced by the feto-placental unit, and some advocate serial measurements of progesterone to monitor pregnancy health.[12] Cross-reactivity of progesterone assays with progestogens must be validated for each commercial test used to measure progesterone.

Oestrogen assays

Oestrogens are broadly produced in equine pregnancy by the foeto-placental unit.[13] The foetal gonads produce oestrogen precursors, which are aromatised by the placenta to produce several oestrogens including oestrone, oestradiol 17α and β, equilin and equilenin. Total oestrogen production increases from approximately day 80 of gestation, peaks at 210 days of gestation and gradually decreases prior to delivery. High maternal serum oestrogen concentrations (usually measured as oestrone sulfate or total oestrogens) indicate a functional foeto-placental unit and are a strong indicator of foetal viability. The usefulness of this tool to detect and monitor pregnancy health has been less well defined than measurement of serum progestagens. Douglas reported that total oestrogen concentration greater than 1000 ng/mL

was consistent with a normal pregnancy between 150 and 280 days gestation.[14] However, an association was made in mares having total oestrogen concentrations less than 500 ng/mL and pregnancy loss. The value of measuring oestrogens as predictors of placentitis was recently investigated by workers at the University of Kentucky.[15] Oestrone sulfate and 17β oestradiol sulfate were measured in normal pregnant mares and mares with ascending placentitis. Oestrone sulfate was not different between groups. 17β oestradiol sulfate dropped rapidly after bacterial inoculation in mares suggesting that this hormone might provide diagnostic information from mares with placentitis. The authors postulated that hormonal changes in naturally occurring infections might be more subtle therefore accurate detection of 17β oestradiol sulfate in mares with placentitis might be more difficult.

Time resolved fluoroimmunoassay technology was recently used to assess steroid (progesterone and oestrogen) hormones in plasma from a large number of late pregnant mares.[2] Using this methodology, the authors measured progestin and oestrogen concentrations with anti-progesterone and anti-17β -oestradiol antibodies. Between 240 and 320 days gestation, mares with abnormal pregnancies had higher progestins and lower oestrogens than mares with normal pregnancies.[2] Further, the authors extrapolated cut off values for progestins and oestrogens at 20 day intervals from Day 200 gestation forward that distinguished normal from abnormal pregnancies. The authors postulated that a one-time measurement of progestins and oestrogens might be possible for predicting an abnormal pregnancy, particularly from Day 241 to 320 gestation.[2] These data suggest an alternative method for monitoring steroid hormones as predictors of abnormal pregnancy.

Biomarkers

Serum biomarkers that reflect the health of the foeto-placental unit are an area of great interest in both human and equine pregnancies. Acute phase proteins, such as serum amyloid A and haptoglobin (indicators of inflammation) have been examined in normal and abnormal equine pregnancies. Using ELISA technology, Coutinho da Silva and co-workers measured serum amyloid A in normal pregnant mares and mares with experimentally induced placentitis.[16] Serum amyloid A concentrations were shown to increase dramatically in the last 36 hours of normal pregnancy. Mares with placentitis demonstrated a premature rise in serum amyloid A. When serum amyloid A (SAA) concentrations were monitored in mares administered treatment for placentitis, concentrations declined in some mares, presumably as a response to treatment. In a similar study, SAA, haptoglobin, fibrinogen and white blood cell counts (WBC) were monitored in mares with normal pregnancies and mares with experimentally induced placentitis.[17] SAA and haptoglobin increased rapidly after experimental infection and stayed elevated until abortion. However, in contrast to the previous study, SAA (and haptoglobin) did not rise prior to foaling in normal, pregnant mares. When the same samples were evaluated using a stall-side serum amyloid A analyser (StablelabÒ, Zoetis Equine, NJ, USA) a rise in SAA was identified within 4 days of experimentally induced placentitis. (Macpherson, unpublished data) The difficulty when using SAA to identify/monitor mares with placentitis is that changes in SAA concentrations are fluid, therefore, sampling at a diagnostic point in time is challenging. Measuring SAA concentrations to detect abnormalities in equine pregnancy is best used as an adjunct to ultrasonography and clinical signs.

Alpha-fetoprotein, a protein member of the albuminoid superfamily, has recently been measured in foetal fluids and plasma from normal, pregnant mares and mares with experimentally induced placentitis.[18] The protein is produced by the foetal liver and has been

used as a predictor of abnormal pregnancy in humans.[19] It has also been suggested that alpha-fetoprotein is predictive of placental abnormalities/insufficiencies, in addition to foetal anomalies such as neural tube defects. Plasma concentrations of alpha-fetoprotein from mares with experimentally induced placentitis were elevated when compared to normal foaling mares.[18] Increased concentrations were most evident in the immediate days preceding delivery which limits the usefulness of the test as a diagnostic tool to initiate treatment for placentitis.

There has been increased interest in broad study of proteins (proteomics) in biologic and disease processes. Kentucky workers performed protein analysis on foetal fluids from a population of normal mares and mares with experimentally induced placentitis.[20] One hundred and thirty proteins were identified in one or both fluids. Unique proteins were more consistently identified in amniotic fluid. Amniotic fluid from mares with placentitis contained 3 highly expressed proteins (haptoglobin, plasminogen isoform X2 and plasminogen-like isoform 1).[20] Haptoglobin was also identified in allantoic fluid but not as consistently.[20] Some proteins were downregulated in amniotic fluid from mares with placentitis including members of the keratin family, para-aminobenzoic acid biosynthesis pathway (folate synthesis) and extracellular matrix proteins.[20]The significance of these findings has not been fully elucidated but may lead to improved diagnosis of placentitis (upregulation of foetal fluid proteins) and/or a better understanding of the pathophysiology of the disease (up and down regulation of proteins).

Treatment strategies for the high risk mare

Treatment of the late pregnant mare is directed at;

1) resolving/reducing the insult;
2) systemically supporting the mare and foetus
3) preventing early delivery.

Often, treatment is palliative. Diagnosis of the primary insult is essential to effective treatment. However, accurate diagnosis of a condition can be elusive, so empirical treatment is often elected based on clinical signs. A simple rule of thumb is to treat the mare to save the foal.

Supportive therapy is the mainstay for all conditions affecting the late pregnant mare. Fundamental to maintenance of a healthy pregnancy is appropriate nutrition, adequate perfusion and musculoskeletal stability. These goals can conflict at times, for instance with a laminitic or metabolic mare, so finding balance is essential. A general approach is to have the mare at ideal body weight, and at an increasing plane of nutrition, as she moves into her final trimester when foetal growth increases by approximately a half kilogram/day.[21] Generally, a well-balanced, highly digestible concentrate ration should be combined with free access to high quality forage. Rarely are supplements necessary. Many high-quality horse feeds are available for the pregnant/lactating mare, the majority of which are based on thorough research and development. Additionally, several dietary calculators are available online such as: https://www.purinamills.com/horse-feed/tools/horse-feed-calculator.

Integral to ideal body condition is the mare's ability to ambulate freely and without pain. A mare with a musculoskeletal condition poses a significant challenge in late pregnancy. Not only may she be unable to access feed freely, debilitating, chronic pain can drastically impair caloric intake. A general approach for managing horses with laminitis/musculoskeletal conditions is to maintain a low-moderate body condition to reduce stress on the laminae and preserve hoof integrity. While this goal is achievable in early/mid gestation, it becomes challenging in the end of gestation and early lactation. Constant monitoring of the mare's body weight and pain level

is required so that dietary adjustments are made to meet the energy demands during this time. Calorie supplementations, such as corn oil, may be required to keep the mare's body weight on target with her stage of gestation. Adequate pain control is essential to ensure maintenance of appetite and attitude.

Conversely, the pregnant mare with insulin dysregulation requires intensive dietary management and calorie restriction. High quality ration balancers, soaked hay and limited access to pasture may be required for the late pregnant, insulin-dysregulated mare even though dramatic calorie restriction is counter intuitive during pregnancy.

Medications in the pregnant mare

Safety data for commonly used equine therapeutic drugs (antimicrobials, anti-inflammatory drugs, sedatives etc) are limited in pregnant mares. Most drug dosing and safety studies for horses have been conducted in geldings, thus bypassing many physiologic changes that occur during pregnancy. The concerns when administering a drug to a pregnant mare are the impact on the developing foetus, as well as the mare. The foetus is particularly at risk given its poor ability to metabolise and eliminate most foreign compounds. Too little is known about placental metabolism of drugs in horses to expect that the placenta provides a barrier to the developing foetus. On the contrary, the presence of placental transport systems suggests that the placental gateway for molecules to access the foetus is greater than once thought. A growing body of work has been published in recent years regarding drugs that are commonly used to treat mares with placentitis.[22-26] As a part of these trials, placental transfer data of antimicrobial and anti-inflammatory drugs have been established (Table 2). Penicillin and trimethoprim sulfamethoxazole (TMS) achieved minimum inhibitory concentrations (MIC) against *Streptococcus equi zooepidemicus* in allantoic fluid of mares with induced placentitis, while gentamicin was detectable at concentrations effective to treat *Escherichia coli* and *Klebsiella pneumoniae* (also implicated in placentitis).[23,24] Pentoxifylline was detected in allantoic fluid of experimentally-infected mares, but flunixin meglumine was not. To date, it is unclear if the sampling method or the placenta were responsible for inability to detect this protein-bound drug. Similarly, ceftiofur sodium and ceftiofur crystalline free acid were not detected in foetal fluids or placental and foetal tissues after administration to pregnant mares.[26] Firocoxib, a potent Cox-2 specific prostaglandin inhibitor has been shown to achieve concentrations in foetal fluids as well as foetal and placental tissues after administration to normal mares.[27] Decision making for medication choices in equine pregnancy should be evidence-based, whenever possible, but many conditions necessitate empirical choices.

Drug	Drug Type	Dose/Route of Admin	[Plasma]	[Placental fluids]	[Foetus]
Potassium penicillin	Antimicrobial	22,000 IU/kg, IV q 6 hours	Equivalent to non-pregnant horses	Therapeutic concentrations	Not tested
Gentamicin	Antimicrobial	6.6 mg/kg, IV q 24 hours	Equivalent to non-pregnant horses	Therapeutic concentrations	Not tested
Trimethoprim sulphamethoxazole	Antimicrobial	30 mg/kg, PO q 12 hours	Equivalent to non-pregnant horses	Therapeutic concentrations	Yes
Ceftiofur sodium	Antimicrobial	4.4 mg/kg, IM q 24 hours	Equivalent to non-pregnant horses	Not detected or very low levels (allantocentesis)	No
Ceftiofur crystalline free acid	Antimicrobial	6.6 mg.kg, IM q 96 hours	Equivalent to non-pregnant horses	Not detected or very low levels (allantocentesis)	No
Doxycycline	Antimicrobial	10 mg.kg, PO q12 hours	Equivalent to non-pregnant horses	Detected (high concentrations?)	Yes
Enrofloxacin	Antimicrobial	5 mg/kg, IV 7.5 mg/kg, PO q 24 hours	Equal/higher non-pregnant horses	Therapeutic concentrations	Yes; Foetal cartilage unaffected
Pentoxyfylline	Anti-cytokine, Vasoactive, xanthine deriv.	8.5 mg/kg, PO q 12 hours	Equivalent to non-pregnant horses	Therapeutic concentrations	Yes
Flunixin meglumine	NSAID	1.1 mg/kg, IV q 12 hours	Equivalent to non-pregnant horses	Not detected (microdialysis)	Not tested
Firocoxib	NSAID	0.1 mg/kg PO q 24 hours (load 3x dose)	Equivalent to non-pregnant horses	Therapeutic concentrations	Yes
Phenylbutazone	NSAID	4.4 mg/kg, PO q 12 hours	Equivalent to non-pregnant horses	Therapeutic concentrations	Not tested

Table 2. Distribution of commonly used equine therapeutics after administration at recommended doses and intervals to pregnant mares.

Placentitis is the most common cause of pregnancy loss in the late pregnant mare (in North America), and the primary reason for medicating pregnant mares. Treatment strategies for mares with placentitis have been directed at addressing infection (antimicrobials), inflammation (anti-inflammatory) and uterine contractility (progestins). Several drugs that are commonly used in mares with placentitis are listed in Table 3. Data examining drug combinations that improve foetal survival suggest that curbing inflammation can be as important as treating infection.[27] This treatment strategy likely applies to all infectious conditions.

Drug	Dosage	Mechanism of action
Potassium penicillin	22,000 U/kg, IV, q 6 h	Antimicrobial
Procaine penicillin	22,000 U/kg, IM, q 12 h	Antimicrobial
Gentamicin sulfate	6.6 mg/kg, IV or IM, q 24 h	Antimicrobial
Trimethoprim sulfa	15 – 30 mg/kg, PO, q 12 h	Antimicrobial
Flunixin meglumine	1.1 mg/kg, IV or PO, q 12-24 h	Anti-inflammatory/anti-prostaglandin (mixed Cox-1 and 2)
Phenylbutazone	2.2 – 4.4 mg/kg, PO, q 12-24 h	Anti-inflammatory
Firocoxib	Administered on a per body weight basis	Cox-2 selective anti-inflammatory
Pentoxifylline	8.5 mg/kg, PO, q 12 h	Anti-cytokine/anti-inflammatory
Altrenogest	0.088 mg/kg, PO, q 24 h	Anti-prostaglandin/tocolytic
Acetylsalicylic acid	50 mg/kg, PO, q 12 h	Anti-inflammatory/anti-platelet

Table 3: Common therapies used to treat mares with placentitis

Delayed delivery and improved foal survival are important endpoints when treating mares with conditions threatening pregnancy. Foal viability after mares were treated with some drug combinations has been assessed using a model of ascending placentitis. Long term administration of TMS and pentoxifylline tended (P = 0.07) to extend gestational length in mares with placentitis when compared to infected, untreated mares.[28] However, foal survival was not improved in treated animals. When progestins (altrenogest; Regumate™) were added to the TMS and pentoxifylline treatment regimen, 10 of 12 (83%) mares delivered viable foals.[29] All five untreated, infected mares aborted or delivered non-viable foals. The treatment regimen of TMS, pentoxifylline and altrenogest has been broadly adopted in clinical practice with varying results. One explanation for variations in efficacy when treating mares for placentitis is the time interval from infection to initiation of treatment. In experimental studies, treatment protocols are implemented immediately after clinical signs are identified in carefully monitored mares. This approach is difficult to adopt in a clinical setting and subtle changes are often missed. Work by Carrick et al.[30] demonstrated the usefulness of early, aggressive ultrasonographic monitoring of mares in an "at risk" population and subsequent early initiation of treatment. In a stud farm population of "at risk" mares, they were examined starting at day 150 to 180 of gestation and were examined at least every 28 days. Treatment using TMS and altrenogest was begun at the first sign of ultrasonographic or physical abnormalities (vulvar discharge, mammary development) and continued until delivery. Live foal delivery rate in treated mares improved from approximately 25%, to over 80% in subsequent seasons using this approach. These data demonstrated that early diagnosis, and early treatment, of mares suspected of having placentitis improved live foal rate.

These concepts of early diagnosis/early treatment of mares with placentitis was further substantiated in a European study of over 4000 mares. Mares were routinely examined for viable pregnancy, using transrectal ultrasound, approximately two thirds into gestatation.[31] One hundred and seventy-seven mares (177/4192; 4.2%) were identified with varying degrees of ultrasonographically-detected placental changes that were consistent with

infection/inflammation. Treatment was initiated, immediately, with varying treatment protocols. Treatments included antimicrobials (TMS, ceftiofur), anti-inflammatory agents (flunixin meglumine, pentoxifylline, aspirin), tocolytics (altrenogest, clenbuterol), in different combinations. Live foals were delivered from the majority (160/177; 90.4%) of treated mares.[31] Furthermore, of affected mares that were bred the same season, 61.1% were pregnant. These data demonstrated two important points: 1. Early detection and treatment of mares with placental abnormalities resulted in a high percentage of live foals born; 2. Fertility of mares with abnormal pregnancies (detected by ultrasound) was within acceptable industry standards.[31] Interestingly, almost all treated mares were administered flunixin meglumine as an anti-inflammatory treatment. Early data, gathered with a microdialysis system, did not demonstrate presence of flunixin meglumine metabolites in the allantoic fluid of treated, pregnant mares. [24] Stated reasons for lack of the drug in the allantoic compartment were 1. The protein-bound drug was too large to pass through the pores of the microdialysis system, and/or 2. The drug did not penetrate the mare's placenta. Given that flunixin meglumine was the most consistently used anti-inflammatory agent in this study, it suggests that the drug is efficacious for treatment of placentitis. Another interesting finding from the same study was that many treated mares delivering live foals were administered ceftiofur (1 gram, IM; presumably ceftiofur sodium).[31] Data collected from foetal fluids and foetal membranes from pregnant mares that were administered ceftiofur products (ceftiofur sodium and ceftiofur crystalline free acid) showed minimal foetal membrane penetration of either ceftiofur product, suggesting that ceftiofur antimicrobials were a suboptimal choice for treating mares with placentitis.[26,32] While the ideal drug combination for treating equine placentitis remains under consideration, compelling information from large field studies provide strong evidence for routine monitoring, and possible treatment, of pregnant mares suspected of having placentitis.[30,31] However, one cannot ignore concerns over increasing microbial resistance to available medications. Therefore, antimicrobial treatments, whenever possible, should be selected based on culture and sensitivity results obtained from purulent discharge or the caudal reproductive tract.

Conclusion

Treating mares for conditions in late pregnancy is not recipe-driven. The challenge of determining the root cause of the problem, providing therapies that are effective and supporting/protecting the health of the mare and foetus is significant for equine veterinarians. Treatments are often empirical. Administration of therapeutic drugs to pregnant mares must be considered carefully and judiciously. Often, the equine veterinarian faces the dilemma of a mare that suffers a life-threatening condition which, ultimately, will threaten the life of the foetus/foal. Therefore, treatment plans for mares requiring pharmacologic intervention should be formulated with good sense and sound judgement. When possible, medications with few to no known adverse effects on a developing foetus should be used. The United States Food and Drug Administration (FDA) provides guidelines for drug use in pregnant women through categorization of drugs into Risk Categories. An adaptation (reprinted, Dr. Steeve Giguere, ACVMIM 2014) of the FDA guidelines, with some equine drugs included, is provided in Table 4. Finally, consistent monitoring of the mare and foetus is necessary so that therapeutic choices can be modified as needed. As is frequently the case, clear client communication is an essential component as outcome for either the mare or foal is often difficult to predict.

Category	Definition	Examples of Equine Drugs*
Category A	Adequate and well-controlled studies have failed to demonstrate risk to the foetus in the first trimester of pregnancy (and there is no evidence of risk in later trimesters)	
Category B	Animal reproduction studies have failed to demonstrate a risk to the foetus and there are no adequate and well-controlled studies in pregnant women	Azithromycin, erythromycin, penicillins, cephalosporins, Metronidazole, amphotericin B, omeprazole, cimetidine, ranitidine
Category C	Animal reproduction studies have shown an adverse effect on the foetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant the use of the drug in pregnant women despite potential risks	Clarithromycin, imipenem, gentamicin, fluoroquinolones, TMS, rifampin, fluconazole, itraconazole, ketoconazole, some NSAIDs, butorphanol, dexamethasone, prednisolone, fluticasone, albuterol
Category D	There is positive evidence from human foetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant the use of the drug in pregnant women despite potential risks	Tetracyclines, aminoglycosides (except gentamicin), some NSAIDs, ACE inhibitors, diazepam, phenobarbitol
Category E	Studies in animals or humans have demonstrated foetal abnormalities and/or there is positive evidence of human foetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits	Misoprostol

Table 4. FDA Pregnancy Risk Categories with selected examples of drugs commonly used in horses (Reprinted and adapted, S. Giguére, ACVIM 2014 Proceedings)[33]

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Diagnosis and treatment of periodontal disease

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Diastemata with periodontal disease is one of the most painful dental disease conditions in horses. It is very common and has been recognised as a significant disease for over 100 years. Although it is very common, it is often underdiagnosed by practitioners due to poor oral examination techniques and underestimating the severity of the disease. Horses rarely show clinical signs even in cases of severe dental disease, and by the time horses do show clinical signs, the disease is very advanced and most likely bilateral.

The most common aetiopathogenesis of cheek teeth periodontal disease in horses is secondary to diastemata with food packing which causes irritation, gingivitis and secondary bacterial involvement. This is unique and different from brachydont species where periodontal disease is regarded as a bacterial infection. Traditional treatments of periodontal disease in brachydont species include scaling and root planning, gingival flap surgery, and guided tissue and bone regeneration, but not all these are practical treatment options for periodontal disease in horses. Neither are all these indicated in horses, as treatment of the diastemata by removing food and preventing re-impaction, will result in resolution and healing of even deep periodontal pockets.

Horses' cheek teeth are rostro-caudally angulated to allow the teeth to push tightly against each other and work as a single functional unit. Diastemata may be primarily due to developmental issues such as teeth erupting far apart or insufficient angulation. This creates a larger interdental space allowing food to pack. Secondary diastemata are due to displaced or rotated teeth. Overcrowding in miniature horses or secondary to overcrowding will also result in abnormal interdental spaces with diastemata. Older horses often develop senile diastemata due to the natural tapering of the cheek teeth apically and smaller erupted crown size consequently.

Figure 1: A general overview of the oral cavity can give a good indication of likely periodontal disease; note the supernumerary 412 with displacement of the 410, 411 and 412 and food evident indicating the presence of diastemata

Localised treatment of deep periodontal pockets can be performed in horses by removing food and debriding the gingiva/ pockets (barbed endodontic files, periodontal forceps, flushing) and ultrasonic scaling of the affected dental surfaces to remove any secondary plaque and calculus. Periodontal medications placed within the pockets such as chlorhexidine, doxycycline or metronidazole has also been used in horses, although once again, very difficult to ensure that the medication stays in the pockets and does not address the main aetiology which is food packing in the interdental spaces. Temporary stenting or bridging of the diastemata with polysiloxane putty, Coe-Pak ™ or harder material such as bone cement or bis-acrylic composite material may help with preventing food packing and allowing the periodontal pockets to heal, although these are always temporary due to the hypsodont nature of horses' teeth and the natural occlusal wear.

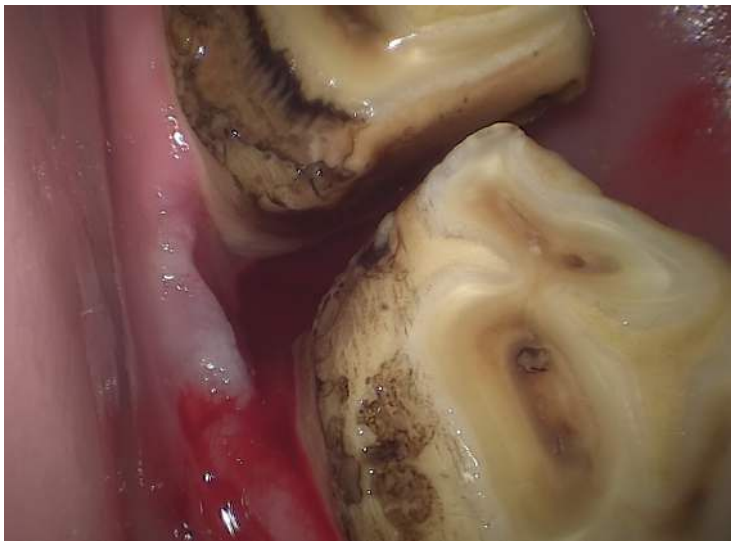

Figure 2: Deep periodontal pocket present after removal of food in this narrow open diastema of the 410/11

Remedial rasping to balance the mouth and remove sharp enamel points, with interdental occlusal odontoplasty (partial widening), changes the occlusal masticatory dynamics and reduces the food packing in the diastemata, and also promotes closure of the diastemata with natural drifting of the teeth together. This together with cleaning out all the impacted food are an essential part of the treatment of diastemata. Successful cleaning requires physical removal of food by using periodontal forceps and endodontic files to help pull out the impacted food. This also causes a degree of debridement of the mucosa/ gingiva. The use of a dental flush will only remove some of the food and used alone is not effective in removing all the food.

In severe diastemata cases in horses, surgical options such as extraction of the affected teeth, may be considered. Careful oroscopic assessment under sedation to determine degree of displacement, rotation or width of diastemata will help to determine if long term closure of the diastemata are likely. In cases where there is greater that 50% of the tooth width displaced, extraction is generally indicated. Radiographic assessment is required in cases with multiple displaced teeth, as this will indicate the teeth with the most severe periodontal inflammation/ disease. Rotation of greater than 30%, will result in irregular

apposition of the mesial and distal dental surfaces with severe food packing and deep periodontal pockets likely to develop and persist throughout the life of the horse. Dental radiographs in young horses with poor dental angulation and multiple open diastemata is essential to determine likely improvement with dental drift and once again, helps to assess the severity of the periodontal disease, with possible irreversible dental compromise. Digitally loose teeth, especially if in good apposition do not necessarily require extraction and in many cases, can be treated successfully.

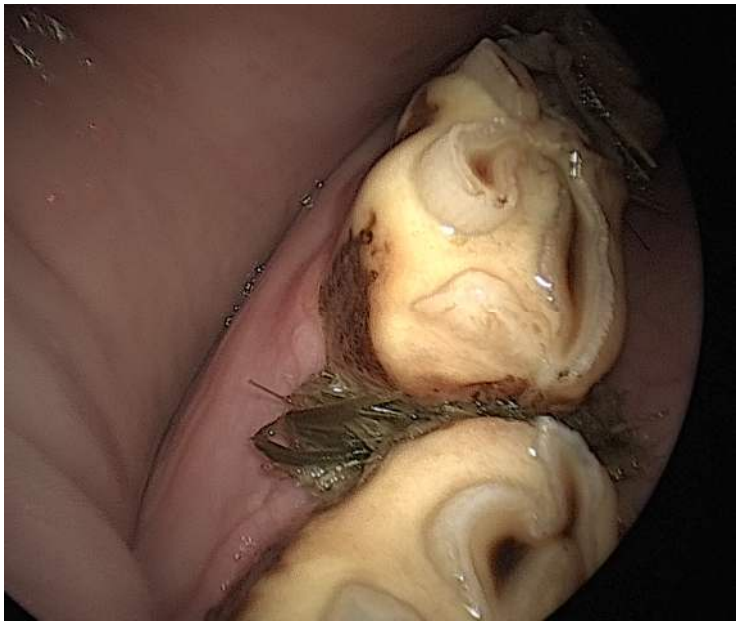

Figure 3: Narrow open diastema between the 307 and 308 with food packing secondary to lingual displacement of the 308

Surgical treatment options for diastemata cases are limited in horses, mostly extraction of the severely affected teeth. Thus, it is only indicated in cases of severe diastemata with deep periodontal disease. In many of these cases, long term resolution cannot be achieved without extraction of carefully selected teeth.

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Principles of endodontic treatment in incisors and cheek teeth

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Endodontic therapy in the horse is a technically demanding and rapidly evolving sub-speciality of equine dentistry. While clinicians can draw upon knowledge from human dental practice, success in equine endodontics depends on adapting those principles to the unique anatomy, pathology, and functional demands of equine hypsodont teeth. These challenges are particularly evident when comparing the treatment of incisors and cheek teeth, which differ substantially in structure, access, and pulpal architecture.

Anatomical and Morphological Foundations

Equine teeth are hypsodont and undergo continuous eruption and occlusal wear throughout life. This means that every layer of the tooth's laminated structure—including enamel, cementum, and dentine—will eventually become exposed at the occlusal surface. As a result, any restorative materials placed within the tooth must be able to withstand attritional forces, and the eventual occlusal exposure of previously subgingival restoration sites must be anticipated during planning.

A critical anatomical consideration in case selection is the Pulp to Tooth Volume Ratio (PTVR). In young horses, a high PTVR indicates that the pulp comprises a large volume relative to the surrounding tooth material. In such cases, a correspondingly large volume of filling material is required to obturate the pulp cavity, raising concerns about long-term mechanical integrity and increasing the likelihood of restorative failure. In older horses, pulp volumes are reduced due to secondary and tertiary dentine deposition, but calcification and canal segmentation can introduce significant technical difficulties.
Key anatomical factors relevant to endodontic treatment include:

- Reduction in pulp volume and increase in calcified dentine with age
- Development and closure of apical foramina (typically simpler in incisors, variable and delayed in cheek teeth)
- Segmentation of pulp canals in cheek teeth, with variable and reducing inter-pulpar communication over time
- Presence of sub-occlusal secondary dentine (SO2D), which may be thin or absent in pathological cases (loss by attrition and lack of formation from dead pulp)
- Anatomical differences in root formation between tooth types
- The presence or absence of biological apexification

These considerations directly influence case selection, technical feasibility, and long-term prognosis.

Pulpal Responses to Disease

The equine pulpodentinal complex remains active and responsive throughout life. The pulp, composed of vascular, neural, and connective tissue elements, can respond to pathological stimuli such as attrition, trauma, or microbial challenge through the deposition of tertiary dentine, including osteodentine.

- Reactionary dentine is laid down by surviving odontoblasts
- Reparative dentine (often osteodentine) forms when new odontoblast-like cells differentiate following injury

The extent and effectiveness of these responses determine whether a pulp remains vital or progresses to irreversible pulpitis and necrosis. Notably, equine teeth may experience

extensive disease progression without obvious overt clinical signs, especially in cheek teeth, where apical changes often present radiographically before clinical symptoms arise. The phenomenon of biological apexification—natural mineralised sealing of the root apex—can occur following pulpal necrosis, offering opportunities for single-stage treatment in some cases and improving overall prognosis if apical disease is well-contained.

Clinical and Technical Considerations

Endodontic treatment of incisors is generally more straightforward, involving single canals with defined apical foramina and direct access. Cheek teeth, however, present substantial challenges due to their caudal location, the limited oral opening of sedated horses, and their deeply positioned, curved or segmented canals. Instrumenting cheek teeth with Headstrom files, reamers, or rotary instruments is constrained by access, canal curvature, and anatomical variability. Most canal systems in cheek teeth do not allow straight-line access, increasing the risk of file separation, ledging, or perforation. Extended-length, flexible instruments are often required, and advanced imaging (particularly CT) is essential for treatment planning and risk assessment. Correct classification is crucial; it determines procedural complexity, material selection, the need for apical sealing, and whether a case is appropriate for treatment.

Materials and Techniques in Equine Endodontics

The success of endodontic procedures in the horse depends not only on anatomical understanding but also on the correct selection and application of dental materials. Unlike brachydont species, equine hypsodont teeth possess a grossly elongated reserve crown, and the pulp cavities being treated are usually within this subgingival crown structure, rather than a true root. Therefore, obturation materials must be capable of eventually withstanding functional occlusal forces as the tooth continues to erupt. For this reason, conventional materials such as gutta percha, suitable in human root canal therapy, are unsuitable in most equine cases.

Irrigants for canal disinfection include:

- Sodium hypochlorite (2–5.25%) – the gold standard for dissolving organic tissue and disinfecting
- Chlorhexidine (2%), iodine-potassium iodide (2%), and EDTA (15–17%) as adjunctive irrigants
- Sterile saline, hydrogen peroxide, or MTAD (human preparation – Mixture of Tetracycline, Acid, and Detergent)

Pulp medicaments, especially when treating vital or recently exposed pulp, may include:

- Calcium hydroxide pastes and hard-setting forms
- CaOH-releasing cements
- Mineral trioxide aggregate (MTA) and bioactive MTA analogues (e.g. Biodentine) for direct pulp capping and barrier formation

Obturation and restoration materials must have high compressive strength and adhesive capability. Suitable materials include:

- Glass ionomer cements (GICs)
- Resin-based composites, both flowable and packable
- Resin-modified GICs
- Novel bioceramic materials, MTA analogues, depending on case specifics

These materials are selected for their ability to bond to dentine, resist wear, and remain stable in the dynamic, eruptive environment of the equine mouth. Restorations often serve

dual roles—as biological seals and as load-bearing occlusal structures. Therefore, restoration planning must anticipate future eruption and attrition, ensuring materials are positioned and contoured to maintain functional integrity without impeding normal occlusal wear.

Prognosis and Ethical Considerations

Endodontic therapy has the potential to preserve equine teeth that would otherwise require extraction, maintain function within the dental arcade, and improve long-term welfare. However, success depends heavily on appropriate case selection, anatomical feasibility, technical execution, and realistic expectations. Clinicians undertaking these procedures must possess a thorough understanding of equine dental anatomy and pathology, the biological behaviour of pulp and dentine, and the principles of restorative material science. High-quality imaging, proper equipment, and extensive training are essential. Given the lack of a large equine-specific evidence base, ethical practice must be guided by transparency, informed owner consent, and a commitment to patient welfare. As techniques are refined and more outcome data becomes available, the field of equine endodontics will continue to evolve and mature.

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COMPARISON OF NOVEL AND EMERGING SPERM ISOLATION TECHNIQUES: EFFECTS ON STALLION SPERM MOTILITY, VIABILITY AND DNA DAMAGE

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Introduction

Successful adoption of equine assisted reproductive technology (ART) requires the isolation of high-quality, functional spermatozoa capable of fertilisation and the initiation of normal embryonic development. The aim of this study was to compare five different methods of sperm preparation and assess the differential effects on sperm quality.

Methods

Spermatozoa were collected from pony stallions and immediately extended with EquiPlus semen extender (n=12). Each ejaculate was split, and processed simultaneously as follows: simple centrifugation (CEN), density gradient centrifugation (DGC) through EquiPure (Nidacon), microfluidic processing using VetMotl™ (VetMotl Inc), electrophoretic isolation using the Felix™ System (Memphasys) and transmembrane selection using the Samson™ prototype (Memphasys). The isolated spermatozoa were then assessed in terms yield, viability, motility (CASA) and DNA damage (Halo).

Results

At 30 min after processing, total motility was highest ($p < 0.05$) in Samson™ (92%) and VetMotl™ (89%), with all four isolation methods resulting in higher motility than CEN (72.9%). The total percentage yield of motile and progressively motile spermatozoa was higher after Samson™, DGC and Felix™ than after VetMotl™ ($p < 0.05$). DNA integrity (% low damage cells) was highest ($p<0.05$) in Samson™, VetMotl™ and Felix™ (mean 89.1%) versus DGC and CEN (mean 79.5%). The total percentage yield of high-DNA integrity cells was highest in Samson™, Felix™ and DGC.

Relevance

This study provides novel alternative methods for successful isolation of equine sperm. This is particularly relevant with the increased uptake of ART by the equine breeding industry. Studies are underway to assess the impact of these methods on embryo development.

INJECTABLE ALTRENOGEST: HOW FREQUENTLY IS IT REQUIRED TO MAINTAIN EARLY PREGNANCY?

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Introduction

Altrenogest (ALT) is often used to support equine pregnancy maintenance. Injectable ALT products are safer and more convenient. Currently, there is limited evidence to suggest injectable products at the recommended ALT dosage (0.3 mg/kg) can maintain early pregnancy in mares with insufficient progesterone.

Methods

Efficacy of IM altrenogest was assessed in fourteen mares aged 8.5 ± 1.8 years, across several pregnancy cycles. Mares were inseminated with fresh semen 12-24h prior to ovulation. Pregnant mares were assigned to a treatment. Group A: two injections of ALT at 7-day intervals, n=10; Group B: two injections of ALT at 5-day intervals; n=10; and Group C: 0.044 mg/kg oral ALT once daily for 14 days (treatment control), n=7. Daily blood sampling and treatment commenced on Day 17 of gestation. Mares were administered a luteolytic dose of prostaglandin one hour after the initial ALT treatment to induce a progesterone deficiency. Pregnancy viability was monitored via trans-rectal ultrasonography four times a week until the end of the treatment period or a viable pregnancy was no longer detected.

Results

Following prostaglandin administration, progesterone decreased by 85.1 ± 4.7% within 24 hours and declined to < 2 nmol/L within 48 hours. Mares in Group B and C had an increased incidence of pregnancy maintenance (9/10 and 6/7 respectively) than those in Group A (3/10).

Relevance to clinical practice

Injectable ALT administered at 0.3mg/kg IM every 5 days was superior to the 7-day interval and comparable to daily oral treatment for pregnancy maintenance in mares with induced luteal insufficiency.

LINKING TECHNOLOGY, ACADEMIA AND INDUSTRY: OUTCOMES OF INTRACYTOPLASMIC SPERM INJECTION IN HORSES AT THE UNIVERSITY OF QUEENSLAND

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Introduction

Ovum Pick-Up (OPU) combined with Intracytoplasmic Sperm Injection (ICSI) is a powerful tool for propagating valuable stallion and mare genetics. We have developed a research-driven equine OPU-ICSI program at The University of Queensland to support industry uptake and application of this technology. This study describes the performance of the program during 2024 and 2025.

Materials and methods

OPU procedures were conducted on mares of varying breeds and ages by collaborative industry partners. Retrieved oocytes were transported to the laboratory within 24 hours and matured *in vitro*. Matured oocytes were injected with a single sperm via ICSI. Presumptive zygotes were cultured in the laboratory, and resulting blastocysts were vitrified or transferred fresh into synchronized recipient mares.

Results

A total of 141 OPU procedures yielded 1,196 oocytes, that were subjected to 41 ICSI sessions. Of these oocytes, 819 (68.5%) matured and ICSI was performed. Cleavage occurred in 539/819 (65.8%), with 22.6% (185/819) forming Day 7–8 blastocysts and 26.4% (216/819) by Days 9–10. The average was 1.53 blastocysts per mare. Outcomes improved from 2024 to 2025: cleavage rates rose from 61.8% to 72.5%, Day 7–8 blastocyst rates from 20.0% to 26.9%, and blastocysts per mare from 1.32 to 1.98. The overall 45-day pregnancy rate following transfer was 70%.

Relevance to Australian clinical equine practice

This academia–industry partnership has established a clinically effective OPU-ICSI program that improves fertility outcomes in high-value mares, enhances predictability in embryo production, and sets new performance standards for Australian equine practice.

RESULTS OF AN OPU/ICSI PROGRAM IN A SOUTHEAST QUEENSLAND REPRODUCTION CENTRE

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Introduction

Ovum Pick-Up (OPU) combined with Intracytoplasmic Sperm Injection (ICSI) is an increasingly utilised assisted reproductive technique in equine practice worldwide. This abstract presents outcomes from the first commercial OPU/ICSI program conducted at a reproduction centre in South-East Queensland, focusing on oocyte recovery, fertilisation, embryo development, and pregnancy rates, as well as breed and age group data.

Materials and Methods

From August–November 2024 and February–April 2025, 65 OPU sessions were performed on client-owned mares at WestVETS Reproduction Centre. Oocytes were collected via transvaginal follicular aspiration and shipped to the ICSI laboratory at the University of Queensland for fertilisation and culture. Frozen semen was used in 91% of procedures. The cohort included Quarter Horses (75%), Australian Stock Horses, Warmbloods, Shires, and Bucking Horses. Mares ranged in age from 2 to 25 years (mean 12.3). Blastocysts were vitrified, transferred fresh, or thawed and transferred, depending on season, recipient availability, and client preference.

Results

The average number of oocytes collected per mare was 8.5; an average of 5.7 were injected. The mean number of blastocysts produced per mare was 1.4. Early pregnancy rates averaged 79%.

Relevance to Australian Clinical Equine Practice

This program confirms OPU/ICSI as a practical and effective option for a range of breeds within Australian equine industries. The outcomes provide benchmarks for advising clients, particularly those with older or valuable mares, and highlight the importance of collaboration between clinics and ICSI laboratories in delivering advanced reproductive services.

PREGNANCY RESULTS AFTER ANTIBIOTIC AND NON-ANTIBIOTIC MEDICATION FOR POSITIVE UTERINE CULTURES

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Introduction

Thoroughbred broodmares are subjected to systemic and intra-uterine treatment guided by antibiotic susceptibility. Antimicrobial selection was compared to 45-day pregnancy results.

Materials and Methods

2947 uterine samples including swabs and uterine lavages were reviewed from the 2024 breeding season. 329 positive cultures were isolated, and pregnancy results recorded for 231 mares. All samples were subjected to routine antimicrobial testing. Non antibiotic treatments included systemic Settle®, intra-uterine administration of hydrogen peroxide, DMSO, and iodine. The most common isolates were *Escherichia coli* (E.coli) followed by *beta haemolytic streptococcus* (BHS) with pregnancy rates of 45% (37/83) and 54% (39/72) respectively.

Results

Higher pregnancy results for BHS positive cultures followed intra-uterine administration of DMSO (OR 2.1; p = 0.2), and systemic administration of Settle® (OR 1.2; p = 0.7). Intra-uterine administration of hydrogen peroxide (OR 0.27; p = 0.01) and penicillin (OR 0.36; p = 0.63) were not associated with an increase in pregnancy rate. *E.coli* In-vitro sensitivity to gentamicin was 47% (54/114) and was positively associated with an increase in pregnancy (OR 2.6; p = 0.07). No association was found with intra-uterine administration of cefazolin (OR 0.86, p = 0.75) despite in vitro sensitivity of 74% (84/114). No association was found with intra uterine administration of hydrogen peroxide (OR 0.35; p = 0.03), DMSO (OR 0.29; p= 0.01) or iodine (OR 0.42; p = 0.15).

Relevance to Australian clinical equine practice

Treatment of intrauterine infections are guided by culture and susceptibility results, however subsequent association with pregnancy rate is poorly understood. This study does not support the intra-uterine of peroxide at this stage with a significant increase in pregnancy rate reported regardless of pathogen detected. Further research is required regarding the dose and intra-uterine absorption of cephazolin, and combination therapies.

THE RETURN TO REPRODUCTIVE COMPETENCE OF THE THOROUGHBRED BROODMARE FOLLOWING CERVICAL REPAIR SURGERY

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Introduction

Thoroughbred broodmares may undergo cervical surgery to maintain reproductive competence, however, there has been little discussion regarding lesion location and post-operative prognosis. This paper abstract aims to quantify the prognostic value of repeat surgical intervention, population factors and lesion characterisation.

Materials and Method

This retrospective observational study includes Thoroughbred broodmares referred for cervical defect repair, between 2000–2020, at Rood and Riddle Equine Hospital. Each mare's surgery reports, age, fertility, lesion size, depth and location, time between live foals and repair history was collated.

Results

441 Thoroughbred broodmares underwent 559 cervical repair surgery. 68.06% of mares produced a live foal after their first surgery, 58.34% after the second surgery and 35.29% after the third surgery. The mean age at first repair was 11.77 years, and the average time between surgical repair and parturition was 14.91 months. 67% of lesions were located between 4'o'clock and 8'o'clock. The least common repair location was at 10'o'clock (5.1%).

Clinical relevance for Australian clinical practice

Broodmares who received surgery were likely to produce a live foal within 15-months. With each year increase in mare age at the time of first surgical repair, the likelihood of live foal production decreased by 14%. For each additional repair performed, the likelihood of producing a live foal decreased by 52%. Lesion characteristics were unlikely to affect live foal production unless located at the 10'o'clock position, which was associated with a 61% decrease in the likelihood of a successful reproduction. Defects repaired between 4'o'clock and 8'o'clock were not likely to affect postoperative foaling rates.

VALIDATION OF A SMART TEXTILE ECG DEVICE FOR USE IN RACEHORSES DURING STRENUOUS EXERCISE

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Introduction

Cardiac arrhythmias are an important cause of poor performance and may contribute to collapse or sudden death. As most arrhythmias are transient, continuous electrocardiogram (ECG) monitoring during exercise is essential for detection. This study evaluated the Myant Skiin, a novel smart textile ECG device, for recording high-speed exercise ECGs in racehorses.

Materials and Methods

Simultaneous ECGs were recorded using the Skiin and Televet devices in 25 Standardbreds and 16 Thoroughbreds during fast work. Maximum speed was recorded for each trial. ECGs were analysed in Kubios; artefact percentage was recorded, and arrhythmias were assessed by a blind observer. Diagnostic quality was compared using Chi-squared tests; mean peak heart rate (HR_{max}) was compared using intraclass correlation coefficient (ICC) and Bland-Altman analysis; arrhythmia agreement was evaluated using Cohen's Kappa and paired t-tests.

Results

Median peak speed was 13.85m/s (Standardbreds) and 17.30m/s (Thoroughbreds). Skiin ECGs had less artefact (0% artefact: Skiin =32, Televet =16; P=0.002). HR_{max} agreement between devices was excellent (ICC = 0.995; mean bias -0.47 bpm). Arrhythmias were detected in 89.6% (26/29) horses. Device agreement for arrhythmia classification was high (93.10%, k=0.8428). While no difference was found in complex vs isolated arrhythmias between breeds, Standardbreds had more premature depolarisations per trial (median 5 vs 2; P=0.015).

Relevance to Australian clinical equine practice

The Skiin device provides reliable, diagnostic-quality ECG recordings during high-speed exercise. Its wearable design enables track-side cardiac monitoring, facilitating arrhythmia detection in racehorses.

ANTIMICROBIAL RESISTANCE AND THE EMERGENCE OF EXTENSIVELY DRUG-RESISTANT ST39*BLACTX-M-15* IN *KLEBSIELLA PNEUMONIAE* ISOLATES FROM NON-REPRODUCTIVE EQUINE SAMPLES

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Background
Antimicrobial-resistant infections are becoming more common in equine practice in Australia. *Klebsiella pneumoniae* is a significant gram-negative pathogen associated with severe and antimicrobial-resistant infections in horses.

Methods
Clinical samples from various non-reproductive equine sources were submitted for diagnostic purposes over three years (2020-2022). Antimicrobial resistance profile and hypermucoviscosity phenotype of *K. pneumoniae* isolates were assessed through broth microdilution and string test. Whole-genome sequencing was performed to investigate genetic characteristics associated with antimicrobial resistance and virulence, as well genetic diversity.

Results
A total of 1,875 samples were submitted, excluding reproductive samples. Ten *K. pneumoniae* isolates were identified (isolation rate 5.3 per 1000 samples). Antimicrobial susceptibility testing revealed that resistance to a broad range of antimicrobials, including third generation cephalosporinses and fluoroquinolones, was common. Multi-drug resistance and extensive drug-resistance was identified in three and four isolates, respectively. *K. pneumoniae* ST39/KL149 was the most frequently identified lineage and was associated with extensive drug-resistance. Hypermucoviscosity phenotype was identified in two ST60/KL5 isolates. Antimicrobial resistance genes against various antimicrobial classes including tetracyclines, sulfonamides, diaminopyrimidines, phenicols, aminoglycosides, fluoroquinolones, β-lactamase and cephalosporines were identified. Furthermore, siderophores loci with clinical risk including yersiniabactin and salmochelin, were also identified.

Relevance to Australian clinical equine practice
The identification of antimicrobial-resistant and virulent *K. pneumoniae* isolates in horses holds significant implications for Australia's veterinary field. Detailed genetic characterization of these isolates improves our understanding of their pathogenetic potential and transmission risk.

THE PREDICTIVE ABILITY OF BLOOD-BASED BIOMARKERS TO DETECT BACTERAEMIA IN HOSPITALISED NEONATAL FOALS

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Introduction

Early and accurate identification of sepsis in neonatal foals improves survival. In human medicine, the neutrophil-to-lymphocyte ratio (NLR), neutrophil-to-monocyte ratio (NMR), monocyte-to-lymphocyte ratio (MLR), and plasma cell-free DNA concentrations (cfDNA) aid in early bacteraemia detection. This study evaluated the prognostic utility of these markers in hospitalised foals.

Materials and Methods

A total of 391 hospitalised foals ≤ 5 days of age with a complete blood count and aseptically obtained blood culture at admission were included from two equine referral hospitals. Physical examination and haematologic parameters, including white blood cell count (WBC) and immunoglobulin G (IgG), were incorporated into logistic regression models, with the area under the curve (AUC) used to assess predictive performance. Plasma cfDNA was measured via fluorometry.

Results

We found that plasma cfDNA, IgG, NLR, and WBC were independent predictors of bacteraemia, and a composite model demonstrated excellent discriminatory ability to identify foals with a positive blood culture (AUC = 0.806). Additionally, plasma cfDNA, IgG, and neutrophil counts were independent predictors of gram-negative bacteraemia, and a composite model demonstrated excellent discrimination (AUC = 0.807). Monocyte count and age predicted gram-positive bacteraemia with a composite model that demonstrated fair discriminatory ability (AUC = 0.67). The NMR and MLR appeared to have a limited relationship with blood culture status.

Relevance to Australian clinical equine practice

These findings suggest that NLR and plasma cfDNA are significantly altered in bacteraemic foals. Combining these markers with other clinicopathologic variables may enable early identification, timely interventions, and improved clinical outcomes in affected foals.

BLOOD CULTURE RESULTS AND ANTIMICROBIAL SENSITIVITIES FROM 87 CRITICALLY ILL NEONATAL FOALS IN SOUTH EAST QUEENSLAND (2009-2025)

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Introduction

Neonatal sepsis represents a major concern in equine medicine, requiring rapid diagnosis and informed antimicrobial selection to improve clinical outcomes. With rising antimicrobial resistance, the use of broad-spectrum antimicrobials must be guided by local sensitivity data to ensure effective treatment, whilst preserving responsible antimicrobial stewardship. This study evaluates bacterial isolates and sensitivity patterns from blood cultures of sick neonatal foals admitted to a referral hospital in South East Queensland.

Materials and Methods

A retrospective analysis was conducted on results of bacterial blood cultures collected from neonatal foals (≤7 days old) at admission to a referral hospital, between 2009 and 2025.

Results

Eighty-seven foals met the inclusion criteria, with a positive culture obtained in 42/87 (48.3%) submissions. A total of 50 bacterial isolates were recovered, comprising 26 Gram-negative isolates (26/50; 52%), and 24 Gram-positive isolates (24/50; 48%). Five of 87 submissions grew more than one bacteria (5.7%). The most frequently isolated bacteria were *Staphylococcus* spp. (15.7%), *Enterococcus* spp. (15.7%), and *Escherichia coli* (13.7%). A statistically significant increase in resistance to commonly used antimicrobials, particularly ceftiofur, was observed over time ($p = 0.02$).

Relevance to Australian clinical equine practice

Gram-negative and Gram-positive bacterial isolates were observed with equal frequency within this population. The observed increase in resistance to commonly used antimicrobials, particularly those deemed of critical importance in human medicine, underscores the need for culture and sensitivity testing to inform targeted, evidence-based therapy.

PREVALENCE AND PREDICTORS OF *SALMONELLA* SHEDDING IN HORSES IN SOUTH-EASTERN AUSTRALIA: A MULTICENTRE STUDY

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Introduction

Salmonella can cause diarrhoea, focal infections, systemic inflammation and death in horses. Hospital outbreaks can have serious financial and reputational impacts. In Australia, anecdotal evidence suggests higher prevalence in hospitalized horses with gastrointestinal disease.

Materials and Methods

A retrospective and prospective case-control study was conducted in five hospitals in Australia between January 2020-June 2024. A total of 2,060 horses were included. Faecal samples were collected for *Salmonella* culture or PCR at admission and during hospitalization. Clinical records were reviewed to determine prevalence, predictive factors, and outcomes. Ethics approval: A22437.

Results

The overall prevalence of *Salmonella* shedding was 18% (95% CI: 16.8–20.1%). Foals aged >2 weeks to <6 months were 1.6 times more likely to shed *Salmonella* compared to adult horses (P=0.004). Horses with surgical colic were most likely to shed *Salmonella* (P<0.001). Inappetence (P=0.02), pyrexia (P<0.001), antimicrobial use (P=0.02) and transport (P=0.04) prior to presentation and, rectal examination (P<0.001), tachycardia (P=0.003), inappetence (P<0.001), SIRS (P<0.001), antimicrobial use (P<0.001), surgery (P<0.01), and nasogastric tubing (P<0.001) during hospitalisation were associated with shedding. Predictive modelling revealed horses presenting with both diarrhoea and pyrexia were 3.4 times more likely to shed *Salmonella* (P=0.02) than those without these signs: either abnormality alone was not useful for prediction. *Salmonella* Muenster was the predominant serotype (67%). Horses shedding *Salmonella* were less likely to survive to discharge (P<0.001).

Relevance to Australian clinical equine practice

Horses with both diarrhoea and pyrexia, particularly surgical colic cases, should be regarded as high risk for *Salmonella* shedding. Biosecurity protocols should prioritize these patients.

CHANGES IN THE FAECAL MICROBIOME OF THOROUGHBRED YEARLINGS DURING PREPARATION FOR SALE

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Introduction

The intestinal microbiome plays a complex role in digestion of food, assisting in the host's nutrient acquisition as well as contributing to immune function. The preparation of Thoroughbred yearling horses for sale is a significant event which may coincide with relocation, changes to diet, treatment with preparative antimicrobials and equine contacts. Using a whole metagenome sequencing approach, we investigated changes in the microbiome and evaluated the distribution of antimicrobial resistance genes in the microbiomes of these horses during sale preparation.

Methods

A pragmatic cohort study was performed where faecal samples were collected weekly from 103 Thoroughbred yearling horses from three studs. All horses were undergoing intensive preparation for sale and preparation strategies differed in each of the studs. In one stud, all horses received prophylactic antimicrobials, and several horses received therapeutic antimicrobials. Extracted DNA from samples was subjected to Shotgun Illumina Sequencing.

Results

Preliminary analysis of alpha diversity (including Shannon and Richness indices) indicated diverse and rich bacterial community in all horses. There were changes in the microbiomes during preparation for sale, these changes were associated with relocation of horses and extended antimicrobial use. Evaluation of the microbiome is an important precursor to the evaluation of the distribution of antimicrobial genes in these horses.

Relevance to Australian clinical equine practice

Understanding how the sale preparation process enables movement of antimicrobial genes via the intestinal microbiome underpins the development of a knowledge-based approach to antimicrobial stewardship.

PROLONGED WIRELESS MEASUREMENT OF INTRAGASTRIC PH AND PHARMACODYNAMICS OF EXTENDED-RELEASE INJECTABLE OMEPRAZOLE IN HORSES

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Introduction

Adaptation of a wireless pH capsule for intragastric pH measurement in horses has a short and variable attachment duration. Prolonged intragastric pH measurement is important for interventional and pharmacodynamic studies. Extended-release injectable omeprazole (ERIO) induces acid suppression for ≤7 days, and 5-day dosing intervals are recommended for improved healing rates.

Materials and methods

A prospective interventional study was performed using 12 horses (Animal Ethics 2024/AE000419). Capsules were attached transendoscopically to the glandular mucosa using either one haemostasis clip (1HAEM; n = 4), four haemostasis clips (4HAEM; n = 5) or a helix tacking device (TACK; n = 9). Intragastric pH was recorded continuously until capsule detachment. ERIO (2g IM; 100mg/mL) was administered 24 hours after capsule placement. Data are reported as mean ± SD or median (IQR).

Results

Capsules remained attached and obtained data for 52.8 ± 12.3h with 1HAEM, 170.2 ± 100.5h with 4HAEM, and 126.4 ± 92.6h with TACK. The percentage of time pH was above 4 (%t pH >4) was 14.3% (7.1 – 23.5) and 82.0% (72.7 – 91.2) in the 24-hour period before and after ERIO administration, respectively. Complete recordings throughout the 5-day dosing interval were achieved in 6/12 horses; %tpH >4 exceeded 66% for 5 days after ERIO in 4/6 horses (66.7%).

Relevance to Australian clinical equine practice

These optimised, non-invasive attachment techniques enabled longer, more consistent intragastric pH measurement and demonstrated a treatment effect of ERIO. %tpH >4 exceeded 66% throughout the 5-day dosing interval in the majority of horses, similar to a previous report.

SERUM DIMETHYL ARGININE IN HORSES WITH CHRONIC KIDNEY DISEASE: A MULTICENTRE OBSERVATIONAL STUDY

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Introduction

In small animals, SDMA detects chronic kidney disease (CKD) with less reduction in glomerular filtration rate (GFR) than serum creatinine (sCr). In absence of studies on SDMA in horses with CKD, it is unknown if the same is true for horses. The objective is to document SDMA concentrations in horses with CKD and describe SDMA concentrations related to their clinical findings, sCr, estimated GFR reduction and CKD grading.

Material and methods

Files of adult horses with renal disease longer than 3 months were searched for clinical findings, SDMA and sCr concentrations. Estimated GFR reduction was based on sCr concentration and ultrasonographic loss of renal architecture. SDMA concentrations were reported for case categories based on clinical findings, sCr, estimated GFR reduction and CKD grading (Olsen, van Galen).

Results

Twenty-one horses were included. Four horses had a normal SDMA (<14µg/dL), five horses had a borderline SDMA (14-19µg/dL) and 12 horses had an increased SDMA (>19µg/dL). SDMA was >14µg/dL in all azotemic horses. Mean SDMA was higher in horses with clinical signs, urinalysis abnormalities, higher CKD grading and higher estimated GFR reduction. All 4 horses with normal SDMA had no azotaemia, grade 1 CKD and a <50% GFR reduction. Four horses had borderline SDMA, and 1 horse had elevated SDMA concentrations with <50% GFR reduction and no azotaemia.

Relevance to Australian clinical equine practice

This study shows that SDMA is not always increased in CKD. However, it suggests that SDMA is an earlier marker than sCr increasing with less GFR reduction.

THE LOOMING STREPTOCOCCUS MENACE: A SWINE PATHOGEN TROTTING INTO EQUINE TERRITORY?

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Introduction

Streptococcus porci was first identified in Spanish pigs in 2010, isolated from pericardium and lymph nodes. *Streptococcus porci* has taxonomic crossover with *Streptococcus suis* infection, which is an emerging zoonosis in Southeast Asia that can cause meningitis in humans by infection via transmission from pigs. Suspected *Streptococcus suis* transmission from horses has been reported. To our knowledge, this is the first reported case of *S. porci* equine infection.

Case History

A three-month-old Thoroughbred colt was presented for post-mortem examination following 4-day management on farm for lethargy, fever and neurological deficits. Pre-mortem cervical radiographs revealed increased radiopacity within the intervertebral foramina from C1 to C3. Twenty-four hours after radiography the foal was euthanised owing to obtundation and persistent recumbency with inability to stand.

Results

Pertinent post-mortem examination findings local to the central nervous system included purulent fluid within the cervical epidural cavity, and caseous purulent empyema of the caudal cranium. Less than half of the cerebral parenchyma was present. The post-mortem findings were consistent with diffuse caudal brain abscessation and parenchymal necrosis. *Streptococcus porci* was isolated from brain and cerebrospinal fluid microbial culture.

Relevance to Australian clinical equine practice

We describe the presentation and post-mortem examination findings of a *S. porci* meningitis in a foal, indicating that horses may serve as a potential reservoir for this pathogen and highlighting potential zoonotic risk. Meningeal *Streptococcus porci* infection has not been previously reported in horses to our knowledge. Owing to taxonomy crossover with *S. suis* and the risk it poses to humans, this report highlights the need for awareness, taking zoonotic precautions, reporting and potential surveillance of this emerging pathogen.

STEM CELL THERAPY PRESERVES LAMELLAR INTEGRITY IN HYPERINSULINAEMIA-INDUCED EQUINE LAMINITIS

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Introduction

Hyperinsulinaemia-associated laminitis (HAL) causes significant lameness in horses due to structural and inflammatory damage to hoof lamellar tissue. This study evaluated the therapeutic potential of cytokine-primed skeletal muscle-derived mesenchymal stem cells (M-MSCs) in an experimentally induced acute HAL model using four Standardbred horses.

Materials and methods

Hyperinsulinaemia was induced using a 36-hour euglycemic-hyperinsulinaemic clamp. The left forelimb received 1.5×10^7 TNF-α and IFN-γ primed M-MSCs via the median artery, while the right forelimb received culture medium only, served as control. Horses were euthanized 48 hours post-treatment. Analyses included histological, immunohistochemical, histomorphometric, and molecular assessments of tissue architecture, M-MSC localization, and inflammatory markers.

Results

Untreated laminitic hooves showed basement membrane degradation, elongated and narrowed secondary epidermal lamellae (SEL), and elevated pro-inflammatory markers (*COX-2, IL-1β, IL-6, IL-8, TNF-α*). M-MSC-treated hooves demonstrated preserved basement membrane integrity, improved lamellar architecture (shorter and wider SEL), and increased anti-inflammatory gene expression (*TSG-6, HO-1, IL-10*). M-MSCs were detected in proximal lamellar tissue vasculature of dorsal sections.

Relevance to Australian clinical equine practice

This pilot study suggests cytokine-primed M-MSCs may offer a novel therapeutic approach for HAL. The preservation of lamellar architecture and modulation of inflammatory responses indicates potential clinical value. However, larger cohort studies and clinical case evaluations are necessary to confirm efficacy and establish optimal treatment protocols.

THE A-PLATE TECHNIQUE FOR MANAGEMENT OF CARPAL ANGULAR LIMB DEFORMITIES

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Introduction

Angular limb deformities (ALD) are commonly observed and if the deformity is worsening or severe enough, surgical intervention is required. Surgical implants include a single transphyseal screw (STS) and screws and wire (S&W) as a temporary transphyseal bridge (TTB). This study describes the Carpal A-Plate; a custom-made, pre-contoured stainless-steel plate, used with 3.5mm locking screws. It was developed to reduce surgical time, minimise tissue trauma and avoid the risk of physeal dysplasia reported with the STS technique. The authors believe a higher rate of sepsis after the S&W technique than is reported in the literature.

Materials and methods

Clinical records of horses undergoing a TTB of the carpus using a Carpal A-Plate were obtained from two veterinary practices between 2020-2025. Variables included: limb(s) affected, surgical time and any complications noted at removal of the A-plate, or that were severe enough to have sought veterinary consultation.

Results

One hundred and four (104) horses were included in the study. Median surgery time for placement of a unilateral A-plate was 22.5 minutes and bilateral 40 minutes. The rate of complications was 16 of 104 cases (15%). Of these, 12 (11%) had surgical site infections (SSI's), three (3%) had over correction of the deviation, and one (1%) had physeal dysplasia causing failure of full correction.

Relevance to Australian clinical equine practice

This study describes a different surgical technique used successfully for treatment of carpal ALD. Although the SSI rate was 11%, there was no incidence of physeal collapse, commonly reported for STS. Surgical time was short, and the authors believe tissue trauma and difficulty in placing this implant was significantly less than S&W technique.

RETROSPECTIVE ANALYSIS OF THE LONG-TERM USE OF 2.5% INJECTABLE POLYACRYLAMIDE HYDROGEL (2.5% IPAAG) IN THOROUGHBRED RACEHORSE PRACTICE: SAFETY ON 701 HORSES, EFFICACY ON 214 HORSES

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Introduction
To evaluate the long-term safety and efficacy of 2.5% injectable polyacrylamide hydrogel (2.5% iPAAG; ArthramidVet®*) in Thoroughbred racehorses through retrospective analysis of clinical records.

Materials and methods
Medical records from a single Australian veterinary practice (2017-2023) were analyzed for Thoroughbred racehorses treated with 2.5% iPAAG. Safety assessment included 701 horses receiving 1,156 injections across 15,050 race starts. Efficacy analysis focused on 214 horses receiving multiple treatments. Performance was evaluated using Timeform ratings, and safety was assessed through catastrophic musculoskeletal injury (CMI) rates, adverse events and status at the end of the study period.

Results
The study documented a CMI rate of 0.0003 per 1000 starts (being 5 in total), significantly lower than the Australian industry standard (0.52/1000). Only two adverse events were recorded (0.17% of injections). The most frequently treated joints were mid-carpal (39%), front fetlock (33%), hind fetlock (9.5%), and tarsometatarsal (9.5%). Average treatment interval was 234 days. Performance data showed an initial improvement in Timeform ratings post-treatment, with maintenance thereafter with subsequent injections. At study conclusion, 38.8% remained in active training, with retirement reasons primarily non-lameness related.

Discussion
This large-scale retrospective study demonstrates that 2.5% iPAAG provides a safe option for long-term joint management in Thoroughbred racehorses, with minimal adverse events and maintained athletic performance. The low CMI rate and sustained racing careers support its clinical application.

Relevance to Australian clinical equine practice
These findings provide evidence for the safety and efficacy of 2.5% iPAAG as a long-term joint treatment option in high-performance Thoroughbred racehorses.

*ArthramidVet®, Innovative Medical Solutions, Cambridge, New Zealand.

USE OF A MONOPOLAR ELECTROSURGICAL TRIANGLE-TIP KNIFE FOR TRANSENDOSCOPIC SALPINGOPHARYNGEAL FISTULA CREATION IN THREE HORSES

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Introduction

Laser salpingopharyngostomy has been reported as an adjunct to medical management for the treatment of equine guttural pouch disease. There are some disadvantages to laser use, which include cost of equipment and potential health and safety issues for the user. This report describes the novel use of a monopolar electrosurgical triangle-tip knife for transendoscopic salpingopharyngeal fistula creation, as an alternative to laser surgery.

Case presentations

Three cases of guttural pouch disease are described. A 26-day-old Thoroughbred filly with severe guttural pouch tympany and empyema, a 6-month-old Thoroughbred filly with severe guttural pouch empyema, and a 9-year-old pony mare with guttural pouch mycosis. Transendoscopic salpingopharyngostomy was performed under standing sedation in all cases. A 9.2mm, 1.5m video endoscope (VET-9215 HD; Aohua) was advanced through the contralateral ventral nasal meatus to the pharynx. The guttural pouch orifice, surrounding mucosa and dorsal pharyngeal recess were topically anaesthetised using 20ml of 2% lidocaine. A monopolar electrosurgical triangle-tip knife (Olympus America, Center Valley, Pennsylvania) attached to a cautery unit (Valleylab force triad) was passed through the 2.8mm biopsy portal and applied in contact fashion to create a fistula through the dorsal pharyngeal recess into the affected pouches.

Clinical outcomes

All cases responded favourably to treatment. A marked improvement in drainage of mucopurulent material was facilitated in the cases with empyema. The procedure was well tolerated, and no complications were observed.

Clinical relevance to Australian equine practice

Transendoscopic salpingopharyngostomy with a monopolar electrosurgical triangle-tip knife is a successful economic alternative to laser management of guttural pouch disease.


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