42nd Bain Fallon Memorial Lectures

Conference Proceedings

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17-21 July 2022

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Bain Fallon Memorial Lectures History

The contributions of the following outstanding equine veterinarians to Australian equine medicine and surgery are acknowledged annually in the presentation of the Bain Fallon Memorial Lectures.

Andrew Murray Bain BVSC MRCVS MACVS

Murray Bain died in Scone in New South Wales on 18 March 1974 after a long and painful illness courageously endured. Murray graduated from the Royal (Dick) Veterinary School, Edinburgh, in July 1937. After service in the Royal Army Veterinary Corps in the Middle East during World War II, and followed by brief periods spent gaining experience of Thoroughbred breeding in Kentucky and New Zealand. He settled in Scone, NSW in 1950 where he worked until his death. In this time he built up a large group practice, based primarily on work with Thoroughbred horses and cattle.

His particular interests were infertility in the mare, diseases of newborn foals and many management problems associated with Thoroughbred breeding. He kept detailed records of all his cases and over the years published many authoritative papers based on his observations. He took an active interest in the postgraduate education and was one of the foundation members of the Post -Graduate committee in Veterinary Science of the University of Sydney. He was a gifted speaker and gave many interesting lectures to veterinary surgeons and horse breeders throughout Australia, New Zealand, America and Great Britain. He was awarded the Seddon Prize by the Australian Veterinary Association for his major contributions to clinical veterinary medicine.

Peter Fallon BVSC MACVS

Peter Fallon died suddenly at his home in Burwood, Victoria, on the 25th of June 1974 on the eve of his departure to attend the Equine Reproduction Symposium in Cambridge. A native of Christchurch, New Zealand, Peter studied Agricultural Sciences at Lincoln University, Christchurch, before transferring to The School of Veterinary Science, University of Sydney, where he graduated in 1948. Following a period as resident veterinary surgeon on two major Thoroughbred studs in Victoria, Peter established a private practice in Tallangatta in northeast Victoria in 1955. Three years later, he moved to Burwood in Melbourne where, up to the time that he died, he admirably served the veterinary needs of many important thoroughbred studs in Victoria and major racing stables in the metropolitan area of Melbourne. A man of great drive, energy and common sense, Peter was always outspoken in his opinions, which were always backed by more than 20 years of hard work, experience and keen observation on Thoroughbred breeding and racing. While not a man of the written word, he nevertheless frequently presented the results of his original research and clinical investigations in his own inimitable style at numerous meetings of veterinary surgeons throughout Australia. As a result, other veterinary surgeons engaged in equine stud practice greatly benefited from his long experience and clinical acumen.

Keynote Speakers

The keynote speakers for the Bain Fallon Memorial Lectures in 2022 are as follows:

Angus McKinnon

Angus graduated from Melbourne University Veterinary School.

After a short time in a mixed rural practice, he received an offer of a residency at the Ontario Veterinary College. As he was a slow learner, he was there for 5 years. That was followed by another 5 years at Colorado State University where he was fortunate enough to learn from the "the big three" in Equine Reproduction of Squires, Voss and Pickett.

After returning to Australia, he established the Goulburn Valley Equine Hospital with Jim Vasey. Recently the Hospital was sold to Melbourne University as part of their teaching program for students. Having the students present all the time and being responsible for their practical training has been a rewarding experience for all of the staff at GVEH.

Along the way he has learnt and forgot quite a few things. He has written many journal articles and published a few books on equine reproduction and ultrasonography. He never expected to be invited to speak again at Bain Fallon, not just because of bad behaviour but due to the frequency of previous invitations. He feels it is a nice gesture to be invited again on the eve of what probably heralds his movement into full time farming and thus he has titled his talks "things I have learnt along the way".

Brian Anderson

- BVSc with distinction 1986 (Massey University, New Zealand), MVSc in Equine Pharmacology and internship in equine medicine and surgery 1989-1991 (Massey University New Zealand).
- Resident in Large Animal Surgery and MS degree 1991-1994 (University of Minnesota USA).
- Diplomate American College of Veterinary Surgeons 1996 in Large Animal Surgery.
- Lecturer and Senior Lecturer in Surgery 1994-2000 Massey University.
- Brian joined the Ballarat Veterinary Practice in September 2000 and became a partner in 2003.
- Specialist in Equine Surgery Victoria, Australia since 2009.

Brian has many years of experience in upper respiratory tract surgery of the horse. He co-authored the chapter on the Larynx in Auer and Stick: Equine Surgery 4th edition. He was the endoscopic consultant for New Zealand Bloodstock Ltd 1996-2000 and has researched, published and lectured on diseases and conditions of the upper respiratory tract nationally and internationally.

Chelsie Burden

Dr Burden graduated from Kansas State University College of Veterinary Medicine in 2011. She subsequently completed an internship at Oklahoma City Equine Clinic followed by a residency and master's degree at the Colorado State University Equine Reproduction laboratory. Dr. Burden became board-certified with the American College of Theriogenologists in 2015. Dr Burden transitioned to Australia (Goulburn Valley Equine Hospital) following her residency. She continued transitioning between the southern and northern hemisphere breeding seasons working at Claiborne Farm in Kentucky, Haras Al Boraq in Morocco, and the University of Florida before settling in Australia in 2017. Dr Burden's interests focus on the problem broodmare and assisted reproductive technology in the mare.

Clinical Paper Abstracts

(In presentation order)

RIGHT DORSAL COLITIS IN HORSES: 33 CASES IN AUSTRALIA

J Flood[1], D Byrne[2], J Bauquier[3], G Ferlini Agne[4], JC Wise[5], CE Medina-Torres[1], K Wood[1], AJ Stewart[1]

[1]School of Veterinary Science, The University of Queensland, Gatton, Queensland
[2]School of Veterinary Medicine, Murdoch University, Murdoch, Western Australia
[3]Melbourne Veterinary School, University of Melbourne, Parkville, Victoria
[4]School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, South Australia
[5]School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales

Introduction

Right dorsal colitis (RDC) is a non-steroidal anti-inflammatory drug (NSAID) induced, protein losing enteropathy in horses associated with high mortality. Despite extensive NSAID use in Australia, reports of RDC are lacking.

Aim

To describe signalment, NSAID usage, clinical presentations, clinical pathology, ultrasonographic findings, treatments, and outcomes of horses diagnosed with RDC in Australia.

Methods

Clinical records of RDC cases were submitted by internists from Australian university teaching hospitals. Definitive diagnosis of RDC was confirmed by biopsy, surgery, or necropsy. Presumptive diagnosis of RDC must have been made by an internist, included a history of NSAID use and met ≥2 of the following criteria: diarrhoea with negative results for infectious diseases; colic with other differentials excluded or right dorsal colon mural thickening on ultrasound.

Results

The study included 33 horses. An overdose of NSAIDs occurred in 83% (19/23) of cases. The most common clinical presentations were diarrhoea (70%, 21/30), colic (62%, 19/31) and tachycardia (53%, 16/30). Common clinicopathological findings were hypocalcaemia (88.9%, 24/27), hypoalbuminaemia (75.9%, 22/29) and hyperlactataemia (76.6%, 13/17). The right dorsal colon wall appeared subjectively thickened in 76.7% (23/30) cases using transabdominal ultrasonography. Mortality rate was 39.4% (13/33).

Relevance to Australian clinical equine practice

Making a presumptive diagnosis of RDC is difficult due to non-specific clinical signs. NSAIDs should be administered within recommended dosage guidelines and client education should be encouraged. Horses with excessive or chronic NSAID administration should have regular monitoring of serum albumin concentration. Compared to previous literature, prognosis for RDC appears to be improving.

SURVIVAL FOR MARES AND FOALS REFERRED TO AN EQUINE HOSPITAL FOR DYSTOCIA: RETROSPECTIVE ANALYSIS OF 33 CASES ADMITTED BETWEEN AUGUST 2020 AND DECEMBER 2021

V Di Sevo, M Bellei, A Adkins, A Hodder, N Collins

Scone Equine Hospital, 106 Liverpool Street, Scone, Australia

Introduction

Equine dystocia is an emergency and threatens survival of foal and dam. This study reports survival outcomes and major peri-operative complications in this population of horses.

Materials and methods

The medical records of 33 mares referred a surgical facility for dystocia between August 2020 and December 2021 were reviewed. Upon presentation, cases were managed by Assisted Vaginal Delivery under sedation (AVD), Controlled Vaginal Delivery under general anaesthesia (CVD-GA) or Caesarean section (C-SEC). Decision-making regarding delivery method was performed by an equine surgeon and equine reproduction veterinarian. Upon delivery, foals were assessed by an internal medicine specialist and transferred to a medicine/ICU unit.

Results

Mares were 9.7 ± 3.9 years old (mean ± SD) and all were Thoroughbreds. Twenty-seven out of thirty-three mares admitted (82%) were discharged from hospital within 2-28 days. Nineteen foals (57.6%) were alive at birth, but nine were euthanised due to congenital defects or post-foaling complications. Ten foals (52.6%) survived to discharge. Foal survival-to-discharge rates for AVD, CVD-GA and C-SEC groups were, respectively 2/5 (40%), 4/7 (57.1%) and 4/7 (57.1%). Delivery method for discharged foals was AVD in 2/10 (20%) mares, CVD-GA for 4/10 (40%) and C-SEC for 4/10(40%). Survival-to-discharge rates for mares in the AVD, CVD-GA and C-SEC groups were respectively: 6/6 (100%), 11/14 (78.57%) and 10/13 (76.9%). Six mares were euthanised due to: 1) pre-existing conditions (2/6), 2) peri-operative anaesthetic or surgical complications (2/6) or 3) post-operative complications (2/6). Five (50%) of the ten foals discharged were born from mares travelling >25km to the surgical facility, and 5/10 (50%) were born from mares travelling distances >25km.

Conclusion

This study reports a survival-to-discharge rate of approximately 80% for mares admitted for dystocia in a referral hospital, and a survival-to-discharge rate for foals of approximately 50%. These results are comparable to previously published reports.

COLIC IN WEANLING AND YEARLING HORSES: 158 CASES (2010-2020)

AE Leather, NM Collins, LA Cudmore, CM Russell and RS Cuming

Scone Equine Hospital, Scone, NSW, Australia

Introduction

Colic evaluation in young horses can be challenging due to their size and variable pain responses. Publications specific to colic in weanlings and yearlings are limited and confined to disease-specific case series or inclusion in surgical studies of broader age cohorts.

Materials and methods

Records of weanlings (aged ≥6 months and <12 months) and yearlings (aged ≥12 months and <24 months) referred to Scone Equine Hospital for colic from 2010-2020 were reviewed. Data surrounding signalment, history, clinical examination and laboratory findings, lesion, medical/surgical classification and outcome were analysed.

Results

The inclusion criteria were met by 158 horses, 52% yearlings and 48% weanlings. The majority were surgical colic cases (53%). The most common surgical diagnosis was displacement of the large colon and transient medical colic was the most common medical diagnosis. Age was associated with colic classification ($p=0.002$), medical cases were yearlings and 59% surgical cases were weanlings. Severity of colic signs ($p<0.001$), total analgesics administered prior to referral ($p=0.001$) and abnormal ultrasound findings ($p=0.002$) were associated with surgical classification. Increased heart rate and colic severity were associated with non-survival ($p<0.001$). In total, 129 horses survived to discharge (82%); long-term survival (>2 years) of eligible horses was 95%.

Relevance to Australian clinical equine practice

This retrospective analysis of colic in weanlings and yearlings provides valuable information for practitioners treating colic in this age group and to assist decision making regarding referral. Short and long-term survival rates for young horses undergoing both medical and surgical treatment of colic are good.

ENDOGENOUS ADRENOCORTICOTROPIC HORMONE CONCENTRATIONS IN APPARENTLY HEALTHY HORSES AND PONIES IN AUSTRALIA

N Bamford[1], A Stewart[2], C El-Hage[1], F Bertin[2], S Bailey[1]

[1]Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, Australia
[2]School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia

Introduction

Measurement of basal adrenocorticotropic hormone (ACTH) concentration is the most commonly used screening test to diagnose equine pituitary *pars intermedia* dysfunction (PPID). This study aimed to investigate the influence of breed on basal ACTH concentrations in an Australian population of horses and ponies.

Materials and methods

Thoroughbred horses (n=127), Shetland ponies (n=131) and ponies of non-Shetland breeds (n=141) from Victoria were studied (Ethics Approval: 1914774.1). Inclusion criteria comprised: aged ≥8 years; no clinical signs consistent with PPID; no current illness, lameness, or medications; and no historical administration of pergolide mesylate. Paired blood samples were collected from all animals within 2 weeks of the autumn equinox and within 3 weeks of the subsequent spring equinox. Plasma immunoreactive ACTH concentrations were determined using chemiluminescent immunoassay (Immulite 1000).

Results

Based on recommendations from the Australian and New Zealand Equine Endocrine Group to interpret ACTH concentrations, during autumn (seasonal upper limit 120 pg/mL), no Thoroughbreds (0%) would have been classified as positive for PPID. However, 61 Shetland ponies (47%) and 33 ponies of non-Shetland breeds (23%) would have been classified as positive for PPID, despite the absence of clinical signs. In contrast, during spring (seasonal upper limit 40 pg/mL), every animal in the study was classified as negative for PPID.

Relevance to Australian clinical equine practice

Clinical correlation is recommended when interpreting basal ACTH concentrations. Given the high proportion of ponies with increased ACTH concentrations during autumn, breed-specific seasonal reference ranges and diagnostic cut-offs might be required to improve the diagnosis of PPID.

THE EFFECT OF SEPARATING EQUINE PLASMA FROM BLOOD CELLS TO IMPROVE SHORT-TERM ADRENOCORTICOTROPIC HORMONE STABILITY

T Johnston, AJ Stewart, KY Yuen, S Hinrichsen, EL Dryburgh, FR Bertin

School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia

Introduction

Pituitary pars intermedia dysfunction (PPID) is a common disease of older horses diagnosed by measuring adrenocorticotropic (ACTH) concentrations. ACTH is poorly stable when left on blood cells; therefore, it is common practice to transfer plasma collected in EDTA into serum (red-top) tubes for transportation. Some laboratories have, however, questioned if this procedure could affect the measured ACTH concentration.

Materials and methods

Blood was collected into EDTA tubes from 16 horses aged 12 to 27 years, including 9 PPID cases and 7 controls. After centrifugation, plasma was either transferred into a cryovial or into a serum (red-top) tube. Samples were stored at 4°C and 20°C with immunoreactive ACTH concentrations measured using a chemiluminescent assay at 2, 24 and 48 hours after collection. Data was analysed using a two-way repeated measured ANOVA.

Results

There was a significant effect of time on ACTH concentrations in PPID-positive horses at 4°C ($P = 0.02$) and 20°C ($P < 0.0001$), and in the overall population at 20°C ($P \leq 0.0001$) with decreasing ACTH concentration overtime; however, no significant effect of transferring plasma into a serum tube was detected in any group, at any time or at any temperature.

Conclusion

Transferring plasma into serum (red-top) tubes does not alter ACTH concentration and serum (red-top) tubes can be used when cryovials are unavailable. Collecting whole blood into an EDTA tube, separating plasma from blood cells, maintaining samples at 4°C and processing samples within 24 hours are recommended to reduce the risk of false diagnoses of PPID.

PROSPECTIVE ASSESSMENT OF CLINICAL SIGNS AND ADRENOCORTICOTROPHIN (ACTH) CONCENTRATIONS IN HORSES TRANSITIONING TO PITUITARY PARS INTERMEDIA DYSFUNCTION (PPID)

NC Kirkwood [1], KJ Hughes[1], AJ Stewart[2]*

[1]Charles Sturt University, Wagga Wagga, NSW
[2]The University of Queensland, Gatton, QLD
*Corresponding author

Introduction

PPID affects 20% of horses >15 years, with development of hypertrichosis, muscle wasting, polyuria/polydipsia and immune dysfunction. Elevated basal ACTH concentration is commonly used for diagnosis, but thyrotropin releasing hormone (TRH)-stimulated ACTH concentration has greater sensitivity, especially in detecting early disease. During the transition to obvious clinical disease, ACTH concentrations may be equivocal.

Materials and methods

Basal and TRH-stimulated (TRH-stim) ACTH concentrations were measured, and clinical signs recorded throughout a 3.5-year period on 7 horses identified as equivocal for PPID based on locally derived seasonally adjusted diagnostic-cut off values. Ethics approval: SVS/562/18.

Results

No horses demonstrated clinical signs of PPID at the beginning of the study. Hypertrichosis developed and basal ACTH increased in 2 horses, with 1/11 negative basal ACTH results in 2018 and subsequent positive results prior to normalisation with pergolide treatment. A gelding with 3/12 negative basal ACTH results in 2018, and variable TRH-stim results developed mild hypertrichosis. A mare with no clinical signs had elevated basal ACTH in winter 2019 and elevated TRH-stim ACTH in autumn of 2018, 2020 and 2021. A mare with no clinical signs had consistently elevated TRH-stim ACTH. A mare with no clinical signs had intermittently elevated basal ACTH but consistently elevated TRH-stim ACTH in February/March. A gelding with no clinical signs had intermittent elevated basal and TRH-stim ACTH and grade 2 pituitary hyperplasia at necropsy.

Relevance to clinical practice

Horses with subclinical PPID may have equivocal or intermittently negative results, but TRH stimulation testing in February or March identifies most transitional cases.

CUTANEOUS XANTHOMATOSIS ASSOCIATED WITH HYPERTRIGLYCERIDEMIA AND DIABETES MELLITUS IN A PONY

A Campbell, W Rogers, PG Harding, AM Cullimore

Ascot Equine Veterinarians

Introduction

Xanthomas are benign granulomatous lesions, containing lipid, that occur in the skin, subcutaneous tissues, tendons or internal organs of mammals. They are commonly associated with pre-existing lipid metabolism disorders and form secondary to hypertriglyceridemia. Reports of cutaneous xanthomas are rare in the horse.

Case History

A 10-year-old Welsh Mountain Pony presented to the referring veterinarian with multiple firm cutaneous masses over the pectorals and limbs. Histological analysis of the lesions was consistent with a diagnosis of cutaneous xanthomatosis. Initial bloodwork demonstrated a moderate hypertriglyceridaemia (67.1mmol/L) and hyperglycaemia (16.7mmol/L. The mare was also reported to have difficulty maintaining condition, despite a very good appetite. On referral, urinalysis demonstrated glycosuria and polydipsia was confirmed. Endogenous ACTH levels were normal. In-feed glucose testing demonstrated an inappropriate insulin response. A diagnosis of type 2 diabetes mellitus was subsequently made and treatment with protaphane insulin (0.75 IU/kg bwt, IM, bid) was commenced.

Clinical Outcome

Insulin therapy facilitated a marked decrease in serum triglycerides (within 5 days) and rapid resolution of the cutaneous lesions (within 3 weeks). Regular repeat measurement of triglyceride levels was continued for 18 months' post hospital discharge. Due to cost constraints, insulin treatment was reduced to once daily. Together with dietary management, triglyceride levels remained appropriately managed and there was no reoccurrence of the lesions.

Relevance to Australian clinical equine practice

Cutaneous xanthomatosis can be the first clinical presentation of severe dyslipidaemia. Successful management of the underlying primary disease is crucial. Skin biopsy is an essential component in the work up of such conditions.

EVALUATION OF AN *HMGA2* VARIANT CONTRIBUTION TO HEIGHT AND INSULIN IN A POPULATION OF AUSTRALIAN PONIES

BL Clark[1], NJ Bamford[2], AJ Stewart[1], ME McCue[3], SR Bailey[2], F-R Bertin[1], EM Norton[3]*.

[1]The University of Queensland, Gatton, QLD

Introduction

Ponies are at increased risk for insulin dysregulation (ID). A missense variant (G>A) in the *HMGA2* gene was previously identified as being correlated with height and insulin concentration in American Welsh ponies. However, the pleiotropic effect of this variant in other pony populations has not been investigated.

Materials and methods

Ponies of various breeds were evaluated for presence of the *HMGA2* variant and its correlation with height and insulin concentrations (University of Melbourne Animal Ethics 2015160.1). Ponies with suspected pituitary pars intermedia dysfunction were excluded from the study, resulting in 238 ponies with mean age of 15 years and representing 8 breeds including 120 (50.4%) Shetland, 66 (27.7%) Welsh, and 26 (10.9%) Australian ponies. DNA was isolated and genotyping assay performed for the HMGA2 variant. Baseline serum insulin was measured utilising an immunochemiluminescent assay. To account for censored data, Tobit regression was performed including covariates sex and age. Pearson's correlations coefficients were calculated between insulin concentration, height, and the additive effect of the A allele.

Results

The A allele frequency was 62% with 112 homozygotes A/A and 71 heterozygotes. There were statistically significant correlations between genotype and height (-0.35; p=2.71e-08), height and insulin concentration (-0.14; p=0.03), and genotype and insulin concentration (0.15; p=0.02). Thus, the A allele was correlated with both height and insulin concentrations, and its presence was not limited to Welsh ponies.

Relevance

A better understanding of genetic contributions to ID will help make informed decisions on how to reduce the risk of ID in Australian ponies.

PREDICTING ATRIAL FIBRILLATION IN RACEHORSES BY P-WAVE CHARACTERISTICS USING A 12-LEAD ELECTROCARDIOGRAM

S Macaspac, L Nath, S Franklin

School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy Campus, SA, 5371, Australia

Introduction: Atrial fibrillation (AF) is the most common cardiac arrythmia affecting athletic performance in racehorses. However, identification of "at risk" horses is challenging. P-wave characteristics have been used to predict AF from sinus rhythm ECGs in human patients. We aimed to compare the maximum (Pmax) and minimum (Pmin) P-wave duration and P-wave dispersion (Pd) in AF-affected racehorses and healthy controls.

Materials and methods: Horses with a history of AF (cases) were identified from stewards' reports. Control horses were matched for age, sex, breed, and exercise status. A five-minute, 12-lead ECG was recorded at rest, when in sinus rhythm. P-wave duration was measured in each lead for five cardiac cycles, and Pmax, Pmin and Pd calculated. Associations were compared using Pearson's correlations and independent t-tests, with statistical significance set at $P<0.05$.

Results: Forty horses (20 cases, 20 controls; 24 thoroughbreds, 16 standardbreds; mean age = 7.1±2.7 years) were examined. Cases had significantly higher Pmax (191.5±17.7ms vs 176.5±23.6ms; $p=0.02$) and Pd (67.4±11.8 vs 45.1±13.1; $p<0.001$) than controls. There was no significant difference in Pmin. A Pd of 53.8ms separated cases from controls with a sensitivity of 95% and specificity of 85%.

Relevance to Australian clinical equine practice: Pmax and Pd were increased in AF-affected horses, which may be attributed to atrial remodelling. Measurement of P wave parameters provides a simple, non-invasive method to accurately identify horses with a history of AF and may be useful to determine horses in sinus rhythm that are at risk of AF, prior to a clinical event.

OSTEOCHONDRAL NECROSIS OF THE FEMORAL CONDYLES IN THOROUGHBRED FOALS: 8 CASES (2008 – 2018)

J Pye, M Spriet, P Dow, S Katzman, B Murphy

Scone Equine Hospital Scone NSW 2337 Australia

Introduction

Necrosis of the femoral condyles is an infrequently encountered cause of severe hind limb lameness in neonatal foals. The objectives of this study were to describe the clinical, imaging, gross and histopathological abnormalities associated with osteochondral necrosis of the femoral condyles and to identify features suggestive of a common pathogenesis.

Methods and materials

8 Thoroughbred foals euthanised with a diagnosis of osteochondral necrosis of the femoral condyles were included. Affected distal femurs were collected, and medical records for each foal were retrieved. Postmortem computed tomography (CT) was performed to characterise lesions of cartilage and bone. The articular surfaces and epiphyses of distal femurs were examined grossly and histologically, focusing on lesions of interest identified on CT images.

Results

The majority of foals presented with one or more concurrent illnesses in addition to hind limb lameness. The characteristic antemortem radiographic and postmortem CT finding was a crescent-shaped osteochondral flap displaced from the affected medial femoral condyle. Synovial fluid cytology from affected joints was considered to be either within normal limits or consistent with mild inflammation. Histologically, all lesions were characterized by osteochondral necrosis and detachment of the articular cartilage. Inflammatory changes including osteomyelitis and polymorphonuclear cells in cartilage canals were found in 6/8 foals.

Conclusions and Clinical Relevance

Osteochondral necrosis was interpreted to be secondary to bacterial colonization resulting in osteochondral inflammation, although definitive evidence is lacking.

ADVANCED IMAGING OF STRESS REMODELLING IN THE DISTAL DORSAL ASPECT OF THE EQUINE THIRD METACARPUS

N Good, GCA Johnston, BJ Ahern, AC Young

School of Veterinary Science, The University of Queensland, Brisbane 4343, Australia

Introduction

Stress-related transverse fracture of the distal diaphysis/metaphysis of the third metacarpal bone (McIII) is a relatively rare, yet important, musculoskeletal injury affecting Thoroughbred racehorses. The presence of distal dorsal cortical stress injury is not well documented as a possible prodromal change for complete fracture propagation.

Materials and methods

A convenience sample of 21 horses euthanised on South-East Queensland racetracks were collected and all limbs examined with radiographic, computed tomographic and magnetic resonance imaging. Descriptive analysis was used to describe the imaging findings.

Results

Periosteal new bone growth was identified at the distal dorsal aspect of McIII in 17% (14) of the 84 limbs and ranged in severity. Three locations were identified: <1cm, centred at 1cm, and >1cm proximal to the distal dorsal sagittal ridge of McIII. No periosteal new bone formation was evident in the distal palmar cortex. One unilateral complete transverse fracture of distal McIII was identified with periosteal new bone evident in both the fractured and contralateral limb. None of these changes were evident in the hindlimbs. Radiography was adequate for the diagnosis of significant periosteal new bone growth, however sensitivity decreased dramatically for detection of subtle changes when compared to computed tomography.

Relevance

Periosteal new bone formation of the distal diaphysis/metaphysis of McIII occurs more frequently than described and does not always develop on the distal palmar cortex as previously reported in the literature. This information can be used to increase awareness of pathology in this location to promote earlier and more accurate detection of potentially catastrophic injury.

USING VETCOMPASS TO CHARACTERISE ANTIMICROBIAL USE IN HORSES WITH HOOF ABSCESSES

KE Bailey [1,2], LY Hardefeldt[1,2], BA Hur[1,2], GF Browning[1,2], JR Gilkerson[1,2]

[1]Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, University of Melbourne
[2]National Centre for Antimicrobial Stewardship, Peter Doherty Institute

Introduction

Currently, knowledge of how antimicrobials are used or the appropriateness of use in Australian horses is limited. Hoof abscesses are a common cause of acute lameness in horses for which effective treatment is largely dependent on establishing drainage and antimicrobial therapy is not indicated unless the patient is immunosuppressed, or cellulitis is present. This study aimed to identify the frequency of antimicrobial prescribing in horses with a hoof abscess using Australian VetCompass equine clinical records.

Materials and methods

Natural language processing (NLP) methods were used to label free text clinical records for the indication of an abscess. These records were then manually reviewed to identify abscesses involving the hoof and their antimicrobial prescriptions.

Results

The NLP methods extracted 2,132 abscess records (1,742 horses) in which a clinical finding of a hoof abscess was manually identified in 1117 records (928 horses) from 21 clinics. No complicating factors were documented in 940 hoof abscess records, but 54% of these had antimicrobials prescribed. Of the 680 records specifying the hoof abscess drainage status, antibiotics were more frequently prescribed than not in those already draining (70%) and those where drainage was established (52%). In non-draining abscesses, less than half (43%) were prescribed antibiotics. The most frequently prescribed antibiotics were procaine penicillin, trimethoprim sulphonamides and long-acting penicillin.

Relevance to Australian clinical equine practice

Although predominantly low importance antimicrobials were prescribed, most records had no complicating factors documented and thus antimicrobials were likely not indicated. This study identified a need to develop antimicrobial stewardship resources to target improved prescribing for equine hoof abscesses by Australian veterinarians.

SUSCEPTIBILITY OF EQUINE PATHOGENS TO CONCENTRATIONS OF GENTAMICIN ACHIEVABLE IN SYNOVIAL FLUID

RL Saltum[1], LY Hardefeldt[1,2], RN Bushell[1], JR Gilkerson[1,2], MS Marenda[1] and KE Bailey[1,2]

[1]Asia-Pacific Centre for Animal Health, Department of Veterinary Biosciences, Melbourne Veterinary School, The University of Melbourne
[2]National Centre for Antimicrobial Stewardship, Peter Doherty Institute

Introduction

Studies measuring synovial concentrations of gentamicin following concurrent intravenous and intra-articular (IA) administration in horses have reported synovial concentrations from 5,720 to 64,535 µg/ml. However, antimicrobial susceptibility testing uses clinical breakpoints based on antibiotic concentrations achieved in serum after systemic administration. For example, the Clinical Laboratory Standards Institute minimum inhibitory concentration (MIC) breakpoint for gentamicin against *Enterobacteriaceae* is <2 µg/ml. Thus, systemic breakpoints, that do not account for the much higher synovial concentration achievable with IA administration, may lead to isolates being wrongly identified as resistant, resulting in use of higher importance antimicrobials such as amikacin. This study aimed to investigate whether clinical equine *Escherichia coli* (*E. coli*) and staphylococcal isolates that appear resistant to systemic concentrations of gentamicin were susceptible to higher concentrations achievable in synovial fluid.

Materials and methods

The MIC was determined using two-fold serial dilutions of gentamicin from 1.0 to 1024 µg/ml against a collection of six *E. coli* and 27 staphylococci previously classified as resistant to gentamicin using the calibrated dichotomous susceptibility method.

Results

The highest MIC for *E. coli* was 128 µg/ml and for staphylococci was 256 µg/ml. All isolates had an MIC value more than 10 times lower than the synovial gentamicin concentration of 5,720ug/ml reported in the literature.

Relevance to Australian clinical equine practice

These results provide preliminary support for the use of a higher synovial breakpoint as a more accurate estimate for the susceptibility of equine synovial pathogens to gentamicin. Further testing of a larger number of equine synovial bacterial isolates is needed to establish the most appropriate synovial breakpoint.

ALPHAVIRUS INFECTIONS IN HORSES IN SOUTHEAST QUEENSLAND

KY Yuen[1], J Henning[1], A Sole-Guitart[1], L McMichael[1], MF Lenz[2], K Caldwell[2], M Coyle[3], H Bielefeldt-Ohmann[1,4,5]

[1] School of Veterinary Science, The University of Queensland, Gatton, QLD
[2] Queensland Racing Integrity Commission, Albion, QLD
[3] UQGC Equine Unit, The University of Queensland, Gatton, QLD
[4] School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD
[5] Australian Infectious Diseases Research Centre, The University of Queensland, St. Lucia, QLD

Introduction

Tentative diagnosis of Ross River Virus (RRV) infection is common in poorly performing racehorses and horses with non-specific viral infection. However, many Old-World alphavirus infections exhibit non-distinguishable clinical signs and the prevalence in horses in southeast Queensland remains unknown.

Material and methods

Blood samples were collected from racehorses in southeast Queensland, Townsville, and Rockhampton in 2019-2020 (n=579), and horses located at The University of Queensland (UQ) Gatton Campus in 2018-2020 (n=146), and screened by virus neutralisation test to determine seroprevalence for RRV, Barmah Forest virus (BFV), and Sindbis virus (SINV), and cross-reactivity to Getah virus (GETV).

Results

In the racehorse population, the seroprevalence for RRV was 40% (231/578), 4.5% (25/559) for SINV, and 1.4% (8/570) for BFV. Of the RRV-positive samples, 69% (161/231) had a titre ≤ 1:160; and 88% (196/223) cross-neutralised GETV. Moderate correlation (Pearson correlation coefficient = 0.70) between RRV and GETV antibody titres was identified. In the UQ horse population, RRV seropositivity reached 78% (72/94) in 2018, of which 67% (48/72) had an antibody titre to RRV of at least 1:160. Sixty percent (15/25) of horses that were born at UQ between 2017-2019 sero-converted to RRV by 1.5 years of age.

Relevance to Australian clinical equine practice

Understanding the seroprevalence of alphavirus infections will guide the identification of risk factors and the role of horses in the epidemiology of RRV infection. Determination of cross-reactivity between alphaviruses allows further investigation into the use of an existing alphavirus vaccine in an alphavirus outbreak situation.

PILUS DIVERSITY IN AUSTRALIAN *RHODOCOCCUS EQUI*

L Wilhelm, J Sung, G Muscatello

Faculty of Science, The University of Sydney

Introduction

Rhodococcus equi (R. equi) is considered one of the most economically significant diseases in the equine industry, causing bronchopneumonia in foals. Pili are typically associated with adhesions to cells or the movement of genetic material between bacterial cells. *R. equi* pili may play roles in immunorecognition and virulence, but their actual function is unknown. Here we describe the first molecular investigation into the presence and variability that exists in virulent *R. equi* pilus determinants.

Materials and methods

This study investigated 45 virulent *R. equi* isolates from foals with pneumonia across NSW and Victoria. PCR protocols were developed to detect and genetically characterise the *RplB* pilin subunit gene. PCR products were genetically sequenced and compared to detect variation in the *RplB* gene. Fisher's exact test was used to explore any associations between pilus alleles and farm or strain type.

Results

Four pilus subtypes were determined based on their allelic variations in the hydrophobic domain of the pilin subunit. Farms often had multiple pilus subtype strains present, and there was a statistical association ($P < 0.05$) between prominent pilus subtypes and restriction fragment length polymorphism (RFLP) *R. equi* strain types.

Relevance to Australian clinical equine practice

The relevance of pilus variability in circulating strains of virulent *R. equi* on Australian stud farms cannot be determined, as the biological and pathological significance of *R. equi* pili have yet to be determined. However, the diversity in the cytoadhesive *R. equi* pilus must be considered if pilus-based vaccine strategies are to be developed in the future.

FATAL HENDRA VIRUS DISEASE IN A HORSE IN 2015 DUE TO A PREVIOUSLY UNRECOGNISED HENDRA VIRUS VARIANT ASSOCIATED WITH MULTIPLE FLYING-FOX SPECIES

EJ Annand[1,2,3,4], BA Horsburgh[5,6], PA Reid[7], B Poole[8], N Brown[1], MC de Kantzow[9], K Xu[10], A Tweedie[1], M Michie[3], J Grewar[10, 11], M Tachedjian[12], A Jackson[1,], N Singanallur[13], K Plain[1,2], I Diallo[14], L Jackson[14], C Secombe[15], ED Laing[16], S Sterling[16], L Yan[16], C Jones[6,17], AC Breed[9], PN Britton[6,17], CC Broder[16], NK Dhand[1,2]*, IL Smith[3]*, JS Eden[5,6]*

1. University of Sydney, School of Veterinary Science, Sydney, NSW, AU; 2. University of Sydney, Marie Bashir Institute for Infectious Diseases and Biosecurity, Sydney, NSW, AU; 3. CSIRO Health and Biosecurity, Black Mountain Laboratories, Canberra, ACT, AU; 4 EquiEpiVet, Geelong, Vic, AU; 5 West Mead Institute of Medical Research, Sydney, NSW, AU; 6. University of Sydney, Sydney Medical School, Sydney, NSW, AU; 7. Private Equine Veterinary Practice, Brisbane, Qld, AU; 8. Cooroora Veterinary Clinic, Cooroy, Qld, AU; 9. Department of Agriculture, Water and the Environment, Animal Health Branch, ACT, AU; 10. Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; 11. JData, Cape Town, ZA; 12. University of Pretoria, Pretoria, ZA; 13. CSIRO Health and Biosecurity, Australian Centre for Disease Preparedness, Geelong, Vic., AU; 14. Biosecurity Sciences Laboratory, Queensland Department of Agriculture, Brisbane, Qld, AU; 15. Murdoch University, School of Veterinary Medicine, Perth, WA, AU; 16. Uniformed Services University of the Health Sciences, Microbiology and Immunology, Bethesda, Maryland, USA; 17. The Children's Hospital at Westmead, Infectious Diseases, Sydney, N.S.W., AU. * Co-senior authorship.

Background

Hendra virus (HeV) causes severe acute respiratory and encephalitic disease mediated by endothelial vasculitis in horses and humans. Sixty-three natural spillovers have been detected resulting in 105 horse deaths, seven human cases, and four human fatalities. Around 1000 horses are tested annually for HeV, mostly from regions within the ranges of the black flying-fox and the spectacled flying-fox, and less than 0.5% of these cases test positive. Causative diagnosis is rarely identified in the remaining cases, including those with high indications of an infectious cause. We hypothesised that some severe equine diseases consistent with HeV, but negative when tested, were due to infection with other paramyxoviruses spilling over from flying-foxes to horses, and plausibly posing zoonotic risks.

Methods

A biobank of samples from horses tested for HeV by the Queensland Biosecurity Sciences Laboratory was constructed and found to be negative. Subject, geographic, clinical and sample details and results were captured using a purpose-built, de-identified SQL database. Cases were classified via a syndromic disease decision algorithm according to likelihood of infectious cause to guide selection and processing for subsequent batched molecular and serological testing pathways that included meta-transcriptomic next-generation-sequencing (RNAseq-NGS) coupled with nested-conventional-PAN-Paramyxovirus RT-PCR screening and a 33-plex microsphere immuno-serological assay screening for both IgM and IgG against emerging infectious diseases.

Results

A previously unrecognised variant of HeV was detected in a case of acute severe disease in a 12-year-old Arabian gelding clinically indistinguishable to that caused by the prototypic HeV. The case, which occurred in September 2015 near Gympie in South-East Queensland, presented clinically with severely 'injected' mucous membranes, tachycardia (75 bpm), tachypnoea (60 bpm), rectal temperature of 38.0°C, muscle fasciculations, head pressing and recumbency, with rapid deterioration over 24 hours resulting in euthanasia on humane grounds. A complete viral genome sequence was obtained by RNAseq-NGS from the blood sample. While the variant is sufficiently consistent in amino-acid sequence and protein structure to be of equivalent pathogenicity (mean 92.5% phenotypic consistency), it was sufficiently divergent in nucleotide sequence (82.9% genotypic similarity) to fail detection by standard molecular surveillance testing for HeV. Updated PCR approaches for routine use to detect both this variant and the formally-known HeV were developed, optimised and made available to state and national human and animal health laboratories. *In-silico* analysis of the variant's receptor-binding protein (RBP or G glycoprotein), using established x-ray structures of the HeV RBP, revealed unaltered epitope structures relevant to the binding of both the Ephrin-B2 & -B3 virus entry receptors and the monoclonal antibody mAb 102.4 used as post-exposure prophylaxis in humans. These findings support the observed consistent pathogenicity and indicate

that equivalent immune protection should be afforded by the Equivac® HeV vaccine against this variant. Comparison of a partial viral sequence identified from a grey-headed flying-fox from Adelaide in January 2013 and genomic sequence of this HeV variant (2015 horse) showed them to be >99% similar.

Recommendations

Equine veterinarians should consider HeV a differential diagnosis in unvaccinated horses anywhere in Australia where flying-foxes are present. Suspect diseased horses should be screened for this variant via routine state biosecurity HeV testing. Biosecurity recommendations should be updated to reflect the link between HeV variants and flying-fox populations while emphasising the protected status and importance of flying-foxes.

DETECTION OF NOVEL HEPATOTROPHIC VIRUSES IN AUSTRALIAN HORSES

CM El-Hage[1], C Fortier[1,2], E Hue[1,2,3], G Sutton[2,3], C. Marcillaud-Pitel[4], K Jeffers[1], N Bamford[1], E Oden[3], R Paillot[2,3,5], CA Hartley[1], S Pronost[1,2,3], JR Gilkerson[1]

[1] Centre for Equine Infectious Diseases, Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, 3010
[2] Normandie Univ, UNICAEN, BIOTARGEN, 14000 Caen, France
[3] LABÉO Frank Duncombe, 1 route de Rosel, 14280 Saint-Contest, France
[4] RESPE, 14280 Saint-Contest, France.
[5] School of Equine and Veterinary Physiotherapy, Writtle University College, Lordship Road, Writtle, Chelmsford CM1 3RR, United Kingdom

Introduction

Two novel hepatotropic viruses, equine parvovirus-hepatitis (EqPV-H) and equine hepacivirus (EqHV), have been reported as the likely cause acute hepatitis in horses. Although these viruses have been detected in horses overseas, to date, they have not been reported in Australia. Our aim was to determine whether EqPV-H and EqHV were detectable in Australian horses and, the prevalence in archived serum samples.

Materials and methods

Sera from 188 Australian horses collected between 2018-2019 were tested for the presence of EqPV-H and EqHV viral nucleic acid. The population included clinically normal horses and horses with signs of clinical disease other than hepatic dysfunction. Validated qPCRs were performed to detect EqPV-H and EqHV. Sequencing for phylogenetic analysis was performed on two NS1 fragments (516 nucleotide{nt} and 587 nt) for EqPV-H and on the NS5 fragment (276 nt) for EqHV.

Results

EqPV-H and EqHV were detected in 6/188 (3.2%), and 21/188 (11.2%) horses respectively. Phylogenetic analysis showed that Australian strains were clustered. Although Thoroughbreds (TBs) were the predominant breed in the population, they were nevertheless more likely to be infected.

Relevance to Australian clinical equine practice

This is the first report of EqPV-H and EqHV in Australian horses. This extends the global map of coverage by these equine hepatotropic viruses, providing local veterinarians with greater information to deal with equine hepatic disease and serological evidence of clinicopathologic abnormalities relating to liver function. The relatively increased prevalence amongst TBs may be reflective of genetic susceptibility or management practices conducive to spread.

TRANSDERMAL EMLA (LIDOCAINE/PRILOCAINE) CREAM FOR INTRAVENOUS CATHETERISATION IN HORSES

B Amiet, J Rainger, J Stewart, S Zedler, S Woldeyohannes, W Goodwin*

University of Queensland

Introduction

Eutectic mixture of local anaesthetics (EMLA) is a lidocaine/prilocaine cream that has been used in the management of pain associated with intravenous (IV) catheterisation in humans and animals. EMLA cream has been demonstrated to be as effective as lidocaine in reducing superficial pain perception in horses. The purpose of this crossover trial was to compare EMLA cream to subcutaneous infiltration of lidocaine when used as a local anaesthetic prior to IV catheterisation in horses.

Materials and methods

Jugular sites of 26 horses were randomly assigned to be treated with 1 g/cm^2 of 5% EMLA cream for 60-minutes prior to IV catheter placement. The contralateral side was treated with 30 mg (1.5 mL) of subcutaneous lidocaine. Aversive behavioural reactions were scored by blinded observers at the time of treatment and subsequent IV catheterisation.
(Ethics Approval: 2021/AE000259)

Results

Aversive behavioural reactions were significantly higher in response to subcutaneous infiltration of lidocaine compared to application of EMLA cream ($p = 0.0014$). Aversive behavioural reactions to IV catheterisation were very mild following treatment with both local anaesthetic agents, however, were significantly decreased following treatment with lidocaine ($p = 0.0132$).

Relevance to Australian clinical equine practice

Lidocaine is commonly used to desensitise the jugular catheterisation site in horses, however, may cause transient pain on injection. EMLA cream represents a less invasive alternative and may offer clinically relevant desensitisation of the skin.

A STUDY OF THE USE OF INJECTABLE ALTRENOGEST FOR THE MAINTENANCE OF PREGNANCY

FF McConaghy, D Searle and NR Perkins

Ceva Animal Health Pty Ltd, Glenorie, NSW Australia

Introduction

This study aimed to support the registration of the long acting altrenogest injection (NV Readyserve®) for the indication of maintenance of pregnancy in mares at risk from pregnancy loss associated with progesterone deficiency.

Methods

Thirteen mares were included in a randomised, blinded, placebo-controlled study. Mares were monitored for ovulation and inseminated with fresh semen ~24hrs prior to ovulation. Mares were treated with altrenogest or placebo injection 3 mL/500 kg every 5 days from day 5 after insemination. Pregnancy testing was conducted on days 15, 21, 28, 35, 45 and 60. Mares were recruited on day 21 based on a positive pregnancy test and administered an abortigenic dose of prostaglandin. On day 60, treatment was stopped and pregnant mares were administered prostaglandin to result in abortion. Injection sites were monitored daily, foetuses were monitored during pregnancy and examined for gross abnormalities at abortion. The reproductive capacity of the study mares was assessed in the following season.

Results

All mares in the control group had aborted by day 28 and all treated mares remained pregnant until day 60. Injections were well tolerated with only 2 minor site reactions and there were no effects on any foetus or reproductive capacity of the mares used in the next season.

Relevance to clinical practice

The study demonstrated that treatment with altrenogest injection prevented abortion associated with prostaglandin treatment. This study was limited by lack of progesterone measurement. The study supports the use of Readyserve® injection for the maintenance of pregnancy associated with progesterone deficiency.

RETROSPECTIVE CLINICAL EVALUATION OF THE GHENT SEDATION ALGORITHM FOR STANDING SEDATION IN THOROUGHBRED MARES UNDERGOING LAPAROSCOPIC OVIDUCTAL PROSTAGLANDIN APPLICATION AT SCONE EQUINE HOSPITAL BETWEEN SEPTEMBER 2020 AND MARCH 2021

M Bellei, M Gozalo-Marcilla*, A Adkins, A Robinson

Scone Equine Hospital, 106 Liverpool Street, Scone, Australia
*University of Edinburgh, Scotland UK

Introduction

The Ghent Sedation Algorithm (GSA) allows for the adjustment of rates of alpha-2 agonist infusions (CRIs) during standing procedures in horses. These results describe its clinical usefulness for standing laparoscopies and the effectiveness of a detomidine-based CRI protocol.

Materials and methods

Retrospective data of eleven mares (10 Thoroughbreds, one Quarterhorse) presenting to the Scone Equine Hospital between September 2020 and March 2021 for laparoscopic oviductal prostaglandin (PGE2) application, receiving a detomidine CRI were included. Initial detomidine CRI rates varied between 3.5-10 mcg kg^{-1} h^{-1} IV and were adjusted by an experienced anaesthetist (MB or AR), if required, at 5-minute intervals throughout the procedure. The adjustments were based on 1) clinical judgement and 2) GSA scores. Bilateral paralumbar infiltration of lidocaine was performed for laparoscope portal insertion. Surgical conditions were evaluated by the main surgeon at the end of each procedure with numerical (NRS) (0 =worst, 3=best) and visual analogue scales (VAS) (0=worst, 10=best).

Results

Mean age and weight were 12.6±4.8 years and 536±45.72kg, respectively. Mares received IV acepromazine (0.019±0.003 mg kg^{-1}), followed by detomidine (0.01±0.0002 mg kg^{-1}) and methadone (0.099±0.0057 mg kg^{-1}). The mean detomidine CRI was 6.4±2.6mcg kg^{-1} h^{-1}. Total surgery and sedation times were 39.72±16.55 and 65.72±14.75 minutes, respectively. The anaesthetist's action agreed with the GSA recommended action in 93 of 107 recorded time points (86.91%), with 3/14 (21%) disagreements, mainly infusion discontinuations near the end of surgery (final 15 minutes). No intra-operative adverse events occurred. Surgeons' median (range) scores for surgical conditions were 2 (2-3) for NRS and 8 (7-10) for VAS. No postoperative complications were observed except for temporary, short-term mild colic post-operatively in two mares. All mares were discharged within 5 days after surgery.

Conclusion

The GSA is a useful tool to assist in fine-tuning detomidine CRIs for standing laparoscopies. The proposed protocol provided adequate surgical conditions for laparoscopies with no intra- and postoperative complications.

URETEROLITHIASIS IN 15 HORSES – CLINICAL AND ULTRASONOGRAPHIC FINDINGS

L. Dillenbeck [1], M.B. Whitcomb[2], B. Vaughan,[2] S.A. Katzman[2], K.G. Magdesian[3]
[1] William R Pritchard

Veterinary Medical Teaching Hospital, School of Veterinary Medicine
[2] Department of Surgical & Radiological Sciences, School of Veterinary Medicine
[3]Department of Medicine and Epidemiology, School of Veterinary Medicine
University of California, Davis, USA

Introduction: Urolithiasis of the bladder and kidneys is well described. Ureterolithiasis has been infrequently reported.

Materials/methods: Medical records of horses that underwent transcutaneous (renal) and transrectal (ureteral/bladder) ultrasound from 2002-2019 were reviewed. Horses with ureteral calculi were included.

Results: Ureteroliths were identified in 15 horses, including 10 geldings, one stallion and 4 mares ranging from 4-35 years (median=20y). Presenting complaints included inappetence (6), abnormal urination (5), colic (4), pyrexia (3), weight loss (3), lethargy (3), neurological signs (2) and laminitis (1). Hematologic abnormalities primarily included neutrophilia (9) and hyperfibrinogenemia (6). BUN and/or creatinine were elevated in 12/15 horses. Urethral stones were removed in 2 geldings prior to ultrasonography. Transrectal ultrasound detected ureteroliths in the proximal (5), mid (8) or distal (3) portion of the right (8) or left (7) ureter. One horse had two ipsilateral stones. Cystoliths were identified in 3 horses. All horses showed significant renal abnormalities, including nephrolithiasis (13), hydronephrosis/pylectasia (7), renomegaly (8) and small end-stage kidneys (7). Six horses were euthanized. Three discharged horses underwent surgical removal via perineal urethrostomy (2) or flank ureterotomy (1). In another horse, spontaneous passage allowed cystoscopic removal. Five additional horses were discharged without removal.

Conclusion/Clinical Relevance: Horses with ureteroliths show variable presenting complaints and were unlikely to be diagnosed prior to referral. Transrectal and transcutaneous ultrasound was necessary to identify all ureteroliths, multifocal urolithiasis and significant renal pathology that accompanied most cases.

Notes

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Keynote Speakers

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Conditions of the Soft Palate

Dr Brian Anderson
ba@bvp.com.au
Ballarat Veterinary Practice
Ballarat, Victoria
Australia

Common conditions of the soft palate may be congenital, inflammatory, or functional. Neoplastic conditions are rare, and occasionally foreign bodies can lodge in the soft palate. The most important conditions for equine athletes are functional, including nasopharyngeal collapse, palatal instability, and dorsal displacement of the soft palate (intermittent or persistent).

Congenital conditions

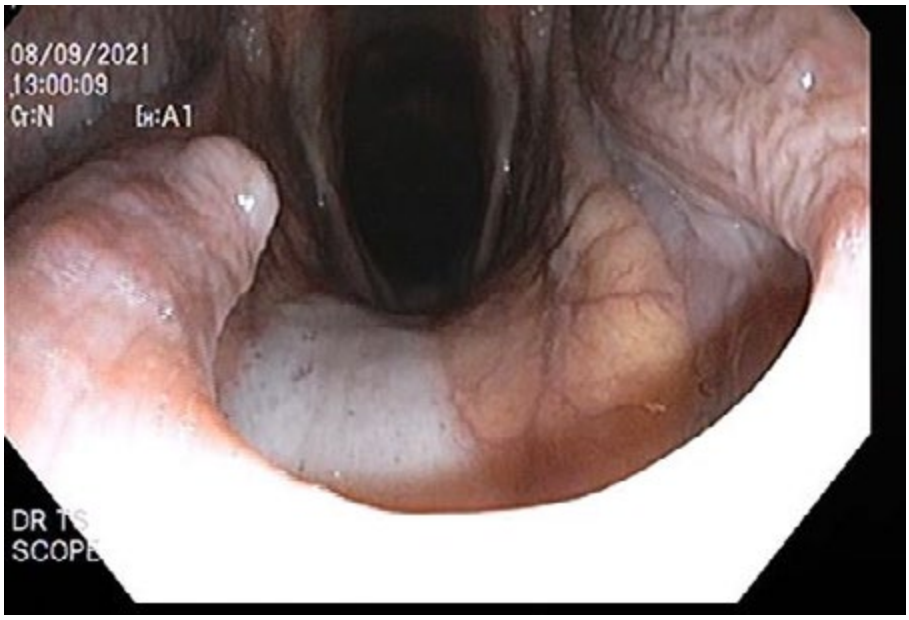
- Cleft palate (palatoschisis)[1-3] is a rare congenital condition occurring in 0.1-0.8% of foals. The cause is an embryological failure of the lateral palatine processes to fuse on midline, and since they fuse from rostral to caudal, portions of the hard and soft or just soft palate can be involved. Clinical signs include nasal regurgitation of milk, coughing, failure of passive transfer of immunity due to inadequate colostrum intake, aspiration pneumonia and slow and suboptimal weight gain due to malnutrition. Diagnosis is by digital or oral examination or by endoscopy. If the soft palate cleft is small (<1/3) and aspiration is minimal, then treat conservatively and monitor closely. Large defects need surgical repair or euthanasia. Surgical repair is complex but can be attempted so long as no more than 20% of the palate is missing and repair of asymmetric defects is not advised. Surgical complications are common and include partial or complete dehiscence of the repair, osteomyelitis of the mandible, salivary fistulas, incisional infection, unsuccessful reoperation and life-threatening pneumonia.

- More common in the author's practice is the diagnosis of cleft palate or hypoplasia of the palate in older horses. Barakzai *et al.* (2014), reviewed 15 cases of congenital defects of the soft palate in mature horses.[1] The mean age was 3.9 years, ranging from 1-12 years, involving various breeds with intended uses (4 breeding, 7 general riding, 2 racing and 2 unspecified). The presentation was for respiratory signs in 12, but 3 had endoscopy for other signs. Clinical signs included nasal discharge, often with food material, saliva and mucopus. Abnormal respiratory noise was not present at rest but present at exercise in the 2 racehorses and 3 riding horses. In the other 10 horses, there was no noise (3), or it was unknown (7).

- Diagnosis was by endoscopy *per nasum,* and 9 horses had a symmetrical palatal defect and 6 asymmetric, 4 were mild (ending <2 cm rostral to tip of epiglottis, < 30% of total soft palate length) 7 moderate (30-50% of soft palate length, 2 severe (>50% soft palate length) and 2 not categorised. Three horses had concurrent epiglottic entrapment, and one horse had epiglottic hypoplasia. Most horses had feed/saliva/mucopus in the trachea to variable amounts. Clinical signs of pneumonia or ill thrift were not present. A 4-year-old racehorse had dynamic endoscopy performed, and excess saliva from the oropharynx obscured the view, but both sides of the cleft palate were drawn medially on inspiration and laterally on expiration. Expiratory ballooning of the coexisting epiglottic entrapment occurred, as did some axial deviation of the aryepiglottic folds.

- Only three of the 15 horses were treated. The other 12 were managed as follows: monitor for signs of aspiration pneumonia and treat as necessary, don't feed for 1-2 h before exercise and wash out mouth prior to exercise. Two racehorses raced, with

one having 7 starts for a win and 2 places. The other was diagnosed as a yearling and at 7 years, had raced 39 times for 4 wins and 14 places. Surgical repair was attempted in 3 horses, including a laryngeal tie-forward and primary repairs in two horses, both of which failed. It was concluded that based on the data from this biased population of "survivors," if as foals the aspiration pneumonia is not too severe, some horses can survive to maturity and perform low level activities and even race with apparent low levels of morbidity.



Cleft palate or hypoplasia of the palate in a 2-year-old Thoroughbred

Palatal Masses, injury, and inflammation

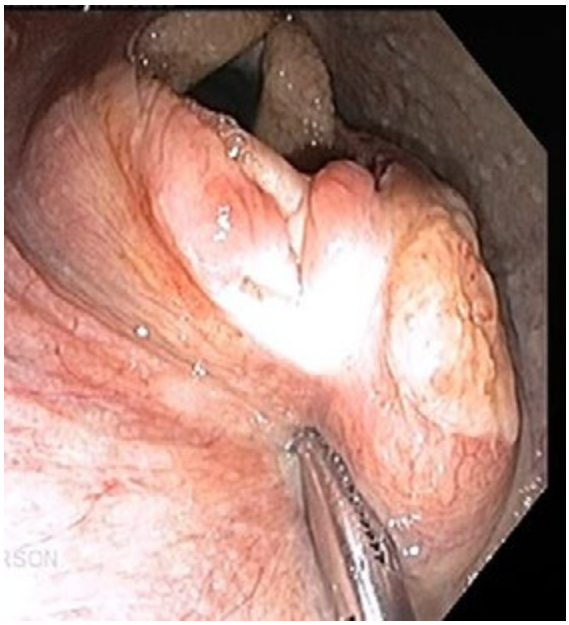
- Any nasopharyngeal mass can potentially involve the soft palate or cause soft palate dysfunction, including extensions from the paranasal sinus, guttural pouch, or oropharyngeal diseases.[2]

- Palatal cysts have been reported in horses with clinical signs of abnormal respiratory noise due to dorsal displacement of the soft palate, increased respiratory effort and dysphagia secondary to persistent dorsal displacement of the soft palate. They are usually located caudally but have been identified rostrally, and diagnosis is via nasopharyngeal endoscopy, lateral-to-lateral radiographs or direct oral examination. The proposed aetiology for these cysts has been obstruction or inflammation of mucous glands i.e., mucocoeles rather than embryonic remnants as suggested for subepiglottic or nasopharyngeal cysts. They are likely congenital. Treatment is by surgical extirpation, laser ablation or possibly injection with formalin. If the cyst lining is destroyed a good outcome is expected. Access via a tracheotomy using a diode laser for caudal palatal bilateral cysts was recently reported.[4]

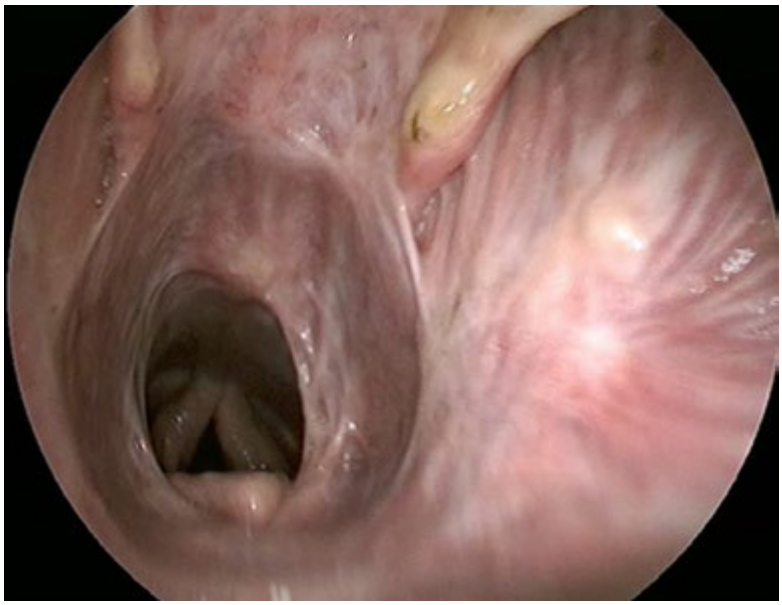
- Palatal granulomas and abscesses occur occasionally. Caudal border ulceration and infection can occur in association with subepiglottic masses or inflammation or because of intermittent dorsal displacement of the soft palate. Treatment typically is with local or systemic antibiotics and anti-inflammatories or occasionally using laser or scissor debridement. Acute or chronic inflammation can result in persistent dorsal displacement of the soft palate, which resolves when the disease is resolved.

- Iatrogenic injury to the soft palate during trans nasal axial division of epiglottic entrapments using an unguarded hook or bistoury has been reported. Surgical repair of these injuries is difficult, and this practice is not recommended.

- Nasopharyngeal cicatrix is a bizarre syndrome originally reported in Southern Texas and Florida, USA but recently reported in NZ[5] with an unknown aetiology which presents as progressive nasal discharge and ends in upper respiratory tract dyspnoea. The features of the condition are:
    - Insidious in onset with repeated exposures resulting in progressive disease over subsequent years
    - Mares are more markedly affected compared to geldings and stallions
    - Signs are worse over summer and into autumn
    - Only occurs in horses at pasture
    - URT endoscopy shows petechiation and ulceration of the nasal septum, serosanguinous to yellow mucoid nasal discharge, yellow plaques and scarring at the level of the guttural pouch ostia with shortening and distortion of the ostia, deformity of the epiglottis, arytenoid chondritis, nasopharyngeal webbing and scarring – cicatrix and ulceration and webbing of the proximal trachea
    - No effective treatment is available to cure the signs, and eventually the severe and debilitating inflammation of the nasopharynx and related structures (larynx, soft palate, guttural pouch ostia) results in progressive scar tissue formation and airway obstruction requiring temporary or permanent tracheostomy.
    - A causative agent has not been identified in the USA or NZ. In NZ, a soilborne fungal or microbe organism is suspected that was inhaled via aerosolised dust (Dr Greg Quinn, personal communication)



Infection and inflammation with granuloma formation on the caudal border of the soft palate



Nasopharyngeal cicatrix – image courtesy of Dr Greg Quinn

Functional disorders of the soft palate

Dorsal displacement of the soft palate DDSP is a functional condition where the caudal border of the palate moves dorsally from its normal subepiglottic position, breaking the oropalatal seal to lie over and cover the epiglottis. The caudal free border sits across the *rima glottidis* and in this position billows during exhalation, causing a characteristic gurgling noise and airway obstruction resulting in a reduction in minute ventilation (breaths per minute), increased hypoxia, and hypercarbia. In racehorses, this is associated with a loss in speed and reduced performance. It is estimated that the condition affects 10-20% of 2–3-year-old racehorses.[2]

There are two forms: intermittent (IDDSP) and persistent. The intermittent form is a dynamic condition, and even with a typical history, a definitive diagnosis requires overground (OGE) or treadmill URT videoendoscopy because a diagnosis using resting endoscopy is unreliable. Persistent DDSP is usually caused by subepiglottic masses or cysts, palatal inflammation or infection, structural changes to the epiglottis or neuromuscular damage to the pharyngeal muscles.

The pathogenesis of IDDSP is not completely known, but recent research and current thought indicate it is multifactorial and involves:[2]

- Structural causes such as palatal cyst, palatal granuloma, subepiglottic masses/cyst, epiglottic entrapment, epiglottic hypoplasia and/or deformity, which result in a mechanical effect by interfering with the relationship with the caudal free border of the palate and the subepiglottic tissue. Such causes contribute to a low number of cases of DDSP
- Caudal retraction of the tongue and open mouth breathing
- Position of the larynx and hyoid bone
- In most cases, however, IDDSP is likely a problem of inappropriate intrinsic muscular contraction of the nasopharynx, resulting in palatal instability due to increased compliance (elasticity) and eventually in DDSP.

Experimentally, blocking the pharyngeal branch of the vagus results in DDSP at rest and exercise. This nerve innervates the palatinus and palatopharyngeal muscles, which both stabilise the soft palate.

Table 16.1 Function and innervation of muscles controlling the tone of the equine soft palate

Muscle	Function	Innervation
Tensor veli palatini	Tenses the rostral aspect of the soft palate	Mandibular branch of the trigeminal nerve
Levator veli palatini	Elevates the palate during swallowing and closes the nasopharynx	Pharyngeal branch of the vagus
Palatinus	Shorten and depress the palate	Pharyngeal branch of the vagus
Palatopharyngeus	Shorten and depress the palate	Pharyngeal branch of the vagus

From: Cheetham J, In: Advances in equine upper respiratory surgery, p91[7]

Injury, fatigue, or neuromuscular dysfunction of the paired thyrohyoid muscles which draw the larynx rostrally and dorsally and the tongue caudally and are innervated by the hypoglossal nerve can result in DDSP. Mimicking the action of these muscles with a bilateral prosthesis is the basis on which the tie-forward surgery was conceived. The hypoglossal nerve also innervates a group of rostral hyoid muscles which move the larynx forward on contraction. Bilateral local anaesthetic blockade of the distal hypoglossal nerve can result in DDSP.

History and Clinical Signs

Typically for racehorses, there is a history of poor performance and a gurgling/vibrating respiratory noise during exhalation, however 20-30% of horses are so called "silent displacers". During training or racing, horses can appear to have suddenly stopped and trainers describe "choking down", "holding the breath" or "swallowing the tongue". Abnormal respiratory noise, which is exacerbated by head flexion, is observed in horses not used for racing.

Diagnosis

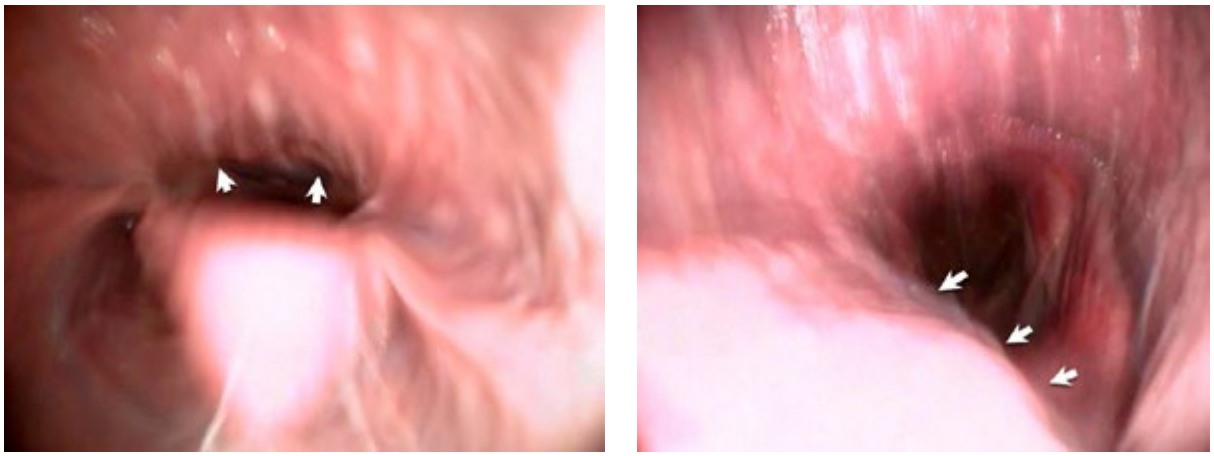
A characteristic history with a typical respiratory noise is suggestive of IDDSP. A resting endoscopic examination should always be done, but the presence of IDDSP at rest is not particularly predictive of IDDSP at exercise. Caudal soft palate ulceration or bruising of the nasopharynx may be suggestive and the structural changes listed above as well as epiglottic abnormalities, especially a flaccid and short epiglottis increase the level of suspicion of IDDSP. Evaluation of the guttural pouches in young horses or horses with concurrent respiratory infection should be performed to look for infection or enlarged retropharyngeal lymph nodes. Neuropathy of the pharyngeal branch of the vagus is suspected to be involved with IDDSP. A definitive diagnosis requires dynamic URT videoendoscopy. There is some evidence that treadmill endoscopy has a higher diagnostic rate for IDDSP compared to overground endoscopy in racehorses but not sport horses. This is probably due to control of exercise intensity in racehorses on the treadmill and more control of head and neck flexion in sport horses during OGE.

In an affected case, dynamic URT videoendosopy reveals some or all of the following:

1. Rostral pharyngeal collapse, aka. rostral soft palate billowing, aka. rostral palatal instability. This is defined as an inspiratory obstruction, and billowing occurs due the inability to resist negative nasopharyngeal pressures. Bilateral transection of the tendon of the *tensor veli palatini* resulted in rostral palatal instability but no DDSP.
2. Palatal instability (PI).[6] This is defined as dorsoventral movements of the caudal portion of the soft palate with flattening of the epiglottis against the dorsal surface of the soft palate. Considered the forerunner to DDSP.
3. DDSP occurs when the caudal free border of the palate displaces dorsally, obscuring the epiglottis and obstructing the *rima glottidis* of the larynx typically when the horse is fatigued or slowing down. DDSP occurs quickly when there are structural changes i.e., epiglottic deformity. By definition, the entire soft palate should be positioned

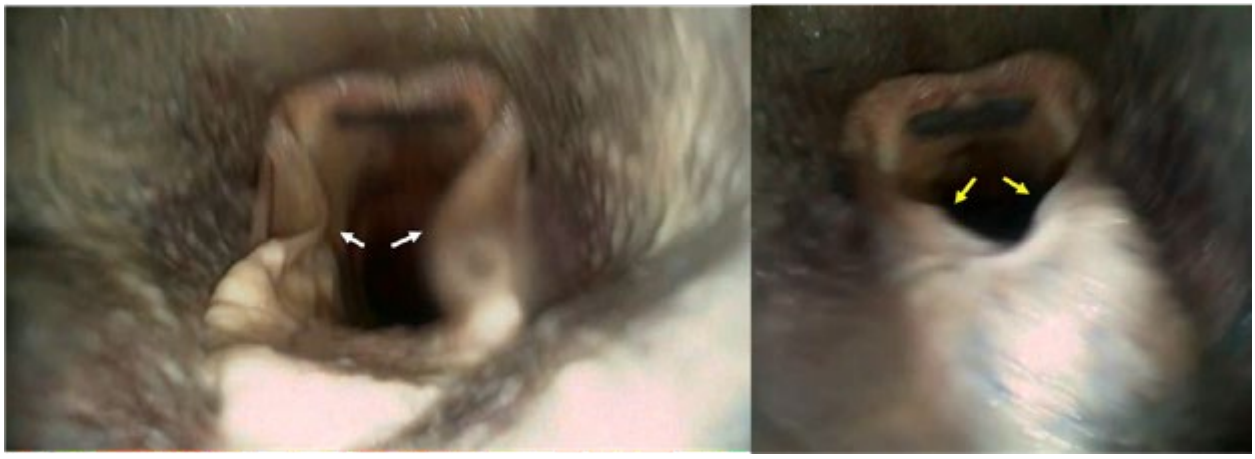
dorsal to the epiglottis for 8 seconds.[8] It may be preceded by PI, or swallowing, or axial deviation of the aryepiglottic folds.

4. Dorsal/lateral nasopharyngeal collapse. Horses presented for a suspicion of DDSP not uncommonly are found to have varying degrees of nasopharyngeal collapse. The cause is unknown but may be due to neuromuscular dysfunction (sensory or inflammatory) or differential pressures between the guttural pouches and nasopharynx.[2] Nasal obstruction may result in increased negative airway pressure exceeding the capacity of the nasopharyngeal musculature to dilate the nasopharynx. The obstruction occurs during inspiration, and the noise is characterised as loud and thick winded, and leads to significant exercise intolerance. Diagnosis is by dynamic URT videoendoscopy, but during the nasal occlusion test collapse may be evident. Both guttural pouches and nasal passages should be examined.



Dorsal pharyngeal collapse (left picture) and right lateral pharyngeal collapse (right picture) in the same horse during dynamic URT videoendoscopy.

Controversy exists as to whether rostral palatal instability is a separate phenomenon to palatal instability and eventually DDSP, or if it is a continuum of events (Dr Sam Franklin, personal communication).



Sequence of events during dynamic URT videoendoscopy: Palatal instability is present in the left picture, with the epiglottis flattened against the caudal part of the soft palate which is billowing dorsally. Mild axial deviation of the aryepiglottic folds is present (white arrows). Dorsal displacement of the soft palate is evident in the right picture when the caudal border of the palate obscures the epiglottis (yellow arrows).

Treatment

The rational treatment of IDDSP would be evidence-based and correct or reduce the causes of the condition. Given that the aetiopathogenesis is incomplete and high-level evidence-based data is limited, it is not surprising that a "shotgun" approach aimed at the factors associated with the condition is often used. Non-surgical and surgical treatments or a combination of the two are used to treat IDDSP.

- Structural abnormalities should be treated first. If nasopharyngeal or guttural pouch inflammation is present systemic and/or topical anti-inflammatory medication is indicated. In young horses a spell (8-12 weeks) combined with a course of anti-inflammatory therapy may help resolve IDDSP. Changes in gear/tack, including using a figure of 8/cross over noseband/dropped noseband to prevent opening the mouth during exercise and/or a tongue-tie (to prevent caudal retraction of the tongue) may be useful. A Cornell collar (throat support device) positions the larynx and basihyoid more dorsally and rostrally. It mimics the permanent effect of a tie-forward procedure and has been shown to be effective in preventing experimentally created DDSP. It is estimated that non-surgical treatments can have a success rate of 53-61% and should be tried prior to surgery.[9]

- Surgical treatments are subdivided according to the proposed aetiology the procedures attempt to address and include staphylectomy, myectomy and the tie-forward procedure.

    - ***Staphylectomy*** is not recommended for IDDSP as the equine soft palate is not abnormally long. It is indicated for caudal structural abnormalities such as a granuloma or cyst or as the second stage following a tie-forward for persistent displacement of the soft palate.[2] Despite this recommendation, a recent report in Swedish warmblood trotting horses showed no difference between a staphylectomy or a tie-forward procedure in terms of race starts, race speed and career earnings.[10] Horses with persistent DDSP have a more caudal larynx compared to those with IDDSP. Treatment is initially with a tie-forward procedure. In 15 racehorses, this resolved the DDSP in 7. [11] In the other 8 horses, a laser staphylectomy was used in addition and resolved the DDSP. Thirteen of the 15 horses returned to racing.

- ***Myectomy*** is a procedure where the caudal "strap muscles" are partially resected to reduce caudal retraction of the larynx.[2] The muscles are the paired *sternothyroideus* and *sternohyoideus* with or without the *omohyoideus*. The procedure can be done standing or under a general anaesthetic, and this is preferred if the omohyoideus muscle is removed. The success rate in uncontrolled reports is 58-71%. A minimally invasive myectomy has been described, and under general anaesthesia, the tendon of insertion of the sternothyroideus muscle is transected 1 cm caudal to the wing of the thyroid, and a 3 cm length of the attached muscle is transected caudally. The reported success rate of this procedure is 58-70%.

- ***Laryngeal tie-forward*** is a procedure in which a bilateral prosthesis replaces the action of the thyrohyoideus muscles by placing sutures between the thyroid cartilage and the basihyoid bone and, on tightening, moves the larynx rostrally and dorsally approximately 4-4.5 cm.[2] Post-operatively the tip of the epiglottis is seen to be more dorsal and rostral ending at the level of the guttural pouch ostia. Radiographs can be used to determine the success of the procedure but are not necessary. The reported success rate varies between 9-46% in national hunt horses and 80-82% in racehorses. In one study involving Standardbreds, the success rate was reported as approximately 66% but with a recurrence rate of 21% within 2 years.[12] As mentioned above, in another study, no difference was found in the success rate between horses treated with a staphylectomy vs. a laryngeal tie-forward. Our experience is that about two-thirds of operated Thoroughbred or Standardbred racehorses have improved race performance post-surgery.

- Rostral palatal instability/rostral nasopharyngeal collapse – nasal laser palatoplasty, oral thermal palatoplasty aim to reduce palate compliance i.e., stiffen the palate, oral palatoplasty aims to increase the tension in the palate. Prognosis appears related to degree of collapse but given there is limited knowledge regarding the condition is considered guarded.[2]

- Dorsal and Lateral Nasopharyngeal wall collapse – treat any primary condition e.g., guttural pouch infection, nasal obstruction, or systemic disease. Consider anti-inflammatory therapy and a spell of 3 months or more. Nasopharyngeal fenestration and alar fold resection can be tried. Prognosis is guarded.[2]

Summary

Functional disorders of the soft palate are the most common obstructive airway conditions in equine athletes. Obtaining a thorough history and performing a physical examination and resting endoscopy of the URT is standard. Definitive diagnosis requires URT videoendoscopy during strenuous exercise. Treatment should be aimed at resolving any underlying condition. In our practice, a tie-forward procedure with or without rostral oral thermocautery and adjunctive changes in tack/gear are the mainstay of treatment.

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Notes

Twins: Their origin, outcome, and management

AO McKinnon

Goulburn Valley Equine Hospital

Origin and outcome of twins

In our practice, the management of twins is mostly confined to Thoroughbred mares on local breeding farms. Other breeds are represented in smaller numbers and generally have a lower probability per cycle. Many breeds are referred later in the year, or after the New Year, for twins that have either been missed or have not responded to veterinarians' or nature's attempts to reduce one. Occasionally twins are identified when the mare is presented for foetal sexing between 60-120 days).

Three things about twins have been known for a long time. Firstly, twins are repeatable within the same mare; secondly twinning rate varies according to breed; thirdly, the more fertile the stallion, the more twins he is expected to achieve. Every year veterinarians involved in Thoroughbred work closely watch the incidence of twins in first season sires as it is an excellent way to gauge the fertility of the horse. Very fertile stallions will have at least a 20% twin rate per cycle in the first 15-20 pregnancies.

Historically, twins have been the single most important cause of abortion. With the advent of ultrasonography, the incidence of abortion associated with twins has decreased dramatically to less than 2% of all abortions. However, in the UK, twins are still one of the major reasons for litigation against veterinarians (J. Pycock, personal communication).

Twins are often disastrous financially. Most twin pregnancies terminate in early foetal resorption or loss, late-term abortions, or the birth of small growth-retarded foals. Mares aborting twins in late gestation frequently have foaling difficulties, damage their reproductive tracts and are difficult to re-breed. If foals are born alive, they are frequently small, demonstrate the effects of intra-uterine growth retardation and have a poor survival rate, with many needing expensive, sophisticated critical care.

Twins are avoidable and should not persist on well-managed breeding farms. Clients understand this and demand the most sophisticated management programs available. The management techniques in use at the Goulburn Valley Equine Hospital (GVEH) are presented below. The hospital is a major referral centre, and each year is presented with multiple referral cases of twin pregnancies that are diagnosed after the cessation of the mobility phase.

Origin of twins

The origin of equine twins is almost exclusively dizygotic. Zygosity refers to the origin of the twin. Dizygotic twins originate from two separate fertilised oocytes with two different spermatozoa, and monozygotic refers to identical twins that arose from one fertilised oocyte.

Monozygotic

Twins in the horse are unlikely to be identical. Almost all cases of twin pregnancy are expected to be dizygotic, and thus related to multiple ovulations, although there are cases of monozygotic twin pregnancies that have been suspected or confirmed in the horse, and most have been related to embryo manipulation (embryo transfer or Intracytoplasmic sperm injection-ICSI). Exactly how monozygotic twin formation occurs is unknown, although pinching by the zona pellucida or damage to the inner cell mass are popular theories.

Time of development probably influences placental arrangements. Four types of placental arrangements are recognised in human monozygotic twins:

1. Dichorionic/Diamniotic: twins are totally separate and share only a common contact area of the chorion. These occur when the embryo divides before blastocyst formation.
2. Monochorionic/Diamniotic: twins share a common chorion but have separate amniotic cavities. These occur when the embryo divides after blastocyst formation and development of the inner cell mass and the trophectoderm.
3. Monochorionic/Monoamnionic: twins separate after the formation of the ectoplacental cone and share a common chorion and amnion.
4. Conjoined twins: twins incompletely separate.

In the horse, one apparent set of dichorionic/diamniotic monozygotic twins has been reported, while all other suspected or confirmed cases of monozygotic twins have been monochorionic/diamniotic. It appears that embryo transfer pregnancies or pregnancies from other forms of assisted reproductive techniques such as ICSI are overrepresented in the reports of monozygotic twins. It is not expected to be possible to determine the true incidence of dichorionic/diamniotic monozygotic twins, as both should be present at the initial ultrasonographic pregnancy diagnosis on or around day 13 after ovulation and would be indistinguishable from dizygotic twins apart from the number of corpora lutea present. In this regard, a study reporting a probable incidence of monozygotic multiple pregnancies of 2.6% should be interpreted with caution as only dichorionic/diamniotic monozygotic pregnancies would be detected at the first pregnancy examination. The identification was based on confirmation of one less CL than the number of vesicles detected which is not always accurate, even in experienced hands.

The reason for the lack of monozygotic twin formation in the horse is probably related to the capsule. The capsule forms in equine embryos aged around 6 days, shortly after their entry into the uterus. When embryos were cultured prior to the formation of the capsule, hatching occurred like the bovine; however, when embryos were cultured after formation of the capsule, the zona pellucida continued to become progressively thinner and finally fell away from the developing conceptus. Other species that do not have a capsule, such as sheep, cattle and humans, all have the ability to give birth to monozygotic twins, which may form from pinching of a hatching embryo by the zona pellucida. Identification of dichorionic/diamniotic monozygotic pregnancies must rely on the use of DNA microsatellite loci as placental arrangements are indistinguishable from dizygotic twins. Monochorionic/diamniotic twins can almost always be assumed to be identical.

<u>Dizygotic</u>

Twin pregnancies originating from multiple ovulations are dizygotic. A strong association between twinning rate and breed has been reported. Other associations that have been reported or suggested are age, reproductive status, season and use of drugs to control ovulation. Twinning appears to have a high degree of repeatability and heritability.

Breed

More twins are diagnosed per cycle in Thoroughbreds versus Standardbreds (Table 1). Twins are more common in Thoroughbreds and Draught-type horses than Quarter Horses, ponies and Arabians. Multiple ovulation rate is correlated with twin twinning rates. Multiple ovulation rates have been reported to be 15-25% in Thoroughbreds, 13-15% in Standardbreds, 24% in Draught Horses, 8-10% in Quarter Horses and Appaloosas, 2-3% in domestic ponies and 0% in Korean wild ponies.

Thoroughbred numbers	Thoroughbred percentages	Standardbred numbers	Standardbred percentages
97/629	15.4	39/635	6.1
236/2436	9.7	46/2086	2.2
510/6123	8.3	123/2690	4.6

Table 1. Identification of twin pregnancies in populations of Thoroughbred and Standardbred mares.

Age

Multiple ovulations have been reported from a slaughterhouse study to be more common in older mares (18% in mares aged ≥ 6yo) than in younger mares (14% for mares ≤ 5yo). The twinning rate has been reported to be 2.8%, 3.4%, 3.6% and 6.8% for mares aged 4-7, 8-11, 12-15 and 16-20yo, respectively.

Reproductive status

Less multiple ovulations are expected on the first post-partum oestrus compared to other cycles. The highest number of twin pregnancies are recorded in barren mares, followed by maiden mares, and then followed by lactating mares.

Season

Although various reports have been published indicating twins were more common either at the beginning or end of the breeding season, many are confounded and not supported by large numbers. It is most likely that season does not play a major part in the probability of twins.

Ovulation induction

We found multiple pregnancies were more commonly detected when an ovulation induction agent was used. It is not clear if a higher probability of twins is a result of more ovulations or a better ability to detect twins due to closer synchrony of ovulation. Twin pregnancy rates were higher when either deslorelin acetate (Ovuplant®) or hCG were administered (19.7 % (29/147) 14.8 % (154/1042) respectively) compared to control non-treated mares (11.8 % (62/527), and overall pregnancy rates were also improved (72.0 % (147/204), 72.0 % (1042/1447), 64.5 % (527/817)) respectively for deslorelin acetate, hCG and control groups).

Despite clear associations in some studies, other reports indicate no effect of ovulation induction on probability of twin detection. Reasons for differences may be related to techniques used to determine when to use drugs to hasten ovulation, dosage of drugs or farm management practices or unrecognised confounding variables.

It has been shown that twins in mares are as likely to result from synchronous versus asynchronous ovulation and that the pregnancy rate per follicle was identical for double ovulations on opposite ovaries as compared to that obtained from single ovulations per cycle.

A point of interest in relation to the origin of twin pregnancies is the recognition that twin pregnancies were more likely to occur ($P<0.05$) if ovulations were bilateral versus unilateral (77% bilateral versus 60% unilateral ovulations), and a similar effect was noted with embryo recovery. The probable mechanism for the decreased frequency of twins in unilateral ovulations may be related to the oocyte capture by the fimbria being disturbed

somewhat in cases of multiple large follicles on one ovary. Apart from the somewhat rare occurrence of trophoblastic vesicles (an absence of the embryo/foetus to develop inside the vesicle/conceptus), there is no evidence of pre-fixation embryo reduction. Thus, the disparity in size between vesicles pre-fixation is almost always due to asynchronous ovulations.

Outcome of twin pregnancies

Understanding the outcome if twin pregnancies are left to develop is important to veterinarians, farm managers and owners. Also important is when to intervene and what probability of success such interventions may be expected to achieve.

The mare is very efficient at reducing twins to a single pregnancy by a competitive absorption of nutrients that is related to size and position of the early pregnancy, and later to the orientation of the embryo proper within the developing conceptus, although another possible influence of embryo reduction is the attachment of the capsule to the other. It has been suggested that the capsule plays a role in initiating adhesion between twins, and twin survival depends on an unencumbered trilaminar yolk-sac wall and a functional vitelline circulation.

Regardless, the implementation of any non-intervention program for the resolution of twins depends on the age at identification, the orientation of the vesicles and any disparity in size.

Pre-fixation

Embryo reduction before or on the day of fixation is not considered an important aspect of the natural correction of twins. The probability of a mare losing one or both vesicles of a set of twins from identification prior to fixation is minimal and approximates that of early embryonic death for the same time period (per vesicle). The recognition of twin pregnancies prior to fixation day (day 16) is dependent on the day of examination relative to the day of ovulation. Asynchronous ovulations occasionally result in a gross disparity in vesicle size, sometimes as much as 4-5 days i.e., identification of a day 11-12 and a day 14-16 vesicle concurrently. In instances such as this, examination one day earlier may have failed to detect the younger of the two pregnancies. Recognition that almost all twin pregnancies occur from multiple ovulations dictates mandatory re-examination of all mares that have two CLs identified and only a single vesicle detected prior to fixation (day 16). Recognition prior to fixation is also dependent on operator experience, resolution of the equipment (≥ 5 MHZ preferred), monitor capabilities, restraint, and other facilities (ability to darken the environment), the presence of uterine cysts and the skill of the examiner. As smaller-than-expected single pregnancies have a higher rate of early embryonic death (e.g., trophoblastic vesicles), management techniques should consider the destruction of the smaller vesicle even if the body of evidence suggests minimal pre-fixation embryonic reduction.

Recognition after fixation (after day 16)

The recognition of unilaterally fixed twins from day 17 through to 21 (prior to a clear recognition of the developing foetus within the vesicle) may be the most difficult time to determine if there are twins present. Ultrasonographically, all that is detectable is a thin line (the apposition of the two yolk sacks) running vertically approximately in the middle of what appears to be a slightly oversized vesicle. Recognition of the foetus(es) within the vesicle a few days later makes differentiation easier. Occasionally, an inexperienced operator may confuse an abnormally orientated 28 to 30-day single pregnancy with 17 to 20-day unilaterally fixed twins. From days 22 to 60, the presence of multiple foetuses, umbilical cords and general excess in the number of visible membranes should alert the practitioner to the likelihood of more than one pregnancy. The junction between two developing

foetuses (after 30 days) results in an ultrasonographically visible common membrane representing the area of apposition between the two allantochorions. This common membrane has been referred to as the *twin membrane* and has diagnostic potential, particularly later in pregnancy when it might not be possible to view both foetuses transrectally (>100 days). After 100 days, careful transabdominal ultrasonography may be necessary to determine the presence of twins.

Days 17 to 40

The outcome of pregnancies post-fixation is dependent upon their size (diameter) and the nature of their fixation. Unilateral (both fixed together at the same corpus cornual junction) fixation reduction is much higher than bilateral (one on each side) fixation reduction. Fortunately, unilateral fixation is much higher (approximately 70%) compared to bilateral (30%). In 28 mares with known ovulatory patterns, synchronous ovulations did not affect the type of fixation (9/17 unilateral, 8/17 bilateral). However, for asynchronous ovulation, the frequency of unilateral fixation (10/11) was greater ($p < 0.01$) than the frequency of bilateral fixation (1/11). The incidence of embryo reduction was greater ($p < 0.01$) for unilateral fixation (14/19) than for bilateral fixation (0/9) and was greater ($p < 0.05$) for asynchronous ovulation (9/11) than for synchronous ovulation (5/17). Practically speaking, this means that if asynchronous ovulations have resulted in significant age differences between vesicles (i.e. > 3 mm diameter at day 15), then rate of natural embryonic reduction is very high. In cases of unilateral fixation, 22 of 22 mares with vesicles of dissimilar size had reduction compared to 19 of 26 (73%) with vesicles of similar size. As a result of work studying the reduction of unilateral versus bilateral twin pregnancies in mares from days 17 to 40, Ginther (1989) proposed that the nutrient intake from the larger vesicle (before the foetus was present) prevented adequate nutrition of the smaller vesicle.[1] Later, the position of the foetus proper and its emerging allantoic sac seemed to determine whether a given conceptus survived or underwent late reduction. The foetus, the vascularised wall of the yolk sac adjacent to the foetus and the emerging allantoic sac were exposed to the endometrium (uterine lumen) in the surviving vesicles. In the vesicles that underwent reduction, much of the corresponding area of the vesicle wall was covered by the wall of the adjacent survivor and is thus deprived of adequate embryonal-maternal exchange and therefore regresses.

In summary, dissimilarity in diameter increases the likelihood of unilateral fixation, increases the incidence of reduction for unilateral fixed vesicles, hastens the day of occurrence of reduction and shortens the interval from initiation to completion of reduction.

The incidence of reduction for bilaterally fixed vesicles is negligible and approximates that of standard early embryonic death in this period.

Of the 85% of reductions by day 40 in cases of unilateral fixed twin pregnancies, 59% of reductions had occurred between day 17 and 20, 27% between day 21 and day 30 and 14% between days 31 to 38. The majority of early reductions occurred spontaneously (by day 20) as compared to reductions after day 20 that were preceded by a gradual decrease in size of the eliminated vesicle. In addition, when twins were dissimilar in diameter (4mm or more), they were more likely to undergo reduction by day 20. Other studies have demonstrated similar results. The hypothesis of an early embryonic reduction mechanism for elimination of excess embryo in mares was not new and had been suggested as early as 1982. However, ultrasonography was necessary to adequately document the occurrence and nature of the reduction.

Day 40 onwards

Ginther and Griffin (1994) examined the natural outcome of bilateral twins (one in each horn) that were viable on day 40 in 15 pony mares.[2] Readers should be aware that pony mares are not necessarily a good model for larger breeds, as the incidence of twins is low, and evidence is suggestive that the larger the breed (Draught, Thoroughbred and Warmblood), the higher the probability of maintaining twins. Fifteen pony mares were monitored by ultrasonography until the outcome of the pregnancy was determined. Sixty-six % (10/15) of the pregnancies suffered from either death of both (80% 8/10) or death of one (20% 2/10) during months two or three. For the remaining 5 pregnancies (5/15), nothing occurred from then until month 8. Between months 8-11, two mares lost one foetus (foetal death was associated with mummification) and two mares lost both. The two mares that lost one pregnancy both delivered undersized weak foals at birth. One mare (7%) delivered live twins at term and two normal foals were born from mares losing the one pregnancy (absorption of the foetus rather than mummification) in month two. In this study, six live foals were born (2 of normal size), from a total of 15 mares and 30 foetuses. This incidence is similar to previous reports wherein of 130 pregnant mares with twins, only 17 live foals (13%) were produced. An interesting observation from the later report was that from the 102 mares that delivered live or dead twins in the previous year, only 37 produced live foals the next seasons. Thus, over two seasons, there was an average of 23% producing live foals. An earlier study was extremely useful in categorising the outcome of twins that managed to survive to later pregnancy. Twinning accounted for 22% of the cases of abortion and stillbirth between 1967-1970. Sixty-two sets of twins and their placentas were examined from Thoroughbred mares. All were considered to be dizygous. Abortion or stillbirth of both twins from 3 months of gestation to term occurred in 64.5% of mares, although most (72.6%) slipped from 8 months to term. In the remaining cases, one twin (21%) or both twins (14.5%) were born alive. Most foals at term were stunted and emaciated, and of the 31 alive at birth only 18 had survived to 2 weeks of age.[3] In this study, twin placentation was divided into three morphological groups according to the disposition of the chorionic sacks within the uterus. Type A placentation was seen in 79% of cases (48 sets of twins). One foetus occupied one horn and most of the body (mean 68% of the total functional surface area), while the other twin occupied only one horn and usually only a small part of the adjacent body. Where the chorions abutted there was a variable degree of invagination of the smaller chorion into the allantoic cavity of the larger twin. These pregnancies frequently ended in abortion or stillbirth of one or both twins. In this group 31/48 lost their pregnancies between 3 and 9 months (64.5%). The gestation length in this group was frequently shorter and at birth the larger twin had a much greater chance of survival than the smaller one. In this group only 6 foals of 48 sets were born alive. Of the 6 foetuses born alive, 5 were the larger twin. Type B placentation occurred in 11% of cases (7 sets) and the placentas were orientated such that the villous surface areas were more or less equally divided and each foetus occupied one horn and half of the body. Both foals were usually similar in size and were usually born alive. Nine foals survived to 2 weeks from 6 sets of twins that made it to term. In this group (7 total) one aborted at 7 months. Type C placentation was seen in 10% of cases (6 sets of twins). In this group, there was a greater disparity between the surface area of the 2 chorions (85% versus 15%). The smaller twin occupying only part of one horn, died earlier on and became mummified. The larger twin was usually born alive with a fair chance of survival. In this group, 3 foals were born alive from 6 pregnancies at term. The authors attributed the loss of twin foetuses and poor survival rates to placental insufficiency. Readers are encouraged to obtain the article of Jeffcott and Whitwell (1973) as, with the current use of ultrasonography, it is unlikely a population of this magnitude of twin pregnancies progressing to an inevitable conclusion will ever be available again.[3]

When one or both of opposite-sex twins are born alive and survive their reproductive potential should not be affected. Cattle that have opposite sex pregnancies commonly

develop the “freemartin condition”, and this is related to vascular connections between both allantochorions and, XX/XY chimerism develops, hormones are interchanged, and ultimately there is masculinisation of the female tubular reproductive tract to varying degrees.

In equine twin pregnancies, interplacental vascular anastomoses were macroscopically visible in ~25% of the cases and resulted in blood chimerism without interfering with fertility, and no freemartins were observed despite evidence of chimerism. This was explained by the relatively late establishment of vascular anastamoses of the allantochorion in the horse (after gonad differentiation has occurred) compared to the cow, where blood vessel anastomses begin as early as 50 days.

It should be clear that our philosophy is that non-intervention is only acceptable when twins are diagnosed as a unilateral occurrence between days 17 and day 40. Then the decision depends on factors such as the value of the foal, the potential for rebreeding and the ability of the veterinarian to manually intervene. Intervention in twin pregnancies is strongly recommended in all other circumstances.

Ultrasonographic management of Twins

It is our responsibility to successfully manage early pregnancies such that no mare delivers or aborts twin foals. In consultation with farm managers, owners, and clients, we must utilise available equipment and technology commensurate with economic constraints and other owner/manager preferences to diagnose twin pregnancies as early as practically possible. It is also the responsibility of the veterinary profession to adequately inform owners/managers/clients of reasons why twins may be not diagnosed.

Twins are still occasionally missed despite multiple examinations. Reasons why twins may not be detected, despite repeated examination are: 1) difficulty distinguishing structures (this may be related to a poor examination environment i.e., too much light, poor display characteristics of the ultrasound unit, mare movement and/or lack of restraint), 2) variable growth patterns especially from asynchronous ovulations, 3) inability to detect heart beats of adjacent embryos, 4) operator experience, 5) resolution of the equipment, and 6) potential confusion of one or both pregnancies with uterine cysts. The most common cause of misdiagnosing the presence of twin pregnancies is examination prior to the time period that a second pregnancy (asynchronous ovulation) may be reasonably expected to be detected. Another reason is examining too quickly or terminating the exam once a single pregnancy has been detected in later (post-fixation) cases of bilaterally fixed (not unilateral) twin pregnancies. Also of note is the propensity of the uterus of early pregnant mares (day 20-35) to bulge forward at the corpus cornual junction, and it is possible with either a single pregnancy or twins to miss a pregnancy at the corpus cornual junction and yet scan the entire uterine horn to the body on both sides without the uterus ever leaving the screen. The potential for this to happen is something that we always try to make less experienced veterinary practitioners aware of. Even experienced equine reproductive practitioners have inadvertently given prostaglandin after a negative pregnancy diagnosis at 30 days only to identify an aborting pregnancy at the next examination. A negative pregnancy test at 30 days should always be reviewed either at the time or a few days later.

Management Techniques

The mare is generally efficient at reducing unilateral twins to a single pregnancy. This is done by a competitive absorption of nutrients that is related to the size and position of the early pregnancy and later to the orientation of the embryo proper within the developing conceptus. However, any non-intervention decision needs to extrapolate the probability of successful reduction based on the age of identification, the orientation and fixation of the

vesicles and any disparity in size. It is not considered good practice to leave twin pregnancies alone in the pre-fixation period unless one or both are too small to manipulate.

Recognition ≤16 days from ovulation

Embryo reduction on or before the day of fixation is not considered an important aspect of the natural correction of twins, and the probability of a mare losing one or both vesicles of a set of twins from identification prior to fixation is minimal and approximates that of early embryonic death for the same time period (per vesicle). As smaller than expected single pregnancies have a higher rate of early embryonic death, management techniques should consider the destruction of the smaller vesicle even if the body of evidence suggests minimal pre-fixation embryonic reduction. The recognition of twin pregnancies prior to fixation day (day 16) is dependent on the day of examination relative to the day of ovulation. Asynchronous ovulations occasionally result in a significant disparity in vesicle size and examination one day earlier may have failed to detect the younger of the two pregnancies. As almost all twin pregnancies occur from multiple ovulations, re-examination of all mares that have two CLs and only a single vesicle detected prior to fixation (day 16) is recommended. Recognition prior to fixation is also dependent on operator experience, resolution of the equipment (≥ 5 MHz preferred), monitor capabilities, restraint, and other facilities (ability to darken the environment), the presence of uterine cysts and the skill of the examiner. Twin or multiple pregnancies recognised prior to the day of fixation should be reduced to a singleton as soon as practically possible (see below).

Recognition after fixation (after day 16)

It should be clear that our philosophy is that non-intervention is only acceptable when twins are diagnosed as a unilateral occurrence between days 17 and day 60, and then the decision depends on factors such as the value of the foal, the potential for re-breeding (endometrial cup formation, stallion availability etc.) and the ability of the veterinarian to manually intervene. Intervention in twin pregnancies is strongly recommended in all other circumstances (see below). Intervention must occur before day 35 if endometrial cup formation is to be prevented and immediate re-breeding is contemplated. When bilateral twins are diagnosed after day 16, then destruction of one should be attempted as soon as possible. The incidence of loss of one of a set of bilateral fixed twins is similar to the probability of loss for a single pregnancy, although is affected by the age of the pregnancy.

When and how should we intervene?

Recognition ≤16 days from ovulation

The first technique for manual crush of the conceptus during the mobility phase utilised manual reduction with good results. The technique involved gentle manipulation of the embryonic vesicle to the tip of one uterine horn and manual rupture. When applied to single pregnancies it resulted in pseudopregnancy, and when applied to twin pregnancies it resulted in a single pregnancy in 7 of 8 attempts. Utilising the same embryo reduction techniques, later studies incorporated treatment of mares with single or multiple intramuscular injections of progestagens administration (hydroxyprogesterone caproate), an anti-prostaglandin (flunixin meglumine) plus progestagens, or given no treatment prior to manual embryonic rupture in the mobility phase.[4] Results were 10/10 (100%) mares maintaining pregnancy in the control group (no treatment, just manual rupture) and 37/40 (92.5%) for treated mares. The amount of $PGF_{2\alpha}$ released was directly correlated with the pressure required to cause embryonic rupture. Flunixin meglumine inhibited $PGF_{2\alpha}$ release after embryonic rupture. Treatment with progestagen plus flunixin meglumine or progestagen singly or multiply was not better than no treatment at all (although it was subsequently shown that the progestagen chosen had no ability to maintain pregnancy in

ovariectomised mares and did not bind to progesterone receptors in the horse). Another report demonstrated that 60 of 66 mares (90.9%) maintained a single vesicle after manual reduction was attempted prior to fixation. Five of the six mares in which the procedure was not successful subsequently conceived. Since 1984 we have used a modification of the technique described originally by Ginther in 1983.[5] With our technique, the ultrasound probe is used to manipulate the vesicles while keeping one or both vesicles in view during the manipulation and more importantly the crushing or rupture of the vesicle. Utilising this technique, it is possible to more accurately and quickly separate vesicles. It was original proposed that when vesicles were in apposition mares be re-examined approximately one hour later. By utilising the probe to manipulate vesicles, separation is achieved (pre-fixation) very quickly in most instances. Commonly, the smaller vesicle is destroyed despite the lack of evidence to support pre-fixation reduction. On occasion, it is necessary to reattempt the embryo reduction technique 24-48 hr after the original evaluation if the smaller of the two vesicles is less than 10 mm in diameter as sometimes these can be more difficult to rupture.

Separation of vesicles should always be possible if the vesicles are still able to be identified as two spherical non-coalesced structures. Briefly, the technique involves separation of the vesicles using the probe. A finger is placed on either side of the probe to help stabilise the vesicle to be moved. Gentle side-to-side movement of the probe with pressure results in the two vesicles becoming separated. The separation is identified by lack of a vesicle under the probe (just the homogenous grey of the uterus). The vesicle can be crushed as close as 5 mm from each other, but it is generally best to separate them at 10-20 mm or more. This is in case the mare moves at the time of increasing pressure. The vesicle is crushed by either gradually increasing the pressure using the probe or a sudden increase in pressure much like a quick flick or snap at the end of the probe. This later technique is quite useful for smaller (day 11-13) vesicles. Over the years we have found that it is hard to teach the "snap" technique. The technique involves gentle pressure until the vesicle is reduced in height at least 50%, then a rapid snap with the tip of probe ablates it. There does not need to be direct pressure against a surface like the pelvis as when the technique is correctly applied the inertia of intestines etc is sufficient to brace against the rupturing of the vesicle. This technique can only safely be applied by placing a finger on either side of probe to restrict the movement of the vesicle while gentle pressure on the probe begins to distort it. At that time a sudden force ("snap") is applied to the tip of the probe. Only people well experienced in rectal ultrasonography should attempt this technique as it can be dangerous for the mare. When the vesicle is crushed, it is not uncommon for its fluid to surround the other. This is not a problem at this stage of pregnancy. Later (≥ day 25) fluid surrounding then other vesicle is thought to be a potential problem.

Generally, separation prior to fixation is not difficult and the crushing technique described above is appropriate for most vesicles between day 13-16. Smaller vesicles are often more difficult to crush, and they should be managed with a rapid "snap" with the tip of the probe (or left alone for one or two days).

Specific variations that can result in vesicles being difficult to crush are when the vesicle is on the bladder, when the mare has an excessively full bladder, or when the vesicle is immediately in front of the cervix. When the vesicle is on a full bladder it will move away from the probe as soon as any pressure is applied, and it must be stabilised and managed by the "snap" technique. A mare presenting with an excessively full bladder is more likely to occur when mares have been held in yards for a few hours prior to being examined and appears uncomfortable in their environment. These mares are best turned out into a yard by themselves after examination as most will urinate within 10 minutes due to stimulus from palpation. Alternatively, the mare can be represented a day later (after being left not yarded). When the vesicle is immediately adjacent to the cervix it is difficult to apply the pressure due to physical restriction to force created by the body of the cervix. In these

cases, it is best to move the vesicle away from the cervix before attempting to crush or re-examine the mare in a few hours.

Most twins in contact will have a horizontal orientation towards each other, however on occasion the twins may be vertically orientated. Gentle pressure usually returns them to a horizontal orientation.

At the GVEH, records were evaluated for 1716 Thoroughbred (TB) mare cycles and 1294 Standardbred mare (STB) cycles. Twins were diagnosed in 245 of 1716 cycles in TB mares (14.3% of cycles) and 46 of 1294 of STB cycles (3.5%). After twin reduction, mares are not routinely examined until the next scheduled examination i.e., 21-25 days post ovulation (detection of the foetus). When mares were re-examined after pre fixation embryonic reduction 10/245 TB mares (4%) had lost the remaining pregnancy and 8/46 STB mares (17.4%) were not pregnant. The number of TB mares losing the remaining pregnancy (4.0%) is similar to 3.7% (63/1716) which was the measured rate of early embryonic death (EED) on the same farms for mares with a single pregnancy diagnosed at day 13-15 and then subsequently found to be empty at the next scan. Interestingly the number of STB pregnancies lost was much higher after twin reduction (8/46- 17.4%) compared to the measured rate of EED (7.1%). This we believe was likely related to economics wherein the STB clients were unwilling to examine for pregnancy early. Thus, twins when detected are likely to be closer to final fixation or fixed already and harder to manage.

A further study from the GVEH demonstrated that between detection and day 45 there were significantly fewer embryonic losses in mares diagnosed with multiple pregnancy (29/633, 4.6%) compared to singleton pregnancy (408/5414, 7.5%, p=0.004).[6]

It is our contention that the procedure has developed to the stage that it is *always expected* that a single pregnancy will exist after pre fixation embryo reduction is attempted. Unless a mistake occurs and the other vesicle is ruptured at the time of initial manipulation, we feel that any failure to survive the procedure is more likely a result of uterine inflammatory changes and/or infection or an embryonic defect rather than a result of the procedure. We believe that this is the most reliable technique available but feel it is important to highlight the experience of the personnel involved. From discussions with farm managers and other veterinarians, it is clear that only veterinarians involved with sophisticated reproductive management such as the routine use of ultrasonography can expect to achieve these types of results. Our strong recommendation to veterinarians and clients is that all mares are examined within 14-16 days of breeding. Expected time to ovulation after breeding will depend on frequency of examination and use of ovulation induction agents such as hCG or GnRH. Factors that may modify this decision are breed, mare value, ability of the stud master or owner to facilitate examination of the mare and on occasion education of the owner.

Recognition after fixation (after day 16)

Days 17-20

In all cases of *bilaterally fixed* twins, one should be destroyed immediately. The mare has an extremely efficient biological embryo reduction mechanism that operates when twins are in apposition (*unilaterally fixed*). Reduction occurred in 100% of 22 mares with asynchronous ovulation (vesicles size difference greater than 4 mm in diameter) and 19/26 (73%) of mares with similar vesicle size (0-3 mm difference). Because the rate of embryo reduction between day 17 and 20 is so high for unilateral fixation, equine practitioners frequently elect to leave these developing pregnancies and determine their outcome later. Our philosophies are that if the two vesicles have coalesced into one larger vesicle with an ultrasonographic visible line in division, they are left totally alone for 5-10 days more particularly so if there is any unevenness in vesicle size. If the individual vesicles

have still retained a spherical orientation or a spherical shape (like a figure ∞), then they can be separated gently with the probe and are crushed either in situ or after being manipulated apart. Due to the nature of our practice, few mares present with this configuration in the Thoroughbred population, however, it is not uncommon in the Standardbred population wherein economics dictate that pregnancy diagnosis is often delayed past the time the mobility phase has ended. Results from our practice with twins in this configuration are reduced compared to pre-fixation intervention procedures. Others have reported good results post fixation. One group reported success in 49/50 cases post fixation. The work of Bowman (1986) more closely parallels our experiences. With bilateral embryo fixation and intervention, he reported almost no losses with 40/44 mares from day 16 to day 30 (90.9%) having a single pregnancy detected on day 45.[7] With unilateral fixation the results were days 16 to 17 (16/18 - 89%) days 18 to 19 (23/24 - 95.8%) days 20 to 21 (8/13 - 61.5%) days 22 to 24 (4/9 - 47.4%) days 25 to 30 (1/4 - 25%). The high incidence of embryo reduction with unilateral fixation and the low incidence with bilateral fixation, makes recommendations with twins in the day 17 to 20 period quite clear.

Day 21 to 30

All cases of bilaterally fixed twins of this age group are manipulated and one destroyed immediately. In most cases we do not attempt to manually destroy one vesicle with unilaterally fixed twins of this age group until after day 30 and before day 35. At this age it is too easy to rupture both vesicles and the maximum success we believe we can expect is 50% (see previous section) which is less than or similar to the mares own biological reduction mechanism.

Day 30 to 35

During the period prior to the formation of endometrial cups, gentle pressure may be placed on one vesicle in cases of unilateral fixation. We do not attempt total ablation at this time as the resulting fluid from ruptured membranes sometimes surrounds the other foetus and effectively separates or prevents placental (chorionic girdle / trophoblast cells) attachments to the uterus. In these cases (total rupture of the vesicle), death of the remaining vesicle is very common. Between days 30-35 we attempt to pinch one vesicle with the probe and create a 'snowflake' effect which is the shedding of cells from the membranes. Demonstration of this effect almost always results in gradual loss of the affected conceptus. The pinching of the vesicle can be likened to membrane "slipping" of bovine foetal membranes except that we use the probe to produce the effect. Occasionally in mares with multiple cysts twins may be missed and then identified at a later time. In general, if they are unilateral, it is best to leave them to the mare's natural embryonic reduction method until day 30, when ablation can be attempted.

Day 36 to 60

Manual manipulation using ultrasound guidance

From day 36 onwards, it is a reasonable assumption that endometrial cup formation and subsequent eCG secretion will prevent many mares from returning to heat after early embryonic death due to varying susceptibility of the supplementary CLs to prostaglandin. Abortion after 35 days is commonly associated with difficulties recycling the mare. In one study, when mares were aborted either between day 26 and 31 or between day 30 and 50, 8/11 became pregnant versus 2/7, respectively. This is similar to the work of Pascoe (1983) who concluded that the administration of a prostaglandin analogue < 35 days of gestation was outstandingly successful as a method of treatment for twin pregnancy as mares could be rebred.

Manual intervention at this time, in our experience, is very good in managing bilateral twin pregnancies (<45 days) and was approximately 65% successful in unilateral twin

pregnancies. More recently (after 2010, although we introduced another technique in 2021), we have introduced some techniques that result in good outcomes for both unilateral and bilateral twins > 40 days. Success improves with use of more subtle pressure and damage to the chorioallantoic membrane rather than complete rupture in one attempt. Demonstration of the 'snowflake effect' without vesicle rupture consistently results in a gradual (48hr) stress of the foetus and ultimate loss of heartbeat. These pregnancies have the foetal fluids that become progressively more hyper echoic and reduce in size without interfering with the survival of the other foetus. Early pregnancies tend to be resorbed without a major increase in echogenicity of the foetal fluids. It is important with these foetuses to always attempt to damage the same one. Multiple attempts, i.e., everyday or every other day for 5 to 10 sessions maybe necessary to elicit the desired response, however, quite frequently we are unable to create sufficient damage for foetal destruction without danger to the other pregnancy. In these cases, rather than creating major trauma (rupture of the vesicle) an alternative approach is sought after day 60. A combination of membrane slip and or oscillation of the foetus are used. If we are unsuccessful in establishing a response at this stage, then the pregnancy is left until after day 100 (see below). Our anticipated success rates are 65% reduction and ~ %30 of cases result in further examination after day 100. With careful manipulation the demise of both foetuses should occur less than 5% of the time.

A variation for foetal destruction that is only possible between day 45-50 is oscillation and dislocation of the foetus from the umbilicus. This technique uses the weight of the foetus on a specific length of umbilicus to create a whiplash effect. The frequency of the oscillation varies from 0.5 to 2 per second. Force is not the key, rather a technique that moves the foetus maximally. The oscillation technique works exceptionally well with bilaterally fixed 45–50-day pregnancies and quite well in unilaterally fixed pregnancies when the one foetus can be isolated. It is not as useful after day 50 as the foetus has such a long umbilicus that it does not oscillate well and thus there is minimal trauma to the umbilicus or foetus. With this technique, colour flow Doppler is extremely useful in that once a significant change in foetal heart rate occurs, the procedure can be suspended until the next day when confirmation of death or survival of the foetus damaged can occur.

An interesting report was the surgical technique for removal of one conceptus from mares with twin concepti more than 35 days of gestational age (Pascoe and Stover 1989). Eight mares had bicornuate pregnancies and 7 mares had uni-cornuate twin concepti. Five of six surviving mares with bicornuate twin concepti, delivered a single viable foal and none of the 7 mares originally with uni-cornuate twin concepti, produced a foal. The poor survival rate of uni-cornuate twin concepti was attributed to disruption of the remaining chorioallantois during surgery.

Transvaginal ultrasound guided foetal puncture for destruction of one of a set of twin pregnancies

Foetal fluids from one foetus were aspirated while observing the relationships of the needle, foetus, yoke sac and/or allantochorion between days 20 and 45. Three of four bicornuate twin pregnancies resulted in a single pregnancy 10 days or greater after interference (similar to or less than our ability to manually destroy one conceptus in this configuration). Three of nine (33%) still had a viable single pregnancy after 10 days when twins were fixed together (between day 20 and 45). These results were disappointing, however they maybe improved with experience and/or antibiotic therapy at the time of intervention. Ultrasound-guided fluid withdrawal between day 40-50 or day 50 and 65 has been studied in single pregnancies; however, these studies did not involve any twins. Macpherson (1995) found that pregnancy reduction was more effective following aspiration of allantoic fluid (83%; 10/12), as compared simple allantoic puncture to 50% (6/12) (P=0.06). Our experiences with transvaginal ultrasound-guided foetal reduction are small

(N=5) however, between 45 and 60 days, the foetus within the vesicle was difficult to position. We only have attempted to directly puncture the foetus, not aspirate fluid, and have abandoned this technique (foetal puncture at this age) due to difficulties involved. All cases ended in loss of both foetuses, usually within three days of interference. The technique of ultrasound-guided transvaginal aspiration of foetal fluids has been excellently described along with results from their work and others.[8] The authors' conclusion was that this technique should be used before day 35. It is our belief that although this technique may well be suitable to use then, it is possible to have similar or better results with the ultrasound-guided manual manipulations described above, which has the added benefit of giving the mare a chance to apply her own natural twin reduction methods. The publication of Journee et al (2013) has confirmed similarly poor success with transvaginal ultrasound-guided twin reduction techniques.[9] In their study, only 33% of mares had a live foal. Although not significant, foetal stabbing appeared better numerically than aspiration 43% versus 29%, (P =0.14). Live foal rates were better when attempts at reduction were performed before 35 days (39.6%) compared to after day 35 (26%) (P <0.05). Both Journee et al. (2013) and Klewitz, Krekeler et al. (2013) reported poor pregnancy rates when attempts at reduction were after day 45.[9,10]

Day 60 to 100

Between day 60 and 100 it becomes more difficult to damage the chorioallantois. In these cases, we identify the most conveniently located (always the smallest) of the twins and repeatedly traumatise it by oscillation, or membrane slip, or attempt to damage the cranium with multiple attempts of single digit percussion. Approximately 50% succumb to this procedure, however it is tedious and time consuming.

A technique of cervical dislocation has been described as useful during this time period.[11] It appears well suited to pregnancies from day 70-90. Two techniques were described: firstly, dislocation of foetal head per rectum, and secondly via a standing flank laparotomy. The authors currently favour flank laparotomy (K Wolfsdorf, personal communication, 2008), personal communication). The technique is straight forward, using standard standing flank laparotomy procedures. Immediately prior to grasping the foetus, the mare is administered propantheline as a uterine relaxant. The chosen foetus is grasped and then the head is removed or dislocated from the neck by pinching it between the thumb and first finger. A distinct popping sensation is evident. In all cases, the foetus that has been manipulated has been alive immediately after and for 24 hours or more. Ultrasonography is useful in demonstrating that the head is well separated from the neck after the surgery.

This technique can be utilised earlier in gestation than trans-abdominal needle injection (after 100 days, see later in this section), potentially reducing prostaglandin-mediated inflammation and possibly enhancing the capacity of the placenta to expand within the uterus and support the remaining foetus

After an initial success rate reported of 2 live foals from 4 mares the authors reported a success rate overall of 64% from 44 cases with the most recent figures of 8 foals from the last 12 procedures (K Wolfsdorf, personal communication, 2008).

We can report one foal from 5 attempts. One mare aborted at 3-4 months after having a discharge and three had no foals. One mare was determined to be negative a few weeks after the procedure. The twin pregnancy was only 60 days old, and the procedure was associated with great difficulty grasping the foal as the uterus had started to contract and resulted in some trauma to the uterus. The heads separated easily, and all cases had two live foals around 3-4 days after decapitation with a clear separation of head and neck. None aborted live twins to our knowledge. It is not known why in our hands such a simple

technique did not meet the same results as others reported. It is possible that a more traumatic neck separation might result in a quicker death of the manipulated foetus. We have not continued to work with the procedure.

In the 2021 breeding season, we developed a technique to damage the foetus against the side of the abdomen. Briefly mares are sedated, and a uterine relaxant (propantheline hydrobromide) administered. The foetus is slowly isolated to the relevant abdominal wall (unilateral and bilateral cases) and then with the probe being placed directly on the isolated foetus a quick snap of the probe against the foetal chest is performed. It is surprising that only one or two attempts seem to be necessary before foetal death is confirmed with colour flow ultrasonography. We did this on 3 unilateral pregnancies last season and in all cases one survived and one died. The destruction of one does not guarantee the survival of the other so we await the results in this year's foaling season.

Days 100 onwards

Probably the most common reason for being presented mares at this late stage of gestation with twins is failure of the aforementioned techniques. Less frequently, twins have been missed in earlier diagnostic attempts and there has been an increase in diagnosis of twins at this stage due to the widespread use of foetal sexing procedures. Frequently mares have been identified with twins late in the breeding season and the owner has adopted a non-intervention approach. Because the possibility of foetal reduction after 100 days is very low, and the probability of abortion or stillbirth is extremely high, an approach was developed to eliminate one pregnancy at a later stage of gestation by Rantanen (1988).[12] The technique involved transabdominal ultrasonographic identification of the twins and intracardiac injection of a lethal substance. The smaller twin was always identified. Initial results with saline and air were unsuccessful but when the solution was replaced with potassium chloride, 7/18 mares (39.9%) had single live foals. We have been utilising this technique since 1988 and can report similar experiences. Our initial success was not very promising (2/10 live foals) until the potassium chloride solution was replaced with 10ml-20ml of procaine penicillin, which has resulted in a live foal rate of 56%. The current procedure at the GVEH is to tranquilise the mare with Detomidine and to identify the smaller foetus or in the case of evenly sized foetuses, the one with more potential for placental expansion. A 6–10-inch, 16g needle with a tip designed for ultrasonographic enhancement is passed through the needle guide biopsy channel into either the heart, lungs or abdomen of the identified foetus. Penicillin is injected, and the foetus monitored for the next 5 to 10 minutes. If the needle delivers penicillin in the chest or abdomen the demise of the foetus still occurs however it just takes a little longer (up to 5 minutes). The apparent advantages of penicillin as we see them are: 1) it can be visualised ultrasonographically as it is injected and 2) foetal death can still be obtained without intracardiac needle placement.

We have placed treated mares on Regumate® and long-term oral antibiotics; however, we have found no difference in foetal survival rates with either treatment compared to those that have received no treatment. At the time of needle puncture mares are treated with systemic antibiotics for 3 days and intravenous phenylbutazone. There has been much discussion about the production of small dysmature foals from successful use of this technique. While this occasionally does occur, care in correct foetus identification (retaining the biggest foetus with the most potential for placental expansion) has lowered the occurrence of this outcome dramatically. This is more readily possible when there have been multiple early opportunities to observe foetal size, position with the pregnant horn(s) and uterine body and placental arrangements. After injection and death of the foetus it gradually becomes mummified. Commonly these foetal remnants can be detected within the placenta at birth.

Late in gestation, twins may be difficult to recognise with transrectal ultrasonography except in those cases with observation of the twin membrane. Abdominal ultrasonography is useful to diagnose twins but is time consuming and difficult for those with less experience.

In the event of failure to diagnose twin pregnancies, occasionally abortion is heralded by lactation late in gestation (>7 months). There are reports of successful maintenance of pregnancy despite premature lactation (>1 month prior to foaling) in cases with twin gestation. Apparently, the premature lactation is induced by foetal death and the beginning of mummification of one foetus and thus is threatening to the remaining live foetus. Four mares with apparent impending twin abortion were able to deliver live single foals concurrent with a mummified twin after supplementation with progesterone was initiated upon recognition of inappropriate lactation late in gestation. In these cases, although foals were born small, they survived and thrived normally. Further work will be necessary to determine which mares will respond best or even at all to supplementation with progesterone/progestagens for initiation of premature lactation.

Induction of abortion

Occasionally, owners elect a wait-and-see technique. This is often due to financial constraints. When twin pregnancies are allowed to proceed to foaling or abortion, there is a significant risk that the mare will develop foaling problems or have difficulty being rebred. After 100 days of gestation, natural foetal reduction is not reliable enough to let the mare continue the pregnancy. Multiple injections of prostaglandin should be used to induce abortion as soon after 100 days as possible. It is not considered advisable for any reason other than finances to induce abortion in earlier twin pregnancies as occasional reductions will occur between day 35-100. Many veterinarians at the time period of 30-35 days administer prostaglandins to abort the mare rather than allow endometrial cup formation and risk missing the opportunity to re-breed the mare in the same season. However, we encourage intervention through manual trauma to the placental membranes, because this can result in good outcomes and manipulations can continue for a few days until the result is clear or destruction of both has occurred.

From all of the preceding discussion it should be obvious to readers that in our opinion the best method of handling twins is early identification and destruction of one during the pre-fixation mobility phase (< day 16).

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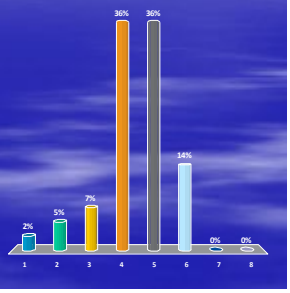
Management of Twins

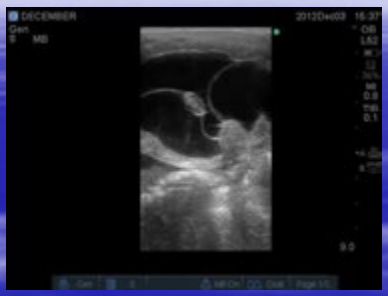
ACT Inaugural Michelle M LeBlanc Lectures
Savannah
Georgia
2019

- Born 15 June 1954
  - Detroit, MI
- DVM Michigan State University June 1977

What is the best age to manipulate and squeeze twins

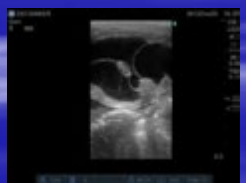
1. Day 11
2. Day 12
3. Day 13
4. Day 14
5. Day 15
6. Day 16
7. Day 17
8. 3-5

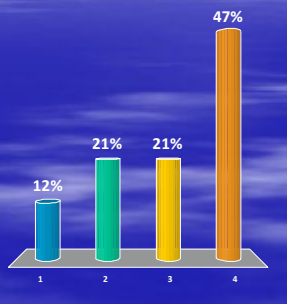




What was the previous slide…?

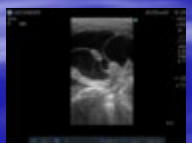
1. Normal day 27 twins
2. Normal day 33 twins
3. Dizygotic twins
4. Monozygotic twins

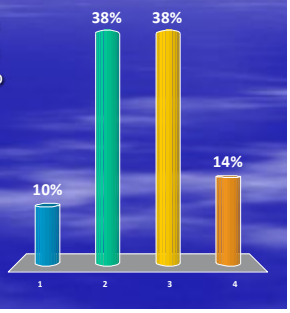




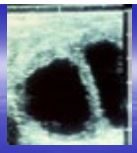
The best description would be..?

1. Diamnionic/Dichorionic
2. Diamnionic/Monochorionic
3. Monoamnionic/Dichorionic
4. Monoamnionic/Monochorionic





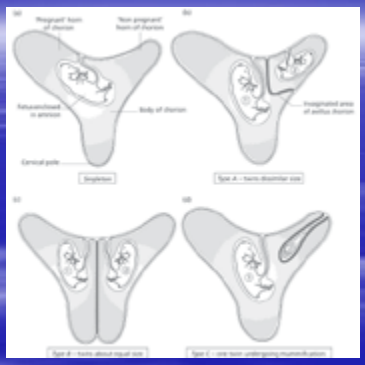
Dizygotic twins



- Repeatable within mare
- Heritable
- Varies with breed
- Stallion fertility
- Ovulation induction ?

Jeffcott and Whitwell 1973

- 22% of abortion or stillbirth (PM)
- 62 sets (all dizygous)
- 72.6% slipped from 8 mo to term
- One twin (21%) or both (14.5%) born alive
- 31 foals alive only 18 were alive at 2 weeks



Type A

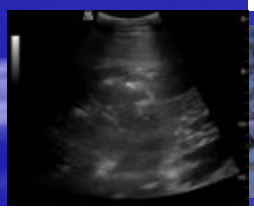
48 sets (79%)
31/48 aborted 3-9 mo
Shorter gestation
6 foals alive (5 bigger one)

Type B

7 sets (11%)
1 abortion and 6 sets alive
9 alive at 2 weeks

Type C

6 sets (10%)
85% v 15%
3 foals alive at term



Results From Initial Breeding Efficiency GVEH Survey

- 1833 Thoroughbred (TB) mares
- 1330 Standardbred (SB) mares
- TB - natural service
- SB - artificial insemination (AI)
- Years 1994-5

Fertility Statistics

	TB	SB
Pregnancies at 15 days	93.6%	97.3%
Pregnancies at 45 days	86.7%	88.6%
Pregnancy rate per cycle	70.4%	62%
Services per cycle	1.04	2.21

Twins

	TB	SB
Twins (multiple) per cycle	10.1%	2.2%
Twins per pregnancy	14.3%	3.5%

Twin Loss After Crushing

	TB	SB
Loss of both (< 25 days)	4.0%	17.4%
Loss of single pregnancies (< 25 days)	3.7%	7.1%

Nath et al Aust Vet J. 2010

Reproductive efficiency in TB and STB mares in Victoria

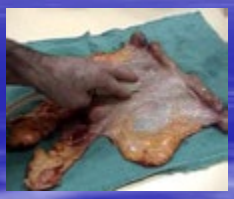
- 3105 TB mares (6123 cycles)
- 1350 STB mares (2690 cycles)
- Pregnancy rate identical (70% per cycle)
- Twins TB 8.3% versus 4.6% per cycle (P<0.001)
- EED (day 13-45)
  Twins 4.58% versus singleton 7.56% (P<0.001)

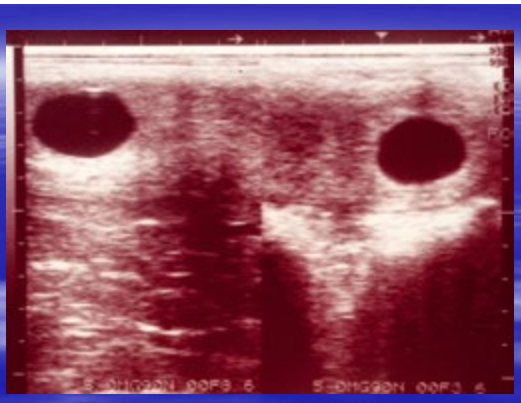
Twin Management
Stage of diagnosis

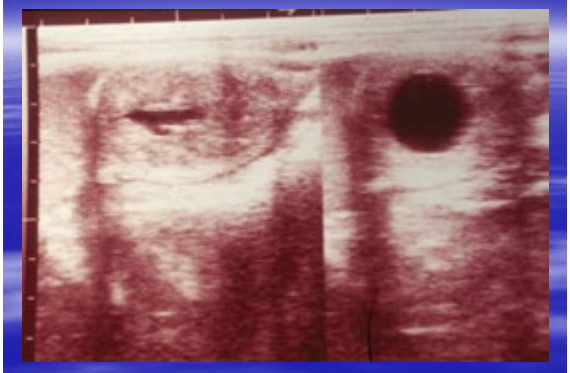
- Unilateral versus bilateral
- Pre-fixation (day 11-16)
- Post-fixation (day 17-25)
- Post-fixation (day > 25)
  - 25 – 60 (35 days*)
  - 60 - 100
  - > 100 days

Management of Twins

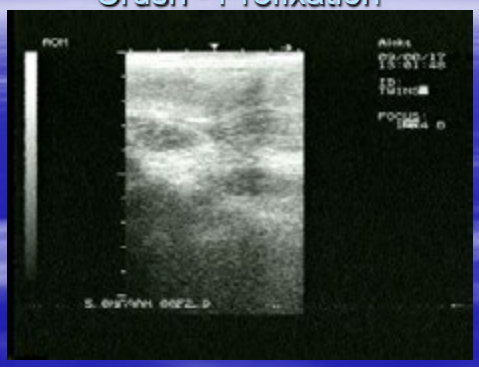
- Pre-fixation day 11-16



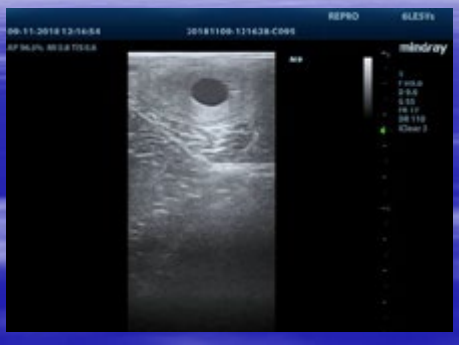




Crush - Prefixation



Crush - Prefixation



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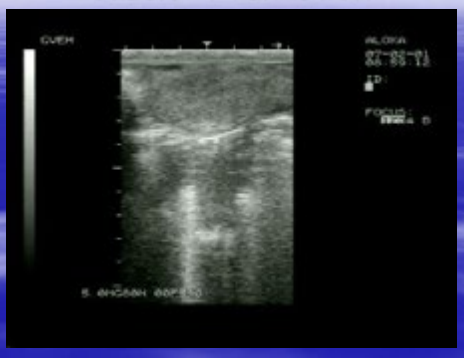

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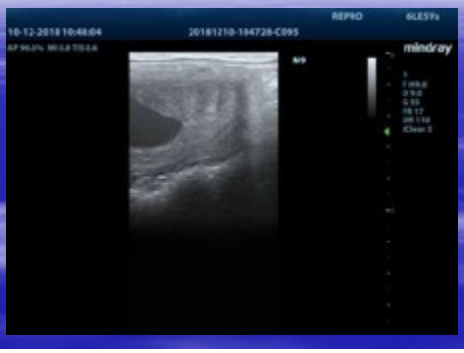

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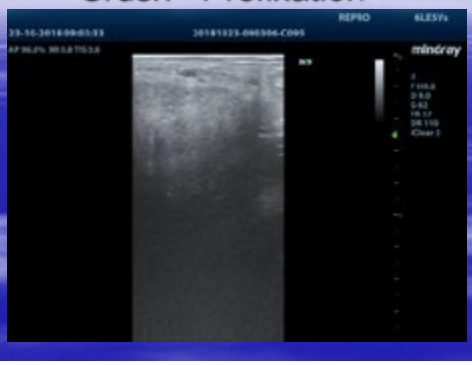
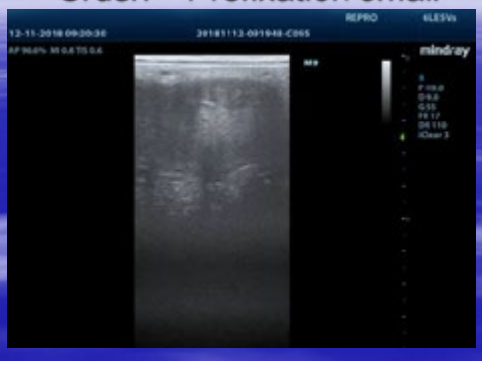
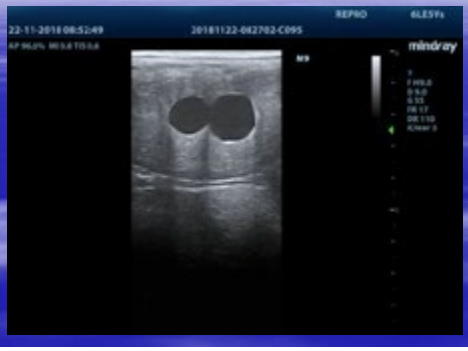


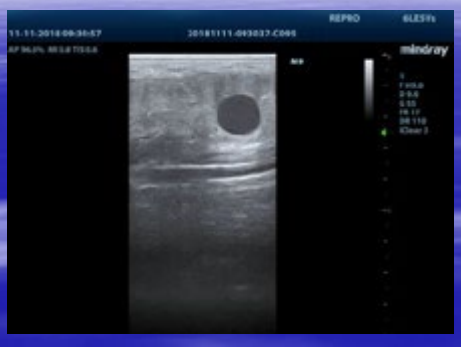
Crush – Prefixation small



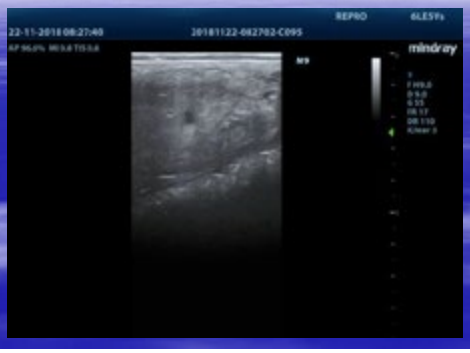
Crush – Prefixation close



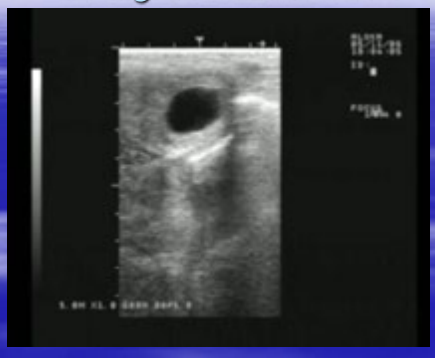
Crush – Prefixation close



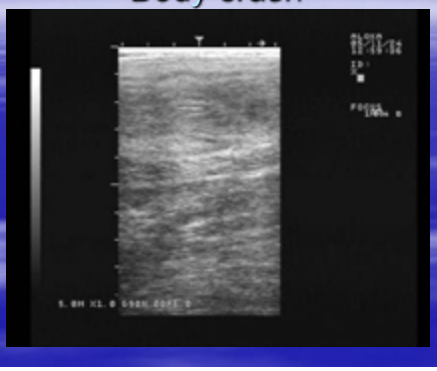
Crush – Prefixation cervix



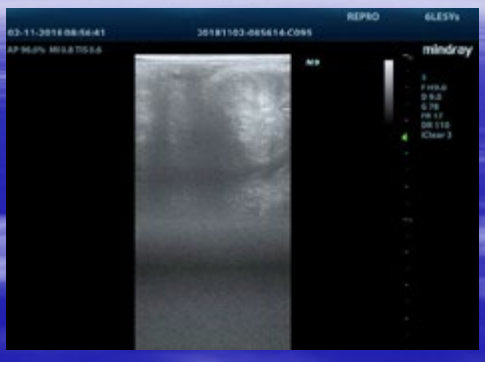
Straight forward crush



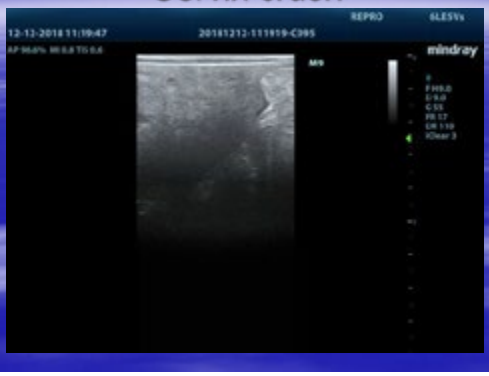
Body crush



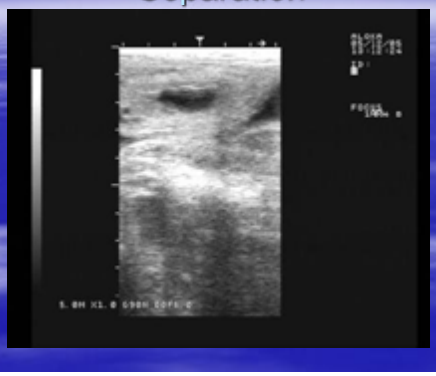
Cervix crush



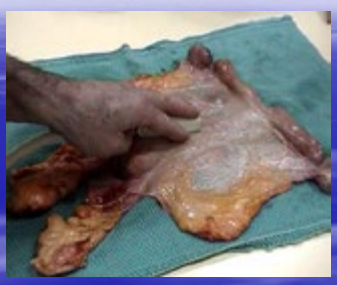
Cervix crush



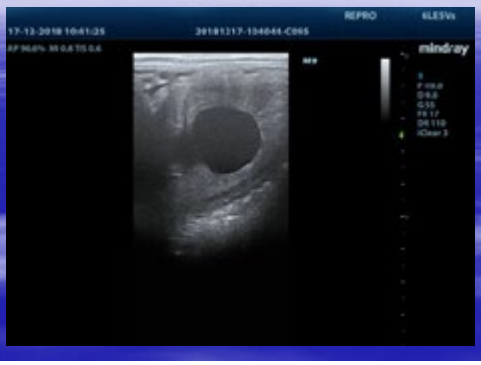
Separation



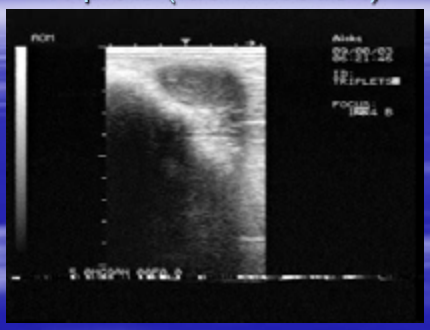
Separation



Separation



Triplets (identification)



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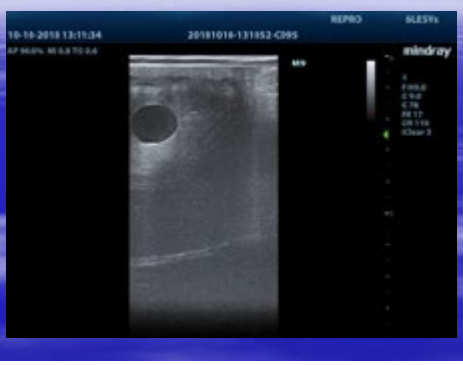

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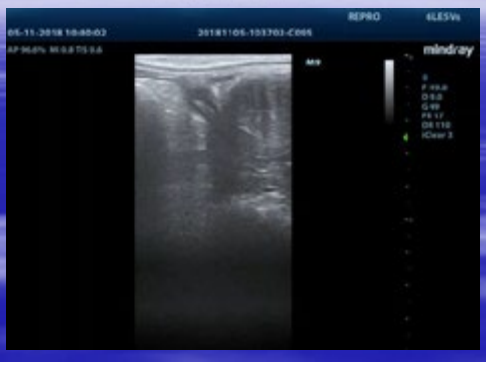
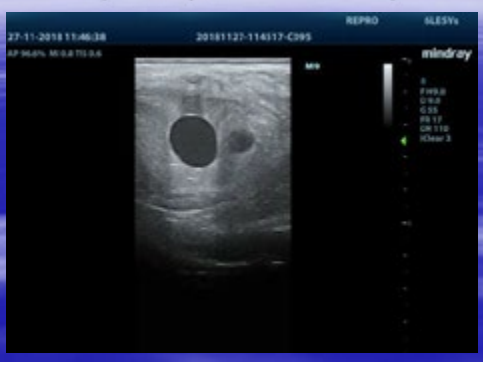
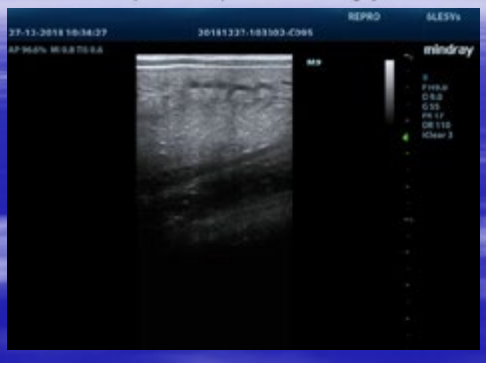


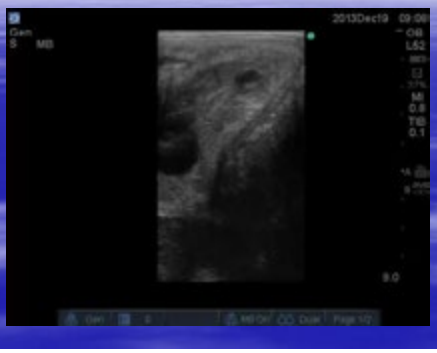
Triplets (Identification)



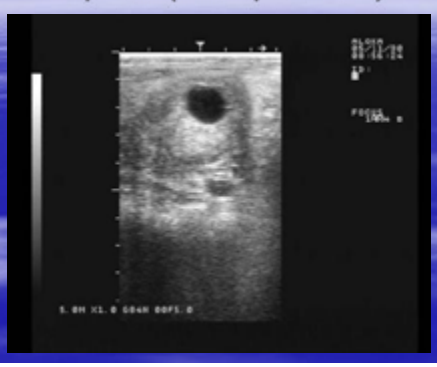
Triplets (in a hurry)



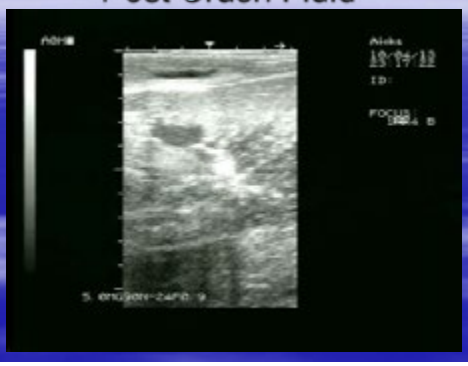
???



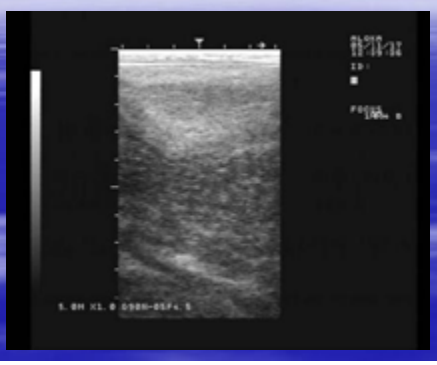
Triplets (manipulation)



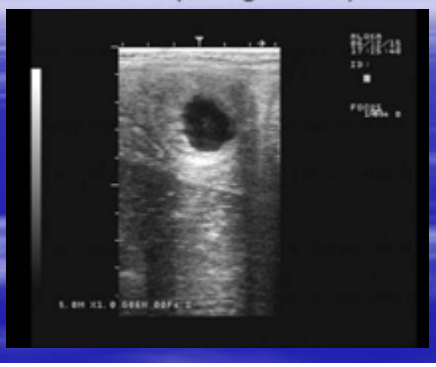
Post Crush Fluid



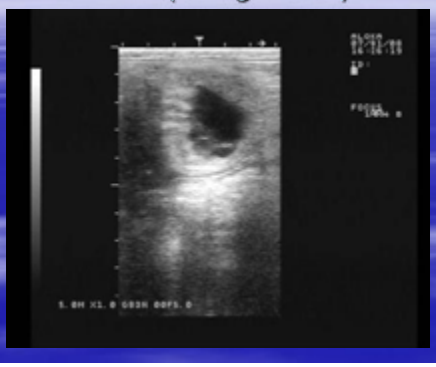
Almost fixed



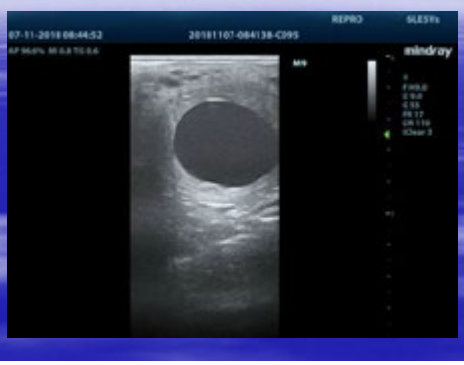
Fixed (dangerous)



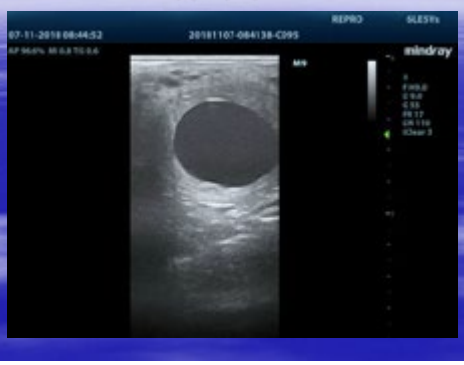
Fixed (dangerous)

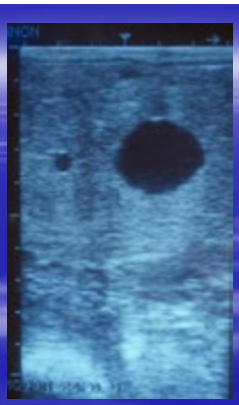


careful

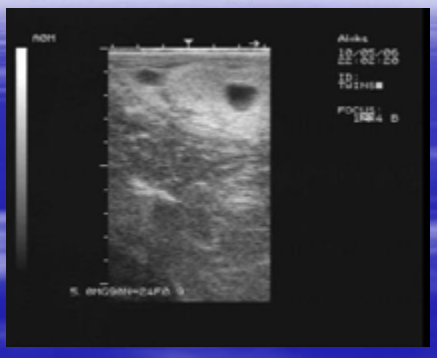


careful

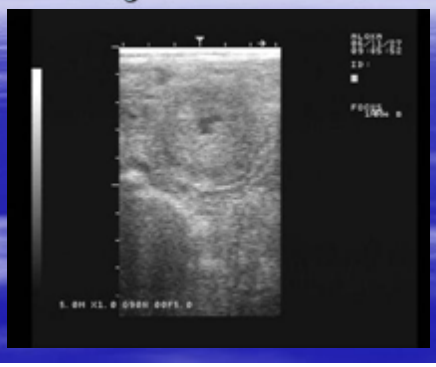


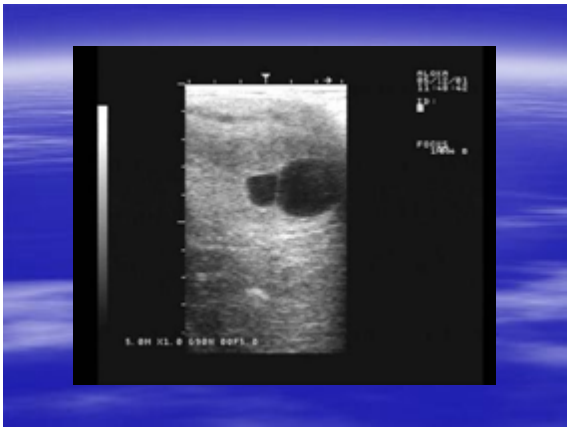


Separation asynchronous

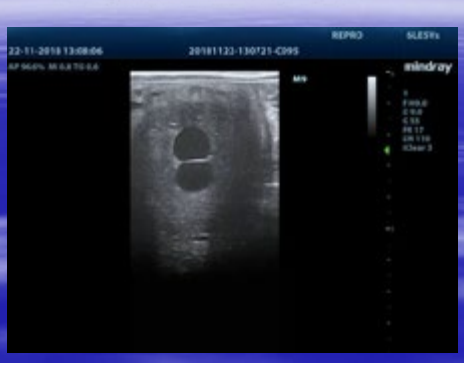


Diagnostic dilemma

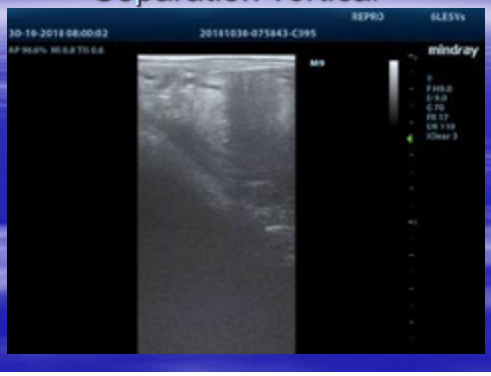


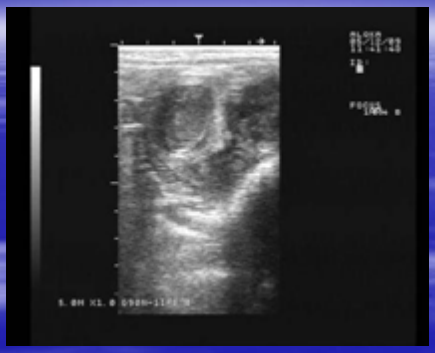


Separation vertical



Separation vertical

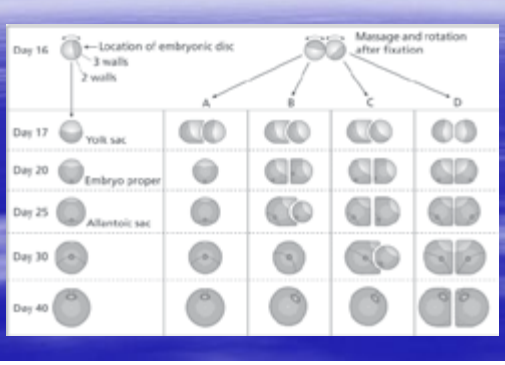


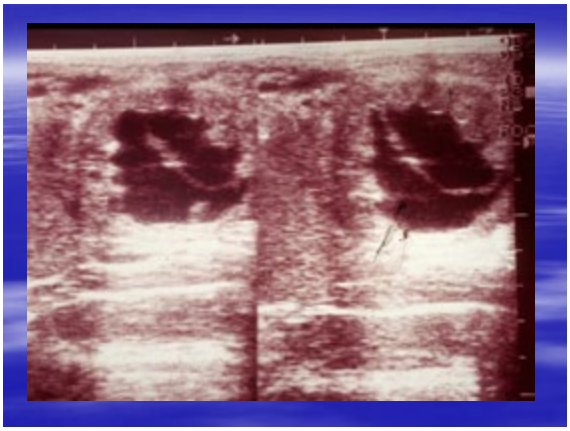


Management of Twins

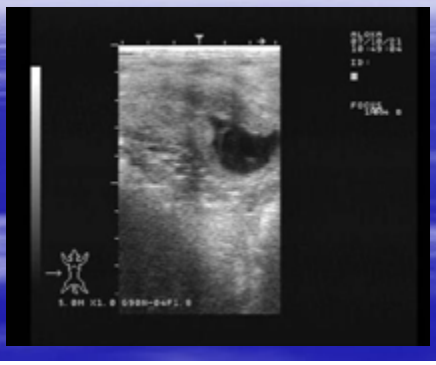
- Post-fixation day 17-25
- Unilateral

Ollie Ginther "Deprivation Hypothesis"

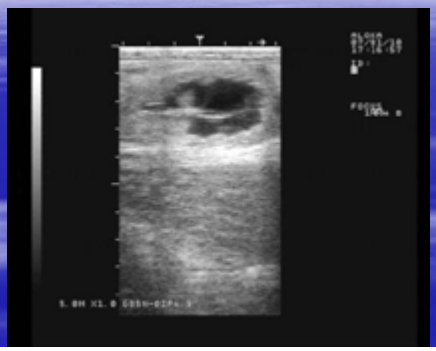




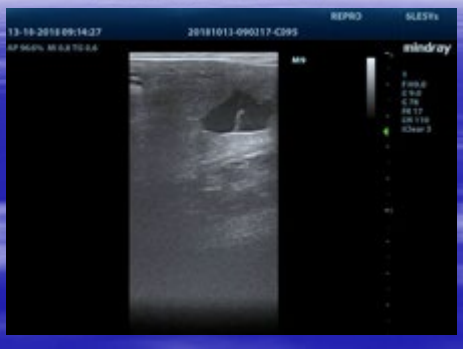
Natural reduction



Natural reduction



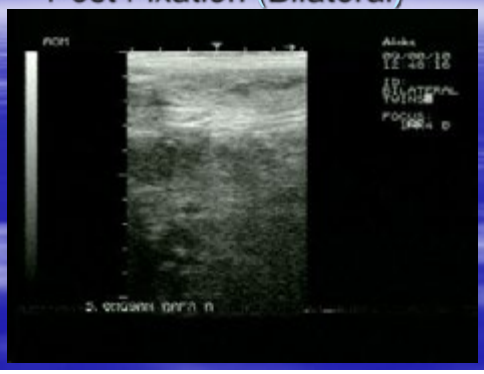
Natural reduction



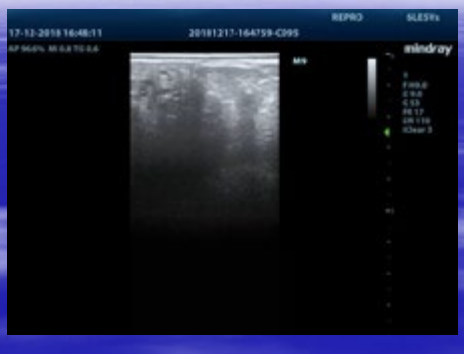
Management of Twins

- Post-fixation day 17-25
- Bilateral

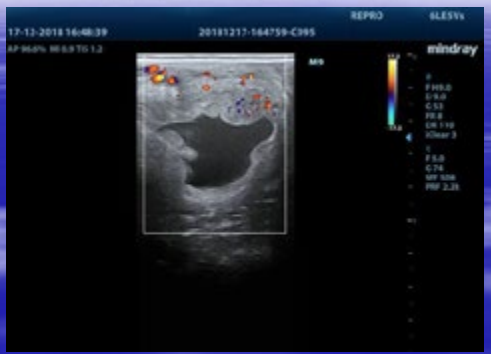
Post Fixation (Bilateral)



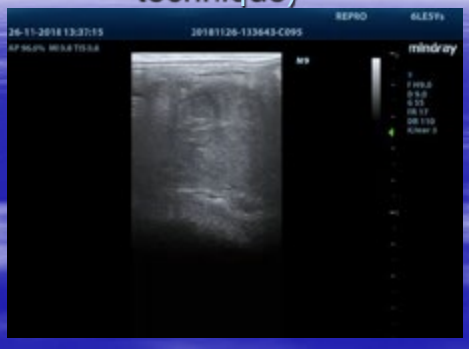
Post Fixation (Bilateral)



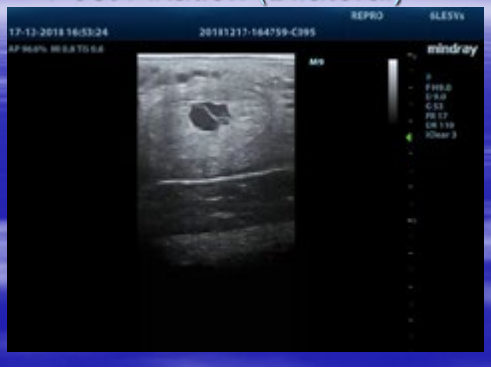
Post Fixation (Bilateral)



Post Fixation (Bilateral technique)



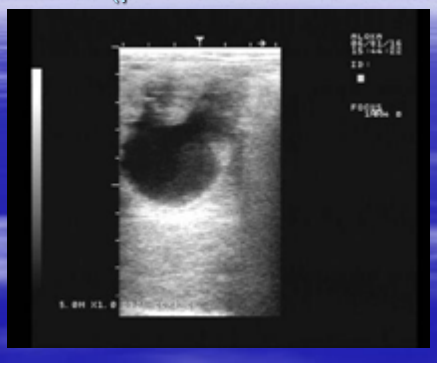
Post Fixation (Bilateral)



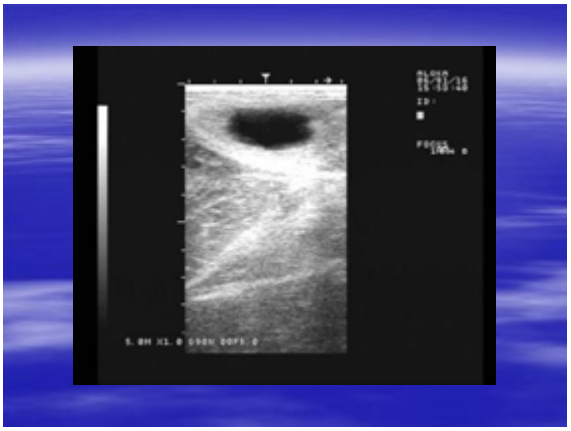
Management of Twins

- Post-fixation day > 25
- Bilateral

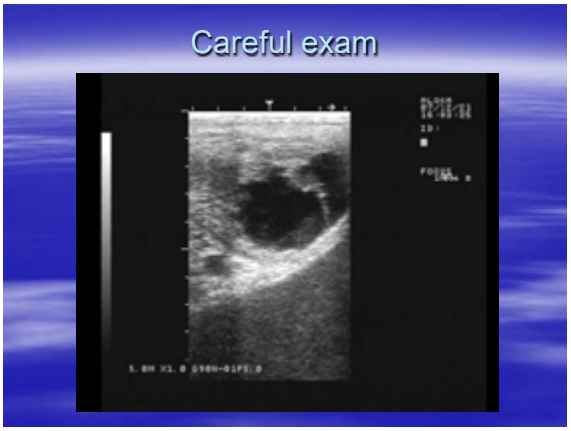
Bilateral (post fixation to 60 days)



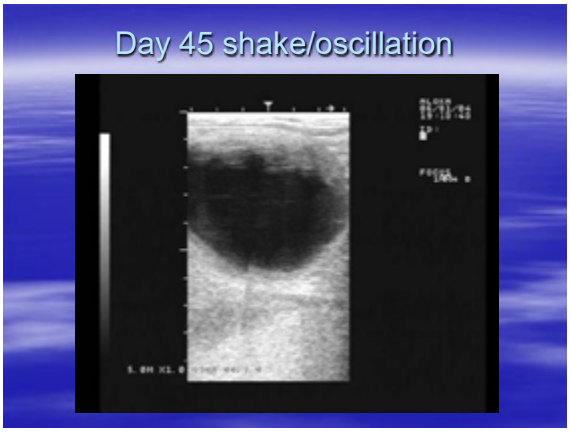
Technique



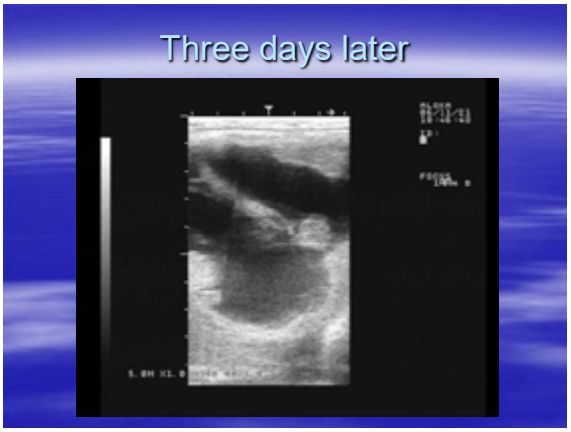
Careful exam



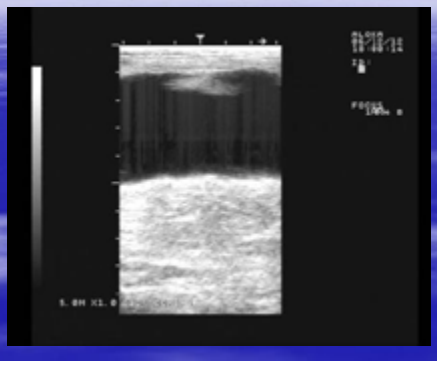
Day 45 shake/oscillation



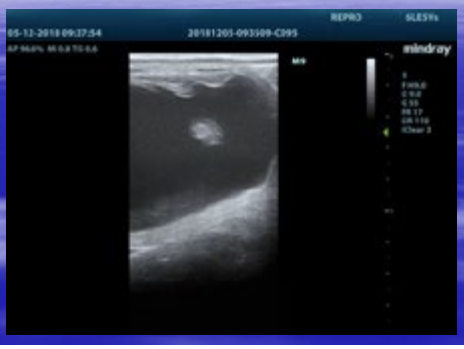
Three days later



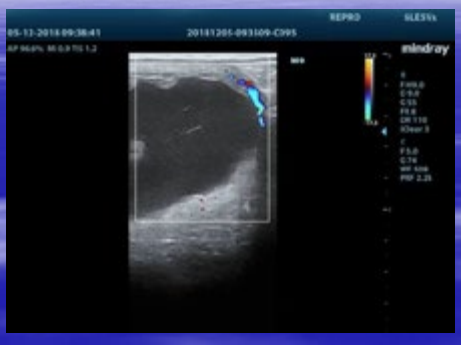
Another shake/oscillation



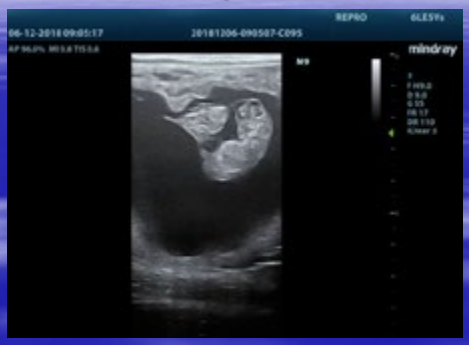
Recent shake/oscillation



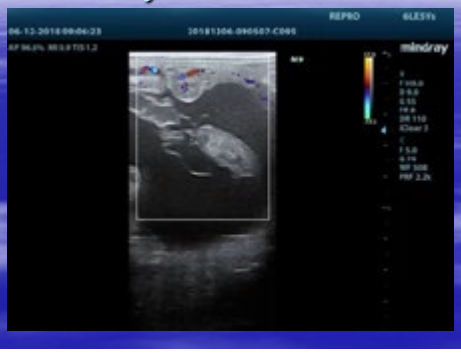
Same Twin Color Flow



One day later

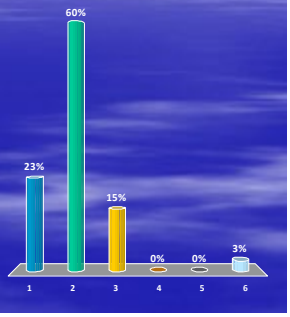


One day later Color Flow



What is the best way to treat a set of bilateral twins at 30 days?

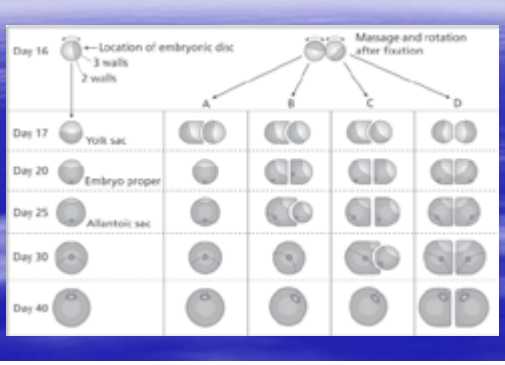
1. Shake
2. Crush
3. Wait until day 45
4. Wait until day 60
5. Transvaginal aspiration
6. Refer

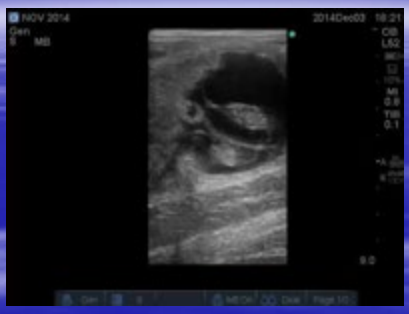


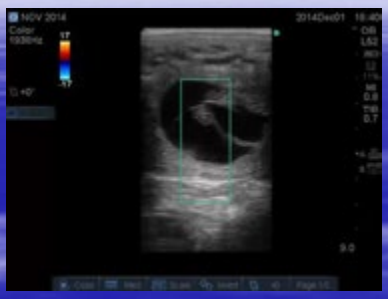
Management of Twins

- Post-fixation day > 25
  - Unilateral

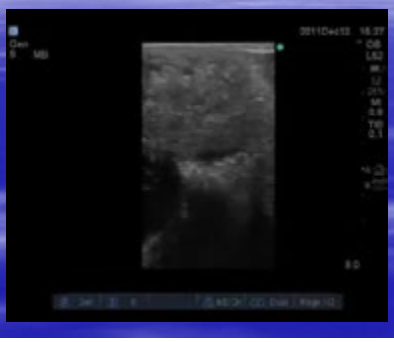
Ollie Ginther "Deprivation Hypothesis"



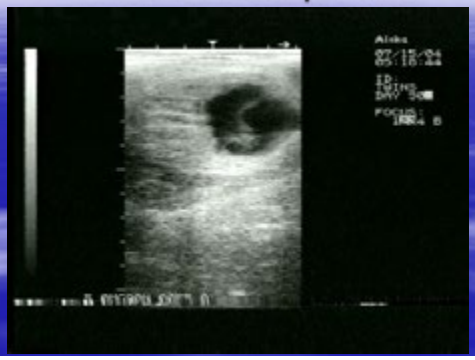




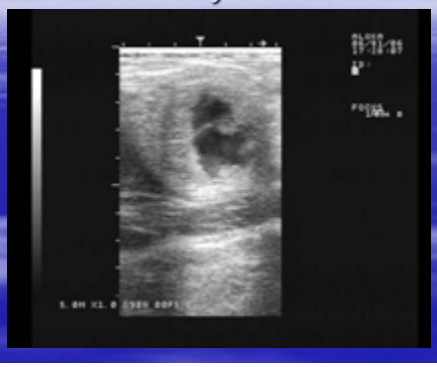
Unilateral reducing



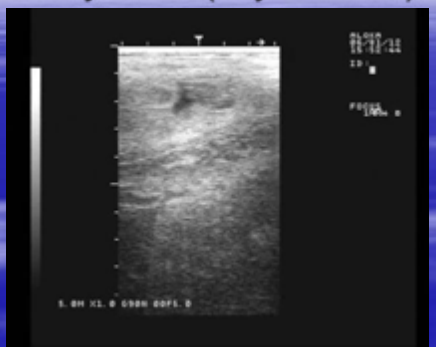
Unilateral Manipulation



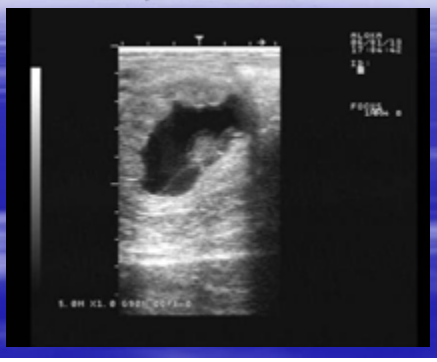
Unilateral day 28 left alone



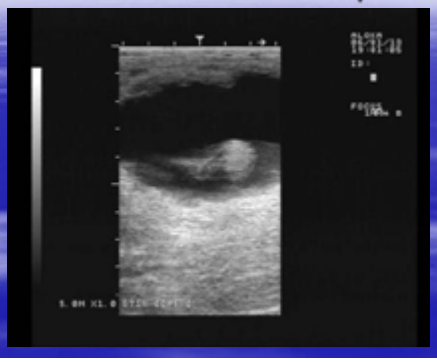
6 days later (day of crush)



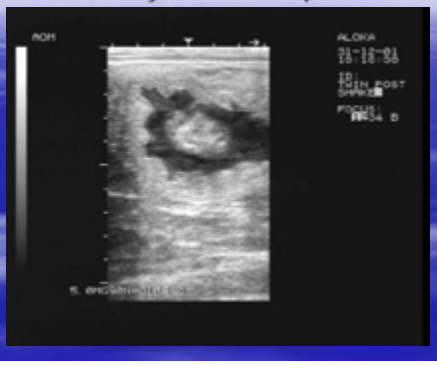
Day after crush



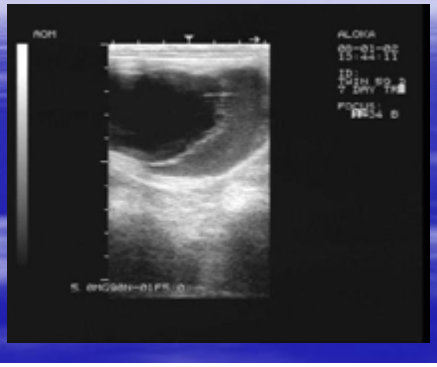
Unilateral shake/slip



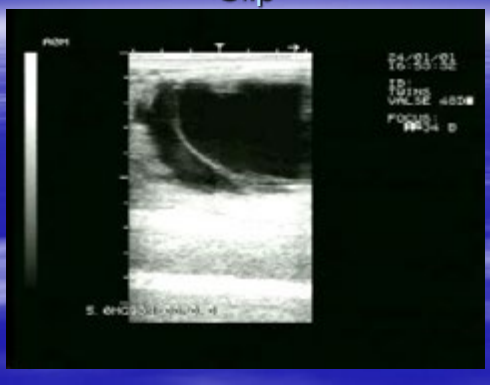
Three days after slip/shake



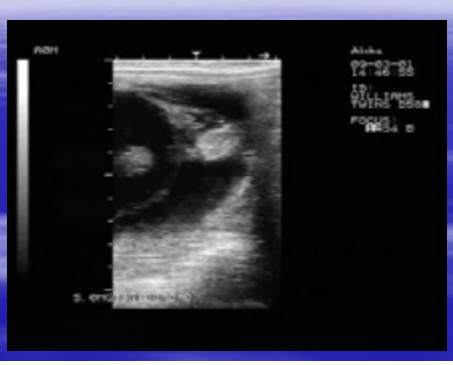
Seven days after trauma (different horse)



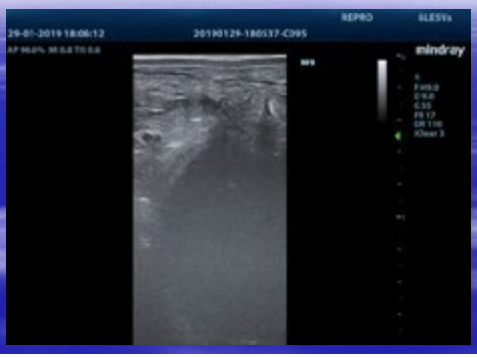
Slip



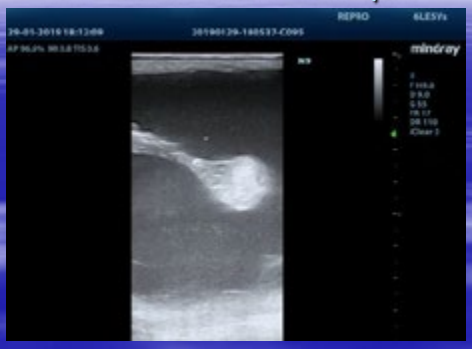
Careful exam of heartbeat



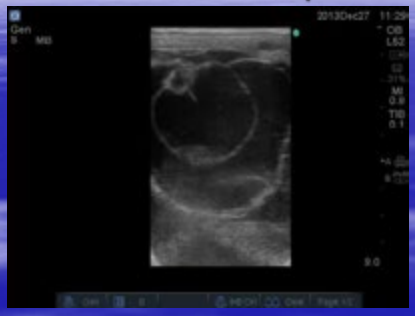
Twins unilateral Day 60 Identification



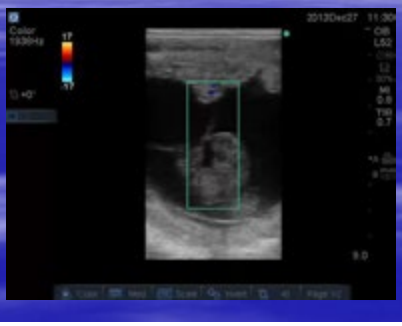
Twins Unilateral technique



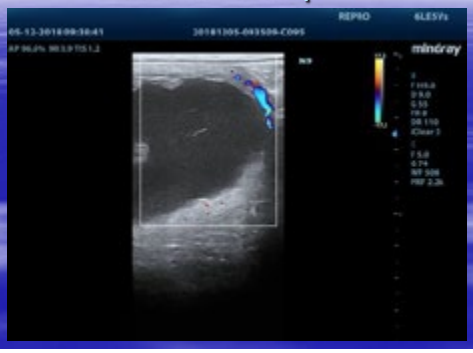
When to stop



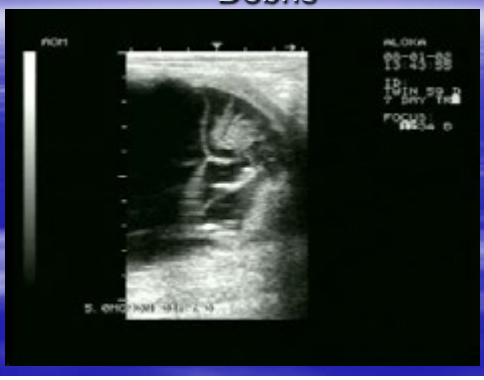
When to stop



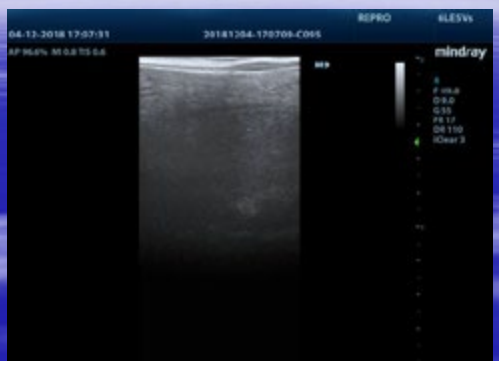
When to stop



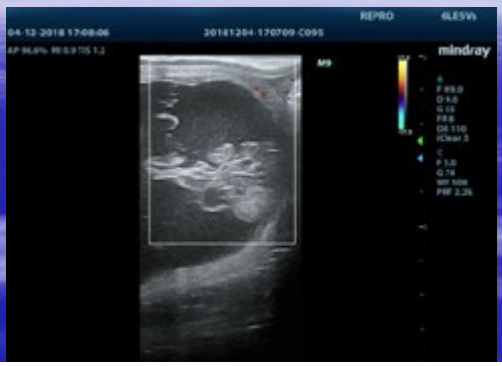
Debris



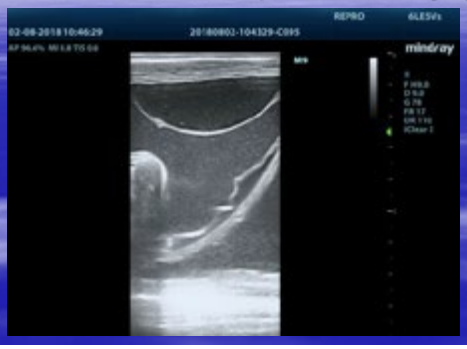
Debris



Lack of blood flow



Twin membrane (after 60 days)



Day 60-100

(Karen Wolfsdorf HDM KY)

Cranio-cervical dislocation:

Isolate one fetus and identify the head:

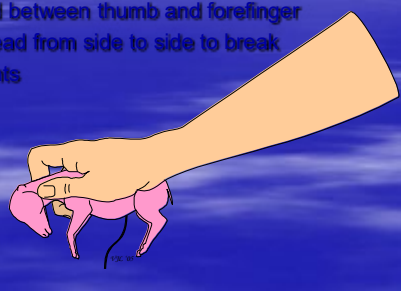
Dome shaped structure with mandible

Follow the neck

Head is about the size of a walnut

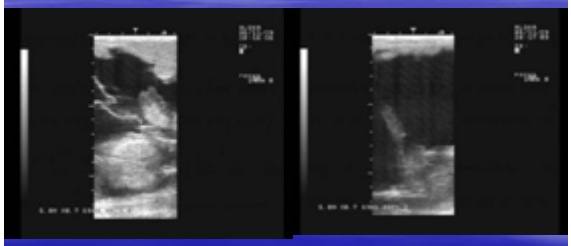
Cranio-cervical dislocation:

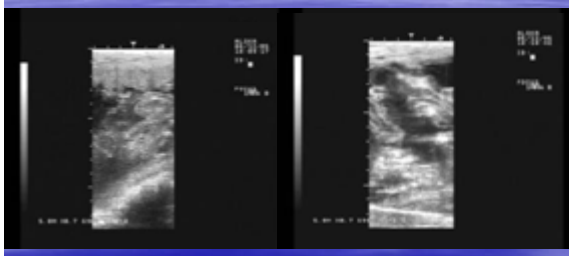
- Stabilize head between thumb and forefinger
- Manipulate head from side to side to break down ligaments



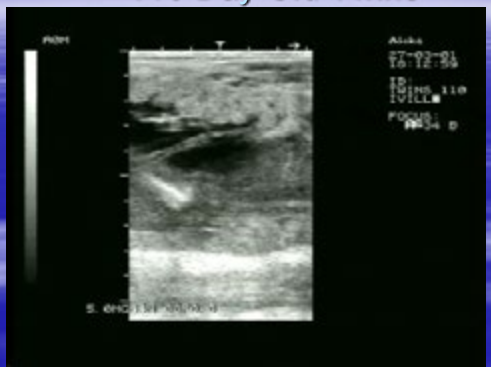
Cranio-cervical dislocation:

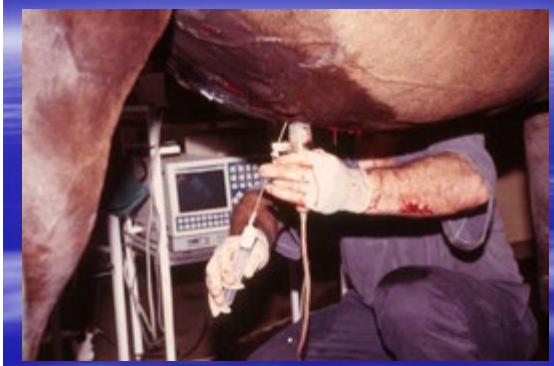
- Dislocation is created by placing thumb at the base of the cranium and applying pressure proximal and dorsally



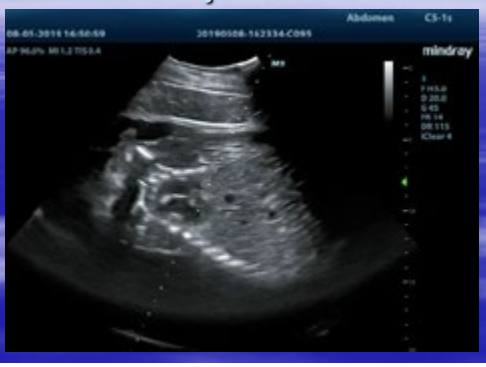


110 Day Old Twins

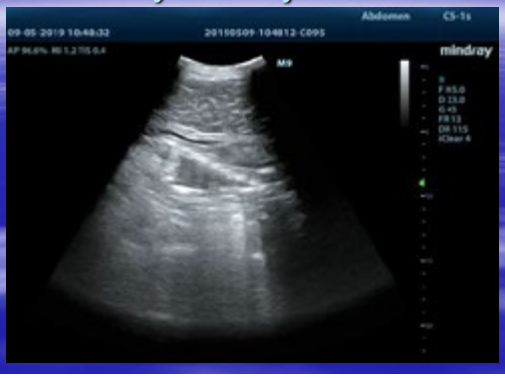




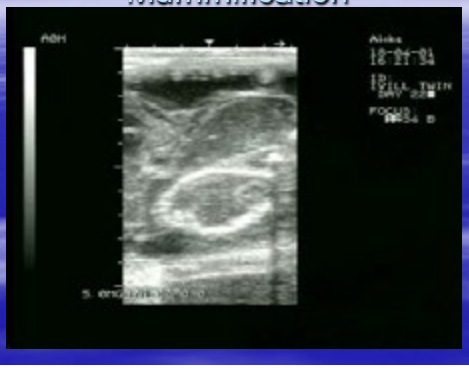
Injection



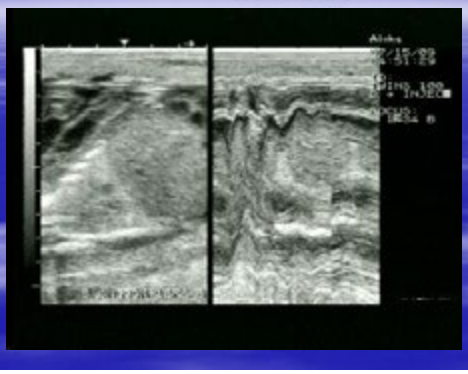
Day after injection

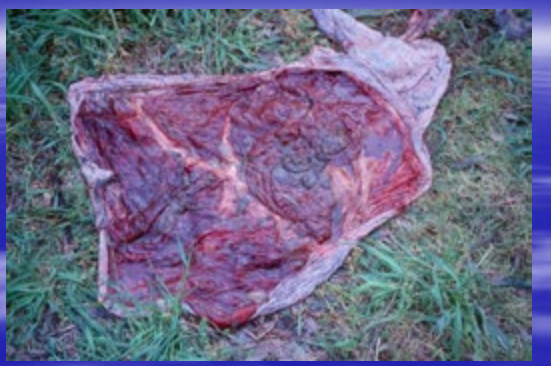


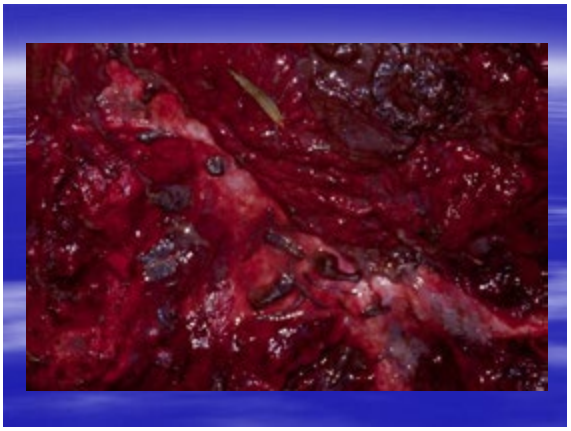
Mummification

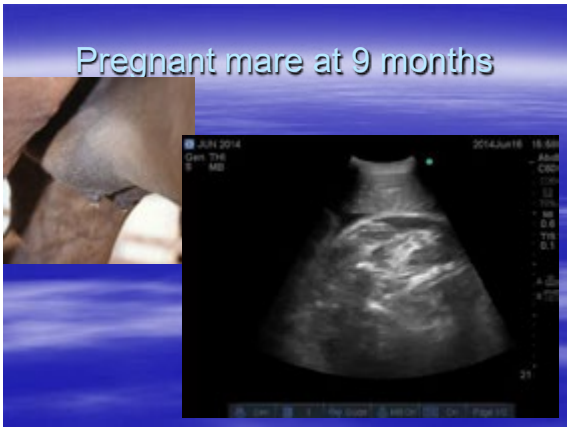


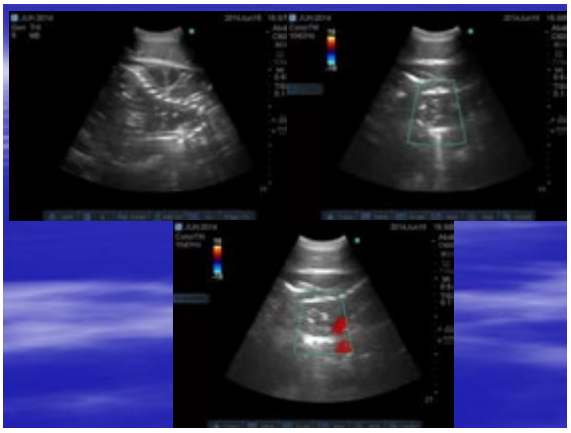
M Mode








Pregnant mare at 9 months



Notes

Peri-parturient problems in the broodmare

Chelsie A Burden
Goulburn Valley Equine Hospital
905b Goulburn Valley HWY, Congupna VIC 3633

Peri-parturient problems in the broodmare can be challenging for the equine clinician and worrisome to the owner. Complications during late gestation require the ability to diagnose and manage the associated condition to maximise the possibility of both mare and foal survival. Pregnancy losses during late gestation could be the result of foetal illness, placental dysfunction, or maternal illness. Pre-partum disorders of the mare can be diagnosed but identifying and monitoring conditions that affect the foetus and the placenta may be more difficult.

Complications in the early postpartum period are common and can be life-threating to the mare if left untreated. This publication will discuss some specific problems encountered in late gestation and the early postpartum period, along with diagnostic and treatment options.

COMPLICATIONS OF LATE GESTATION

Every mare at risk or presenting with clinical complications in the late gestation, should be systematically evaluated. A detailed history should be taken with a focus on current and past reproductive history. A review of previous pregnancy complications, birthing abnormalities and details of the current pregnancy should be discussed. A thorough clinical examination of the mare should be performed, including physical examination, examination of the mammary glands and vulva, and transrectal palpation. Further diagnostic procedures may be performed including endocrinology, transrectal ultrasonography, transabdominal ultrasonography and/or vaginal examination.[1]

Indications for examination include systemic illness, mammary development or lactation, vulvar discharge, increased abdominal size or shape and overdue pregnancy.

Placentitis

Placentitis in mares poses a significant threat to foetal and neonatal viability and is a leading cause of foetal loss in the horse.[2,3] Ascending placentitis is the most described aetiology and results from contamination of the caudal reproductive tract. The most frequent bacterial pathogens implicated in equine placentitis are *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Klebsiella pneumoni*ae, and *Pseudomonas aeruginosa.*[4] While ascending bacterial placentitis is common in the mare, systemic sources of infection and inflammation can occur. One such consideration is mucoid or nocardioform placentitis. While little is known about the source and pathogenesis of nocardioform placentitis, various organisms have been identified from the mucoid debris found in the dependent surface of the chorion.[5,6]

The most common signs of placentitis in mares are premature udder development with or without milk production and vulvar discharge. Observation of clinical signs and diagnosis of equine placentitis often occurs well after establishment of disease. A mare presenting with clinical evidence of placentitis should undergo further examination using ultrasonography. Endocrine evaluation may also be valuable in diagnosis and monitoring of disease progression.[3]

Transrectal ultrasonography of the caudal allantochorion in late gestational mares provides an excellent image of the placenta close to the cervical star for diagnosis of potential ascending placentitis by measurement of the combined thickness of the uterus and placenta (CTUP) and evaluation of the echogenicity of the amniotic and allantoic fluids.[2,7] The ultrasound probe should be positioned lateral from the cervix to image a ventral uterine vessel. CTUP measurement should be measured between the vessel and the start of the allantoic fluid.[7] It is important to obtain all CTUP measurements from the ventral aspect of the uterine body in a location that the amnion is not directly adjacent. It is recommended that three measurements should be taken and averaged for monitoring of placentitis progression. Normal values for CTUP have been established and are correlated to stage of gestation (8 mm between days 271 and 300; 10 mm between days 301 and 330; and 12 mm after day 330).[3,8] Increases in CTUP have been associated with placental abnormalities such as placentitis. In some cases, hyperechoic fluid (purulent material) can be noticed separating the uterus and the placenta. In these cases, measurement of either the uterine or placental thickness is meaningless as one is no longer measuring the combined unit.[2]

Transabdominal ultrasonography examinations of the foetus and placenta in mares that are considered at high risk during late gestation should be routinely performed. One method for placental evaluation is systematic examination of the four quadrants - right cranial, right caudal, left cranial, and left caudal.[9] Normal values for the combined thickness of the uterus and the placenta (CTUP) were established (minimal CTUP of 7.1 ± 1.6 mm, and a maximum CTUP of 11.5 ± 2.4 mm). While this measurement is variable and likely dependent on stage of gestation, when placental thickening and partial separation of the allantochorion from the endometrium is observed, placentitis originating from haematogenous infection is likely. Additionally, a pocket of hyperechoic fluid that can be seen at the base of the lowest area of the uterus is highly suggestive of nocardioform placentitis.[6] In addition to diagnosing placental disease, transabdominal ultrasonography is useful for monitoring foetal health. The foetal heart rate can be easily identified, and a foetal heart rate recorded.[3,9] Further evaluations of foetal health, such as vascular resistance measurements, can be utilised but are beyond the scope of this publication.

Endocrine evaluation for foetoplacental well-being in the mare has many potential applications but also presents challenges. Steroids, particularly progestogens and oestradiol appear to be good biomarkers for diseases such as placentitis; however, normal values for particular laboratories need to be established due to differences and cross reactivities of specific immunoassays.[10] Foetal-placental progesterone is rapidly metabolised to 5α-pregnanes. Mares with placental pathology may have increased plasma concentrations of progestagens as a result of stress to the foetal-placental unit.[2,10] Oestrone sulphate in maternal serum has been used to monitor foetal well being.[10] However, this test has not consistently been useful to detect early signs of placentitis. Serial blood samples are required to detect abnormal trends. Decreasing, or consistently low progestin concentration in the last month of pregnancy is associated with abortion, premature rise in progestins may be an indicator of foetal stress, oestrone sulphate concentration demonstrates foetal viability, and a sharp decline in oestrone sulphate may reflect a compromised foetus.[11] Alpha-fetoprotein (AFP), a major protein constituent of equine allantoic and amniotic fluids, may also be a possible biomarker for placentitis in the mare since elevated concentrations of AFP were observed in mares with experimental placentitis.[12] Acute-phase proteins such as serum amyloid A (SAA) demonstrate a rapid and large increase after experimentally induced placentitis, but SAA is not consistently elevated as a diagnostic in clinical cases.[10] Monitoring of changes in SAA concentrations may however be predictive of outcome after initiation of treatment.[13,14]

Treatment of mares with evidence of placentitis is directed at resolving microbial invasion, decreasing inflammation and uterine contractions, and increasing blood flow to the potentially compromised foetus. Systemic treatment can include antibiotics, exogenous progestagens, anti-inflammatories, and tocolytic agents.

If a vaginal discharge is present and the cervix open, speculum examination and culture of the exudate provides identification and sensitivity of the organism; appropriate systemic or local treatment can be initiated. Antimicrobial therapy using penicillin (22,000 IU/kg, q 6 h), gentamicin (6.6 mg/kg, q 24 h) or trimethoprim sulfamethoxazole (30 mg/kg, BID) can be considered as these therapies resulted in minimum inhibitory concentrations (MIC) of these drugs across the placenta and foetal fluids.[15,16]

Anti-inflammatory considerations may include flunixin meglumine (1 mg/kg every 12 hours), pentoxifylline (8.5 mg/kg twice daily), and more recently firocoxib (0.1 mg/kg, PO, daily) administration.[3,17]

Treatment with progestins has long been advocated to promote uterine quiescence in mares with uterine pathology. Altrenogest (0.088 mg/kg SID) was able to prevent prostaglandin-induced abortion.[18]

Tocolytic drugs, such as clenbuterol, enhance utero-placental blood flow in some placental insufficiencies and inhibit labour. In the horse, clenbuterol has been used both intravenously and orally during dystocias to produce uterine relaxation for manipulation. However, there is a transient increase in maternal and foetal heart rate during treatment throughout pregnancy.[19]

Clinical observations suggest that long term therapy with a combination of antibiotics, anti-inflammatories and altrenogest can positively impact pregnancy outcome with delivery of healthy foals in mares with placentitis.[3,20]

Uterine torsion

Uterine torsion is an infrequent complication of pregnancy that usually presents as colic during late gestation. Severity of clinical signs is dependent on degree of rotation, level of vascular compromise, and potential intestinal involvement. The cause is unknown, although it is postulated that uterine torsion may develop secondary to rolling as a result of colic or trauma, or due to vigorous movements of the foetus, during the later stages of gestation.[21] Uterine torsion is not only detrimental to the foetus due to vascular compromise, but a torsion greater than 360º puts the mare at risk for uterine rupture and/or haemorrhage.

Diagnosis is based primarily on rectal palpation of the broad ligaments. Torsion of the uterus can occur clockwise to the right: the left broad ligament is stretched horizontally over the top of the uterus and the right broad ligament courses tightly ventrally or vertically under the uterus toward the opposite side. Transrectal and transabdominal ultrasonography can aid in assessment of uterine compromise by enabling determination of uterine wall thickness, placental integrity, vascular distension, foetal viability, and condition of the foetal fluid.[22] If there is concern about intestinal involvement, abdominocentesis may be performed to aid in determining the appropriate mode of replacement or prognosis.[23]

Treatment is correction of the torsion, and options include rolling the mare, or surgery.[2] Rolling the mare under general anaesthesia may be a less expensive alternative to surgery but can lead to uterine rupture in mares close to term. The technique may predispose to separation of the chorioallantois from the endometrium with subsequent abortion, premature

birth, or death of the foetus.[24,25] The procedure is performed with the mare lying on the side to which the uterus is torsed and then rotating the mare's body around the uterus while the uterus is held in place with a wood plank. Surgical correction may be performed by either standing lank or midline laparotomy. In calm mares with an uncomplicated torsion, six weeks or more from term, a standing lank laparotomy is the treatment of choice.[1] In mares close to term, an incision in both flanks may be necessary or a ventral midline laparotomy performed. A ventral midline approach is preferred in late term mares or those with suspected concurrent gastrointestinal lesions. If a Caesarean section is deemed necessary, it can be performed at the same time.[26-28] Surgical correction is the most effective treatment for uterine torsion and was found to result in the highest survival rate of foals. Survival was significantly better when uterine torsion was corrected via standing flank laparotomy, compared with ventral midline coeliotomy. Furthermore, the prognosis for mares and foals is best when uterine torsion occurs at <320 days of gestation.[25]

Hydrops

Hydrops is the accumulation of fluid withing the allantoic cavity of the placenta (hydroallantois) or the amniotic cavity (hydramnios). These conditions are uncommon in the mare and the prognosis for pregnancy is considered poor. The conditions are associated with a high proportion of congenital abnormalities, especially those of the foetal head, and many affected mares abort spontaneously.[1] Hydroallantois is more common than hydroamnios, with rapid abdominal enlargement occurring over a period of 10 to 14 days. In contrast, hydroamnios develops gradually over several weeks to months during the second half of gestation.[29] Mares present clinically depressed and uncomfortable. In a normal pregnancy, the allantois contains 8 to 15 litres of fluid and the amnion 3 to 5 litres; in a case of hydroallantois, the allantoic volume can range from 120 to 220 litres.[30] Ventral oedema may be present and the mare may show laboured breathing and difficulty walking.

The presenting clinical signs are suggestive of hydrops. Transrectal palpation reveals a grossly enlarged uterus with the inability to palpate the foetus in most cases. Ultrasonography can be used to confirm the diagnosis, rule out twin pregnancy and assess foetal viability.[1] In late gestation, maximal allantoic fluid depth is abnormal at more than 221 mm. Maximal amniotic fluid depth is abnormal at more than 185 mm.[31] Transabdominal ultrasound can be used to confirm the presence of excessive volumes of fluid and differentiate between the two cavities; foetal viability can also be observed.

Hydrops conditions increase the risk of uterine rupture, abdominal hernia, or prepubic tendon rupture. As the mare is highly unlikely to deliver a live, healthy foal at term, treatment is often termination of the pregnancy to ensure the health of the mare and avoid further complications.

The sudden removal of abdominal fluid associated with termination of the pregnancy or foaling may result in hypovolaemic shock and death of the mare. Supportive fluid therapy may be needed at the time of foaling or termination of pregnancy to maintain blood pressure. If possible, the fluid should be drained gradually prior to removing the foetus. Manual dilation of the cervix and puncturing of the chorioallantois offers the best chance of slow fluid drainage. A stomach tube can be used to siphon the fluid slowly, and the foetus manually delivered once palpable.[32] Mares managed with transcervical gradual fluid drainage demonstrated higher survival rate compared to those not managed with transcervical drainage.[33] The mare should be monitored closely during and after the procedure. The mare may also be at increased risk for retained foetal membranes. Future breeding soundness is not adversely affected in mares with uncomplicated resolution of a hydrops condition.[33]

Body wall defects

A degree of ventral oedema is normal in late gestation, especially in mares with reduced exercise. This oedema results from the restriction of lymphatic drainage and venous return by the weight of the pregnant uterus. It is important to differentiate this physiological oedema from inflammatory oedema secondary to tearing of the body wall musculature or prepubic tendon.[11] Conditions causing severe distention of the body wall, such as hydrops, twins, severe ventral oedema, or trauma, may result in rupture of the prepubic tendon, abdominal wall rupture or an abdominal hernia; many cases occur with no apparent predisposition.

Mares usually present with severe ventral oedema and are reluctant to move. Unilateral oedema may suggest partial prepubic tendon rupture or localised damage to the body wall. A mare with prepubic tendon rupture will have an elevated tail head and tuber ischii, due to the lack of ventral support of the pelvis, which results in lordosis and a sawhorse stance. The mammary gland may be shifted cranially from its normal position, and mammary secretions are often contaminated with blood. Although a mare with an abdominal hernia or rupture may have an enlarged abdomen, her tail head and tuber ischii will be in a normal position. Acute progression of either condition can result in severe pain categorised by distress, colic, tachypnoea, tachycardia, and sweating. Internal haemorrhage can occur, leading to shock and even death.[11]

Treatment depends upon the extent of the rupture, the pain experienced by the mare, stage of pregnancy, foetal viability and the value of the mare and foal. Termination of the pregnancy may be best option in cases of acute progression; however, medical support the mare until parturition, or a time when the foal is mature enough for parturition to be induced, are realistic approaches.[34] Mares should be restricted in movement and abdominal support applied to support the abdominal musculature. Analgesics and NSAIDs should be administered to effect and broad spectrum antibiotics are recommended.[11] If the foal has passed 315 days of gestation, then foetal maturation and induction of parturition can be considered. Premature foetal maturation can be achieved by starting the mare on 100mg dexamethasone phosphate daily.[35] Parturition is generally initiated within 5 days. If the mare's condition deteriorates or compromise of the foetus is an important factor, induction of parturition or elective Caesarean should be considered. Assistance with parturition is always necessary due to the inability to properly contract the abdomen.
Mares with a history of abdominal wall rupture should be considered for embryo transfer on future breeding attempts.

POST-PARTUM COMPLICATIONS

A mare presenting for post-partum complications should be fully evaluated even if the cause seems obvious (e.g., retained foetal membranes). A complete history should be taken, including past reproductive history, any known complications of the pregnancy, the time when the foal was delivered, if the foaling was assisted or complicated, when the foetal membranes were passed, and any treatment that was given around the time of foaling. A thorough clinical examination of the mare should be performed. If available, examination of the foetal membranes should also be performed to note any pathology of the chorioallantois, amnion or umbilical cord and to ensure that the chorioallantois is intact. Further examination may include transrectal palpation and ultrasonography, transvaginal examination, transabdominal ultrasonography, abdominocentesis and bloodwork. While a mare may present for multiple reasons in the post-partum period, common complications are further reviewed in this publication.

Haematoma and abscess formation

Perivaginal bleeding can occur following parturition and result in haematoma formation. The mare may present with mild discomfort of the haematoma, or it may be an incidental finding on routine post-foaling examination. Haematoma formation is not difficult to diagnose with ultrasonography.[11] Treatment may not be necessary, but large haematomas can be drained following clot formation to avoid abscess formation.

Haemorrhage

Haemorrhage in the mare typically occurs during parturition, with problems noted immediately post-partum and is a common cause of mare fatality. Rarely, haemorrhage can occur pre-partum either spontaneously or following trauma associated with exercise or transport, or uterine torsion or rupture. Rupture of the middle uterine artery is the most frequent cause of haemorrhage in the mare, but rupture of the external iliac, utero-ovarian and vaginal arteries may also occur. Haemorrhage is a significant cause of peri-partum complications in the mare. Usually older, multiparous mares are of greater risk as rupture is associated with atrophy and fibrosis of the arterial wall but can occur at any age or parity.[36,37]

Most uterine artery bleeds are contained within the broad ligament, resulting in haematoma formation; however, the bleed can occur within the uterine wall, enter the uterine lumen or directly into the abdominal cavity. Haemorrhage initially contained within the broad ligament can subsequently rupture and leak into the abdomen.

The clinical signs associated with haemorrhage depend on the location and severity of the bleed. Mares with broad ligament haematomas will show variable signs of discomfort resulting from tension on the ligament and serosal surface of the uterus in proximity. Signs of colic, sweating, tachycardia, tachypnoea, ataxia, and pale mucous membranes are apparent as a bleeding event progresses. Continual blood loss leads to rapid onset of haemorrhagic shock and is often fatal.

Following the initial examination, if a ruptured artery is suspected, the mare should not be disturbed any more than is necessary.[38] Careful rectal palpation and ultrasonography can reveal bleeding in the broad ligament(s) or uterus but is often very painful for the mare.[38,39] Haematology maybe performed but has limitations in the acute phase of haemorrhage.[40] If the mare survives the acute phase, a drop in PCV is usually observed. Transabdominal ultrasound is useful to detect the presence of free fluid in the abdomen along monitoring of progression of the bleed (active swirling, clot formation, resorption). Abdominocentesis can confirm haemoabdomen and in cases of broad ligament haematoma, show evidence of increased protein concentrations.[41]

In all cases of suspected haemorrhage, keeping the mare quiet and contained until examined is vital. With active, ongoing internal haemorrhage, the treatment approach will be further dictated by the facilities available for monitoring and treatment.[38] Mares with subtle signs of haemorrhage based on physical examination may only require conservative therapy. Box rest, a calm environment, and pain relief are recommended; some mares may need light sedation. Broad-spectrum antimicrobials are administered to reduce the incidence of infection and abscess formation. Mares with more significant blood loss and signs of hypovolaemic shock necessitate aggressive treatment. IV fluids, hypertonic saline, and blood transfusions can be balanced to maintain a stable blood pressure to avoid clot disruption. Aminocaproic or tranexamic acid that are reported anti-fibrinolytic agents can administered to aid in clot stabilisation.[42,43] Yunnan Baiyao is a Chinese herbal preparation that is used as an adjunctive treatment of bleeding.[44] In one study, administration of Yunnan Baiyao at a dosage typically

used in clinical practice had no effect on in vitro measures of platelet or von Willebrand Factor (vWF) function and no enhancement of fibrin-clot formation or stability.[45]

Mares that survive acute haemorrhagic shock have a good prognosis for survival and can produce foals following recovery.[37]

Retained foetal membranes

Retained foetal membranes (RFM) represent one of the more common postpartum problems in mares. Foetal membranes are considered retained in mares if they are not passed within 3 hours post-partum.[46] Although the overall incidence is low (2%– 10.6% of mares), the consequences can be severe.[46,47] Retained foetal membranes can lead to life-threatening sequelae such as metritis, sepsis, and laminitis.[46-48] Several factors are associated with a higher occurrence of RFM, including mare age, previous history of RFM, breed, and peripartum complications (abortion, dystocia, placentitis, prolonged gestation, and hydropic conditions).[49] However, the root cause for foetal membrane retention in mares is not clear. Whatever the cause of retained membranes, the consequences can rapidly progress from mild (endometritis) to severe (metritis, laminitis, endotoxemia, death). Therefore, prompt expulsion of foetal membranes is a priority in the postpartum mare. In some situations, there are clear advantages to actively removing membranes from mares at risk for retention or metritis (placentitis, dystocia, abortion). However, proactive membrane removal in the normal post-foaling period is a controversial topic in equine veterinary medicine.[50]

A variety of methods to aid in removing retained membranes in the mare have been described. The most common method to aid in foetal membrane removal is administration of oxytocin in the early postpartum period.[48] Oxytocin can be administered in bolus injections (5–20 IU, intravenously [IV] or intramuscularly [IM]) and repeated every 30 minutes to every 2 hours for the first 6 hours after foaling or until complete expulsion of the fetal membranes is achieved.[48,51] Alternatively, 60 to 100 IU oxytocin can be mixed in 1L of lactated Ringer's solution or 0.9% saline solution and administered slowly (30–60 min) IV. Membranes that are hanging at the hocks or below should be tied in a knot to avoid the mare standing on them while providing a weight to support expulsion. When the membranes are intact, the chorioallantois can be distended with dilute betadine solution or 0.9% saline (Burns technique). Fluid is infused into the chorioallantoic cavity, retained for a period of 15 to 30 minutes to facilitate membrane release. The fluid is then siphoned out or expelled along with the intact membranes. Large volume uterine lavage is commonly used in both normal post foaling mares and mares with retained membranes. Other facilitated techniques utilised for removal of RFMs include umbilical vessel infusion causing vascular distension of the membranes and controlled manual removal techniques.[52,50] Umbilical infusion is performed by catheterisation of an exposed umbilical vessel to allow distension of membrane vasculature and detachment of the chorioallantois from the endometrium.[52]

While controversial, controlled manual removal of foetal membranes is another method of membrane removal that has been used for many years. Techniques that have been described for manual foetal membrane removal include grasping the externalized free portion of the membranes and applying controlled traction, placing a hand between the endometrium and chorion to separate the attached membranes, twisting of the allantochorionic membrane into a tight cord, and placing a wooden ring between the chorion and endometrium and advancing the ring to separate the membranes from the endometrium. [53,54] A simple and safe method was described, in detail, with data supporting the use of the procedure.[55]

Metritis

The overall incidence of metritis is low but increases substantially with foaling trauma and/or retained foetal membranes. Inflammation of the uterine wall allows bacteria and toxins to enter systemic circulation causing bacteraemia and septicaemia. Clinical signs are generally evident within 48 hours post-partum. The mare may be depressed, anorexic, febrile, have vulvar discharge or show signs of laminitis indicating the need for examination. On bloodwork, metritis is often association with pronounced neutropenia.[56]

Treatment is aimed at reducing intrauterine contamination along with systemic broad-spectrum antimicrobial and anti-inflammatory therapy. Intravenous fluids may be warranted in advanced cases. Large-volume uterine lavage should be repeated until the recovered fluid is free of contamination. A homemade saline solution can be made for lavage by combining 90g of table salt with 10 litres of water. Lavage procedures are often performed daily or twice daily until clinical signs resolve. Along with large uterine lavage, oxytocin therapy is useful for uterine clearance. Supportive care for laminitis is recommended.

Uterine rupture

Uterine rupture in the mare is a rare but severe condition in the mare that most often occurs in association with dystocia, foetotomy or uterine torsion. Complications associated with uterine rupture include visceral herniation, peritonitis, haemorrhage, shock, and death. In rare cases, the uterus may rupture before term - the mare may not show much pain and if the foetus escapes into the abdomen and haemorrhage is not severe, uterine involution may occur without external signs. The more common place for rupture is the dorsal surface of the uterine body but the tears can occur anywhere and are not uncommon at the tip of the horn.

Immediate diagnosis is difficult unless a tear can be felt during a transrectal internal examination. In the early stages, before significant abdominal contamination has occurred, there may be no obvious outward clinical signs. If peritonitis develops, the mare will become increasingly ill over 24 to 72 hours with fever, inappetence, reduced gut motility and abdominal pain. Diagnosis is usually suspected if the mare is depressed and inappetant a day or more from foaling. Occasionally mares may show colic. Commonly a complete blood examination will reveal a leukopenia.[57] Ultrasonography and abdominocentesis are two extremely useful diagnostic tests. Abdominocentesis will reveal signs consistent with septic peritonitis.[41]

Medical management is possible if the tear is identified early, and minimal contamination of the abdominal cavity has occurred. Broad-spectrum antimicrobials (penicillin and gentamicin), flunixin meglumine, oxytocin and IV fluid therapy is reported with success.[58] Uterine lavage is contraindicated as lavage fluids may enter the abdomen leading to further contamination. Large uterine tears will require surgical repair, and immediate surgical correction is recommended along with aggressive medical management for peritonitis. Dorsal tears may occasionally be sutured by hand through the vagina and uterus. Other modalities such as laparoscopy are also occasionally beneficial depending on location of the tear. As peritonitis can also be caused by traumatised and devitalised bowel, if a uterine laceration is suspected but cannot be detected, then laparoscopy or laparotomy may be necessary to differentiate the cause.[59] In general, it is best to do an exploratory laparotomy and identify the tear(s) and suture them. This technique also allows concurrent flushing of the abdomen.

The prognosis for mares with uterine ruptures is related to the size and location of the tear and to speed of recognition and appropriate treatment. Unfortunately, many mares present several days postpartum with significantly advanced peritonitis.

Uterine inversion

Inversion or intussusception of the tip of the uterine horn may result in colic symptoms in the post-partum mare. While the condition can occur spontaneously, discomfort following foetal membrane removal may suggest horn inversion.[50,55,56] Transrectal palpation may identify a shortened uterine horn with a tense mesovarium ligament.

Intrauterine manual reduction can be performed. In the author's experience a smooth muscle relaxant such as propantheline bromide is useful to replace the inverted horn. Uterine fluid infusion can aid re-positioning of the horn. Small doses of oxytocin are then utilised to promote tone and uterine involution.

Uterine prolapse

Uterine prolapse is an uncommon but serious condition in the mare. Although it can occur spontaneously, prolapse is more common following dystocia or removal of retained foetal membranes.[50] Uterine prolapse often occurs immediately after foaling but can occur hours to days postpartum. Involvement of the bladder, gastrointestinal tract and concurrent haemorrhage should be evaluated upon initial examination.

Management should involve timely cleaning and replacement of the prolapsed tissue. If down, the mare should be left in the recumbent position. If standing, the mare may require heavy sedation. If the mare is fractious or straining heavily, an epidural, or even general anaesthesia may be considered. For epidurals, we prefer the following mixture: xylazine (0.17mg/kg), lidocaine (0.15 mg/kg; 3mL of 2% lidocaine solution) and ~6 mL of Lactated Ringer's solution to a volume of 10 mL.[11] In our clinic, a large clean surface such as bed sheet or extra strength garbage bag are used to lift the uterus. This is a two-person task with one person supporting the weight while the clinician replaces the uterus. Caution is used with manipulating the uterine surface to avoid perforation of the tissue. If the foetal membranes are retained, removal at the time of replacement will decrease straining after replacement and improve cleanliness. A Caslick stitch is generally performed following replacement. After replacement, the mare should be closely monitored for signs of recurrence and internal haemorrhage. The mare should be restricted, and metritis managed with systemic broad-spectrum antimicrobials and anti-inflammatory drugs.

Prognosis following uterine prolapse depends on concurrent haemorrhage and damage to the uterus during replacement. One study concluded mares that died had severe haemorrhage. In the same study, 74% of mares survived to hospital discharge.[60] Future fertility is associated with degree of uterine damage.

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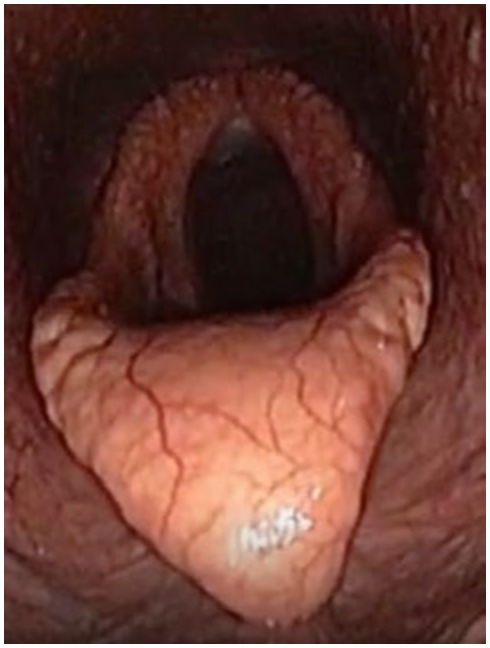
Notes

Conditions of the epiglottis

Dr Brian Anderson and Dr Cyrus Ho
ba@bvp.com.au
Ballarat Veterinary Practice
Ballarat, Victoria
Australia

The epiglottis is the most rostral cartilaginous component of the larynx.[1] It is a triangular structure with a broad base directing rostrally to its tip and has serrated edges on either side. It sits above the soft palate with the caudal free margin of the palate closely opposed to the epiglottic base. Its dorsal convex surface is covered by a tightly adherent mucous membrane caudally contiguous with the aryepiglottic folds and ventrally with the subepiglottic tissue (see below). A prominent blood vessel is observed on either side with small perpendicular branches that course to the left and right lateral margins which are serrated. The ventral surface is covered by a loose mucosa, the subepiglottic tissue which facilitates smooth dorsal and caudal movements of the cartilage to occlude the trachea while swallowing.[1,2] Disorders of the epiglottis frequently result in abnormal respiratory noise, often secondary to dorsal displacement of the soft palate, exercise intolerance and poor performance.[1,3] Other symptoms can include coughing, nasal discharge and dysphagia.[1,3]



Endoscopic image of a normal epiglottis.
Note the dorsal vasculature, leaf-like appearance and serrated margins.

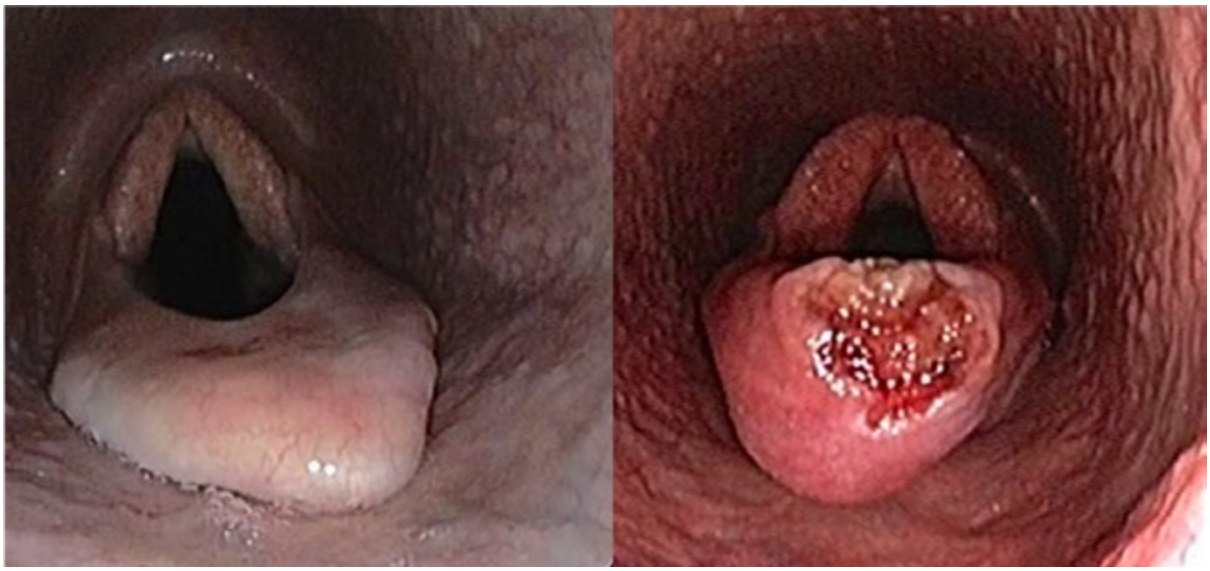
Table 1 Conditions of the epiglottis in horses.[4,5]

Athletes	Non-athletes	Neonates/ foals
Epiglottic entrapment Epiglottitis Subepiglottic cysts Dorsal epiglottic abscess Epiglottic hypoplasia, flaccidity, or other deformities Epiglottic retroversion Axial deviation of the aryepiglottic fold	Epiglottic entrapment Dorsal epiglottic abscess Subepiglottic granuloma Epiglottic hypoplasia, flaccidity, or other deformities Epiglottic retroversion Axial deviation of the aryepiglottic fold	Subepiglottic cysts

Epiglottic entrapment

Epiglottic entrapment (EE) occurs when the loose subepiglottic tissue ventral to the epiglottis becomes positioned above the dorsal epiglottic surface, entrapping the epiglottis and appears continuous with the aryepiglottic folds to which it is contiguous.[1,2] It is commonly observed in the Thoroughbred and Standardbred racehorse.[4] The predominant clinical signs include upper respiratory tract noise and exercise intolerance (although not always), and it can be found as an incidental finding during routine endoscopy. Coughing and nasal discharge are reported less frequently. Diagnosis is generally by resting endoscopic examination, but intermittent EE may require dynamic URT videoendoscopy. Typically, the outline of the epiglottis is visible above the soft palate, but the serrated edges and dorsal vasculature of the epiglottis are no longer visible. The entrapment can be described according to severity and chronicity as follows:

1. Intermittent or persistent
2. Thin or thick
3. Narrow or wide, using the length of the epiglottis as a reference where less than half of the length of the epiglottis is regarded as narrow
4. Non-ulcerated or ulcerated, where the severity of ulceration and the amount of accompanying granulation and fibrous tissues varies

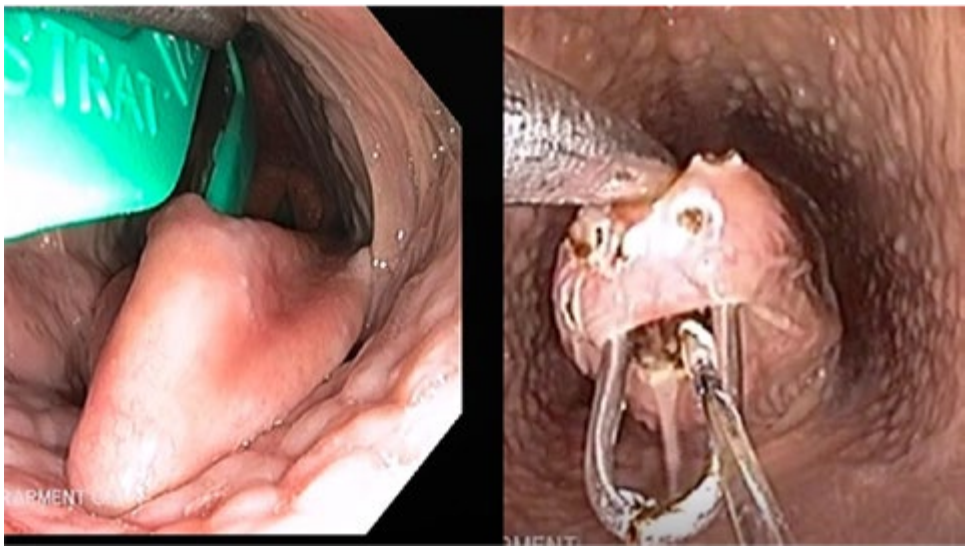


Endoscopic images of a simple epiglottic entrapment on the left, and an ulcerated and thickened epiglottic entrapment on the right.

The aetiology of EE has yet to be elucidated, but it has been associated with dorsal displacement of the soft palate (DDSP) and epiglottic hypoplasia (see later).[1,3] Frequently after correction of EE, the epiglottis is found to be deformed or foreshortened. Whether this a reason the EE occurred, or a consequence of chronic entrapment is not known. However, in these cases, the risk for recurrence of the entrapment or IDDSP is increased. In addition to endoscopic examination, radiography may be used to evaluate the thyroepiglottic length, epiglottic thickness and contour.

While EE can be found incidentally in well performing horses, surgical treatment is routinely recommended as soon as a diagnosis is made because it is more difficult to treat chronically inflamed EE's. Current surgical recommendations are to use "tissue sparing techniques" where possible because excessive resection of subepiglottic tissue can result in granuloma formation, ulceration and fibrosis which can alter the function of the epiglottis or soft palate.[1,3]

Several techniques for axial division have been described in the literature. They are trans nasal laser axial division (with or without resection of redundant subepiglottic tissue using scissors), trans nasal/ transoral endoscope-guided axial division with a curved hook bistoury, trans nasal endoscope-guided axial division with a shielded hook bistoury, transoral hand-assisted axial division with a hook bistoury, and trans nasal electrosurgical axial division.[1-3] The decision of which technique to be adopted depends predominantly on surgeon's preference and experience, equipment, the characteristics of the entrapping membrane and potential post-operative complications.[1-3] Simple, uncomplicated entrapments respond favourably to axial division techniques with healing times generally about 3 weeks. Pre- and post-operative broad-spectrum antibiotics and anti-inflammatories are recommended, as are oral and/or nasal throat washes for 7-10 days. If possible, a grass only or wet hay diet for the first 3 weeks gives the best results. A repeat endoscopic examination is recommended 3 weeks after surgery. Recurrence of the entrapment in routine cases is approximately 5-15%, and for IDDSP similarly. The prognosis for athletic activity is usually excellent. Complicated entrapments (thick, ulcerated, fibrotic, epiglottic hypoplasia or deformity) are more difficult. In these cases, treatment with systemic corticosteroids, broad spectrum antibiotics, NSAIDs and throat wash prior to surgery to reduce as much as possible the inflammation that is present can be useful. There is frequently a higher rate of recurrence of the entrapment and development of IDDSP or persistent DDSP. The prognosis in these cases is often guarded for future athletic activity, and healing times can be 6-8 weeks or longer.[6-9] Horses requiring subepiglottic tissue resection to resolve the entrapment are reported to have a shortened career length and less earnings compared to those that did not.[8]

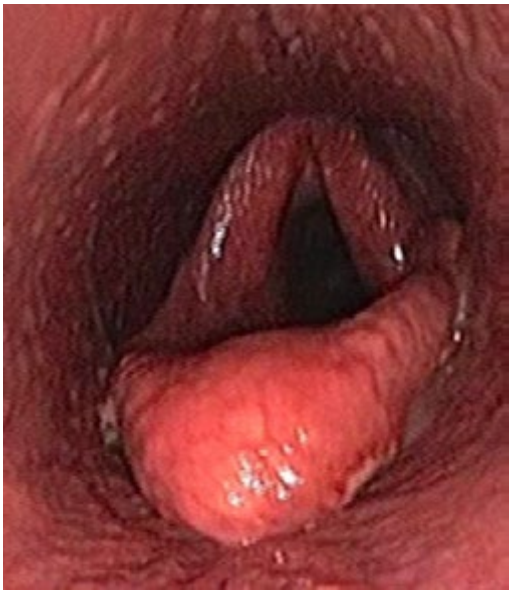


Surgical treatment can involve per os axial division with a hook (left image) or laser division with the horse standing (right image).

Epiglottitis

Epiglottitis is the inflammation of the epiglottis characterised by mucosal oedema, hyperaemia as well as thickening of the epiglottis. The aetiology of epiglottitis is not well understood. Primary epiglottitis is rare. It is often secondary to other pharyngeal and/or laryngeal disorders such as epiglottic entrapment and IDDSP, with similar clinical manifestations, e.g., respiratory noise, exercise intolerance and coughing. Epiglottitis is not uncommon following surgery to treat epiglottic entrapment, but it is usually temporary. It is more commonly observed in adult racehorses, although primary epiglottitis has been reported in a foal. Diagnosis is via endoscopy. Dorsal elevation of the epiglottis is often appreciated because of the mucosal oedema of the lingual surface of the epiglottis.[1,3] Severe inflammation could lead to marked elevation of the epiglottis and thus obstruction of the upper airway necessitating a temporary tracheotomy.[10] Chronic inflammation, especially if the mucosa of the epiglottic tip is eroded, may lead to epiglottic deformity.[1,3,11]

Treatment includes strict rest, systemic non-steroidal anti-inflammatories, and topical anti-inflammatories and antimicrobials in the form of pharyngeal throat spray, with or without corticosteroids, delivered using a nasal catheter for 7-14 days. Endoscopic reassessment of the epiglottis is recommended after the course of treatment prior to returning to work. Horses generally respond with medical treatment, but long-term complications can occur in up to 50% of affected horses and include epiglottic deformity, epiglottic entrapment, IDDSP or persistent DDSP.



Mild epiglottitis following surgery for epiglottic entrapment. Note the rounded lateral margins and more rounded and swollen tip.

Subepiglottic cysts

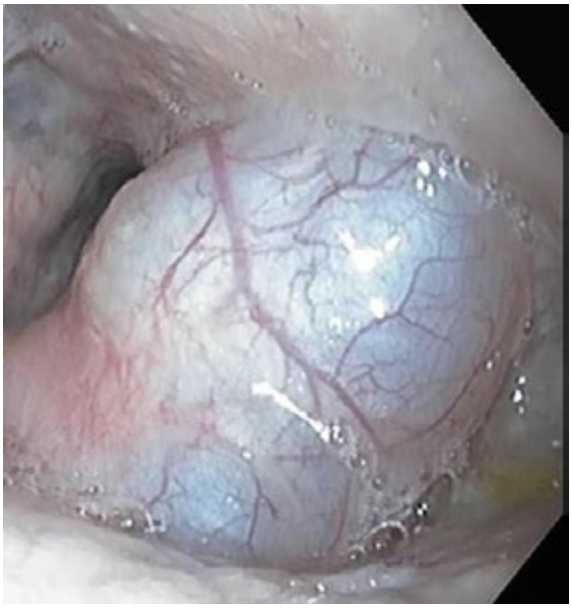
Subepiglottic cysts (SEC) are uncommon and can occur in both adults and foals, causing upper airway obstruction.[1,3] SECs can be congenital or acquired. Congenital cysts are thought to develop from a remnant of the thyroglossal duct and as such are found in neonates, foals, and young Thoroughbreds and Standardbreds identified during presales endoscopy. Acquired cysts are thought to occur secondary to inflammation and/or trauma and this might explain their presence in adult horses without a history of previous respiratory tract problems.[1,12] A common clinical sign is increased respiratory noise during fast exercise during inspiration and expiration. However, in one report only 27% of the horses diagnosed with a SEC had a history of respiratory noise. This is likely related to age as horses over 1 year of age were more likely to have a respiratory noise. Other clinical signs include dysphagia, nasal discharge, coughing, and dyspnoea.[3,13] The size of the SEC and the age of the horse could influence the presentation of the clinical signs. Horses with large cysts are more likely to develop dysphagia compared to those with smaller cysts.[3,13] Young foals are prone to aspiration pneumonia from untreated SEC.[3,13]

Diagnosis is by resting URT endoscopy. SECs appear round to oval in shape, smooth, pink, fluctuant and are covered with mucosa, usually located ventral to one side of the epiglottis.[1,3] As they loosely attach to the ventral epiglottis and sit dorsal to the soft palate, the epiglottis is often elevated asymmetrically.[1,3] SECs can be found together with an EE in 20-36% of horses, or in association with IDDSP or persistent DDSP. Making the horse swallow or the use of an epiglottic elevator may be needed to facilitate the diagnosis because it is not uncommon that the cysts can intermittently slide under the soft palate.[1,3] Radiographic examination of the larynx can be performed to localise and characterise the cysts in terms of size. Histopathology is required to ascertain the cause, congenital or acquired.[1,3,13]

Complete surgical excision or destruction of the secretory cystic lining is key to complete resolution of a SEC.[1,3] The cysts often recur within days if drainage is the only procedure. The cystic mucosa quickly reforms, and the cyst is refilled by the secretory cells. The following approaches can be used:

- Oral approach using a wire snare (obstetrical wire) or transendoscopic electrocautical wire snare. Laser ablation of the cyst lining or laser resection en bloc can also be performed.
- Trans nasal endoscopic guided laser resection
- Formalin injection per os or via an endoscope passed up the nose.
- Submucosal resection via a laryngotomy

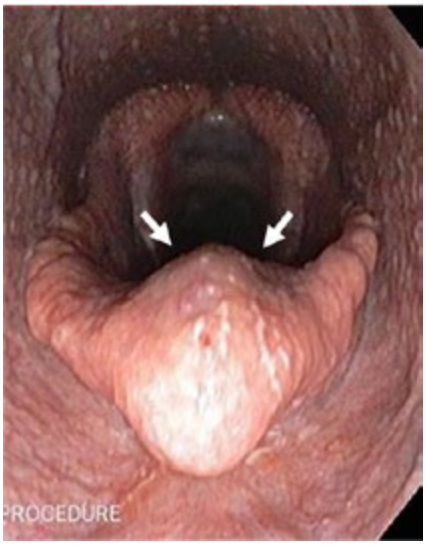
Oral approaches are preferred over a laryngotomy because of less post-operative wound care and reduced convalescent time.[15] Common complications reported include excessive subepiglottic swelling and cicatrisation.[15] Post-operative care involves systemic antimicrobials for 3 days, systemic and topical anti-inflammatories for 2 weeks, strict stall rest for 2-3 weeks. Follow-up endoscopy at 3 days and 2-3 weeks post-operatively should be performed to evaluate the healing process at the surgery site.



Intra-oral endoscopic image of a subepiglottic cyst in a foal obscuring the epiglottis

Epiglottic abscess

Dorsal epiglottic abscess is a rare condition that causes coughing, respiratory noise, and exercise intolerance. On URT endoscopy a round to oval, smooth, well-defined, elevated mass is often observed on the dorsal surface of the epiglottis.[3,16] Aspiration of the mass can confirm an infection and the fluid retrieved sent for culture and sensitivity. Treatment is achieved by transendoscopic drainage of the abscess using a contact Nd:YAG or diode laser, followed by endoscopy-guided debridement using bronchoesophageal grasping forceps. The abscess cavity should be lavaged with dilute disinfectants (chlorhexidine or iodine) in saline. Aftercare includes stall confinement, broad spectrum antibiotics, NSAIDS and throat wash. Uncomplicated healing occurs in 10-14 d and prognosis for return to athletic activity is usually good. Septic chondritis is a possible sequela as is epiglottic deformity.



Epiglottic abscess (white arrows)

Epiglottic chondritis

Septic chondritis of the epiglottic cartilage is a rare condition with affected horses showing clinical signs of respiratory noise, coughing and dysphagia.[16] The aetiology is unknown but bacterial infection of the epiglottic cartilage is likely secondary to epiglottitis, ulceration or dorsal epiglottic abscessation.[16] Diagnosis is made with endoscopy, histopathology and/or microbiological culture and sensitivity. Necrotic cartilage involving the rostral third of the epiglottis is evident and frequently there is purulent exudate discharging from an area of epiglottic mucosal ulceration.[16] Treatments involve mechanical debridement with a gauze swab using brochoesophageal forceps or debridement with scissors. Systemic empirical antimicrobials (penicillin and gentamicin), systemic and topical anti-inflammatories (phenylbutazone and throat spray) are warranted after surgical debridement, in addition to 4-8 weeks of box confinement and light hand-walking. Follow-up endoscopy is recommended 6-8 weeks after initial debridement. It is not uncommon that cartilage shortening and deformity result as complications, therefore the prognosis for athletic performance is considered poor.

Subepiglottic granuloma

Subepiglottic granuloma often occurs after acute trauma or ulceration of the subepiglottic tissue on the ventral aspect of the epiglottis. Excessive granulation tissue forms in response to infection and/or chronic irritation.[1,5] A common presenting clinical sign is coughing, as well as exercise intolerance and possibly dysphagia depending on the size of the granuloma.[1] The diagnosis is made endoscopically but may require epiglottic elevation or even oral endoscopy under general anaesthesia. Dynamic URT videoendoscopy is recommended to rule out IDDSP or intermittent EE in horses diagnosed with subepiglottic ulceration, especially if there is a history of abnormal respiratory noise.

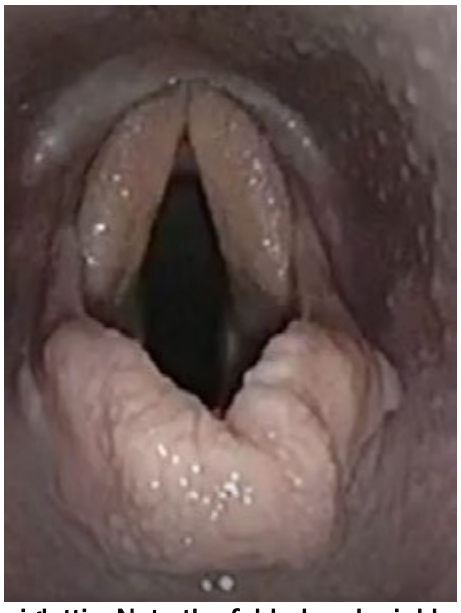
Treatment is either medical or surgical. Topical and systemic antimicrobial and anti-inflammatories are recommended, and strict rest with no work for four to six weeks is required. Working in the presence of an ulcer may prolong the healing time and encourage excessive granulation tissue formation, promoting the size of the granuloma. If medical therapy fails, then excision through the nasopharynx, oropharynx or via a ventral laryngotomy is indicated. Transoral transendoscopic laser resection or trans nasal transendoscopic laser resection are alternative approaches. The use of a transendoscopic electrocautical snare is also possible. Post-operative care includes topical and systemic antimicrobial and anti-inflammatories with strict stall rest of 6-8 weeks. Prognosis for returning to athletic function has been reported as good if there is no recurrence of concomitant IDDSP, however, it is not uncommon to see non-healed subepiglottic ulcers for many months later.[1,5]

Epiglottic hypoplasia, flaccidity, or deformity

Hypoplasia is the underdevelopment or incomplete development of a tissue or organ. Epiglottic hypoplasia refers to an epiglottis which appears to be short and narrow and sometimes thin. It is more pronounced from the midbody to the tip. It can also refer to an epiglottis with a normal or shortened length but flaccid in nature that matches the contour of the soft palate. On endoscopic examination, frequent DDSP or persistent DDSP is observed or can be induced with nasal occlusion, swallowing or changes in head position. Oral endoscopy and digital palpation during general anaesthesia can be useful to further define the problem. Epiglottic hypoplasia has also been associated with epiglottic entrapment.[3,17] Epiglottic deformity is diagnosed when the epiglottis is shrunken, wrinkled and/or folded dorsally and axially uni-/bi-laterally, and this could be primary or secondary to other underlying abnormalities, such as trauma, chronic epiglottic entrapment and chronic epiglottitis.[1,18]

Lateral radiographs of the larynx can be used to measure the thyroepiglottic length, and contrast pharyngography may be applied to assess the thickness and contour of the epiglottis as well as the relationship between the epiglottis and neighbouring anatomical structures. The length of epiglottis in apparently healthy Thoroughbred and Standardbred horses is found to be 8.56 ± 0.29 cm and 8.74 ± 0.38 cm, respectively.[17] The length of a hypoplastic epiglottis could be as short as 6.59 ± 0.33 cm in Thoroughbred horses as reported previously.[19] Endoscopy is used to assess the epiglottic margin as well as flaccidity. While epiglottic hypoplasia and flaccidity appear to predispose some horses to IDDSP, the precise mechanism is not clear. The apparent flaccidity of the epiglottis in mature horses can change depending on larynx position and contraction of the hyoepiglotticus muscle. In some horses, the epiglottis may appear to be flaccid on resting endoscopy but become rigid at exercise during dynamic URT videoendoscopy. When epiglottic retroversion is experimentally induced, DDSP does not occur despite the fact the epiglottis does not "hold down the palate". For this reason, epiglottic augmentation (stiffening of the epiglottis with polytetrafluoroethylene) has fallen out of favour. [20-22] Both resting and dynamic URT videoendoscopy to assess a hypoplastic/flaccid or deformed epiglottis is recommended.

The appearance of the epiglottis in young thoroughbreds observed during presale URT endoscopy can vary form apparently normal to hypoplastic. While severe forms of hypoplasia are a risk for future athletic performance, it appears that an increase in "maturity" of the epiglottis in terms of length and rigidity occurs as the horse gets older.



A deformed epiglottis. Note the folded and crinkled appearance

Epiglottic retroversion

Epiglottic retroversion is a rare epiglottic disorder responsible for respiratory noise during inspiration. The epiglottis retroverts into the opening of the glottis at inspiration and returns to its normal position over the soft palate at expiration, resulting in an inspiratory gurgling noise. It has been reported in both Thoroughbred and Standardbred racehorses.[23-25] The aetiology of this disorder is not well understood. Retroversion has been induced by local anaesthesia of the hypoglossal nerve, indicating that a loss of neuromuscular function of the hyoepiglotticus or other muscles controlling the position of the basihyoid are involved.[26] Dynamic URT videoendoscopy is required for the reliable diagnosis of epiglottic retroversion.[23,24] Epiglottic augmentation was attempted in two horses with the use of polytetrafluoroethylene paste, and one Standardbred gelding was able to return to racing with several wins at a lower level of competition and the other Thoroughbred filly was retired because of unresolved respiratory noise after surgery.[25] Glosso-epiglottic mucosal resection is another technique that attempts to tighten the subepiglottic tissues to reduce the chance of collapse but did not result in successful athletic function after surgery.[27] Epiglottopexy is a surgical technique to stabilize the epiglottis by using a suture to mimic the function of the hyoepiglotticus muscles where the suture passes between the subepiglottic fascia and rostral thyroid cartilage. This approach appears to be the only surgical technique for which horses were able to return to compete at previous competition levels.[28]

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Notes

Caudal reproductive tract surgery

AO McKinnon

Goulburn Valley Equine Hospital

Introduction

The procedures below represent techniques used at the Goulburn Valley Equine Hospital. This chapter is not intended as an exhaustive, heavily referenced treatise of all available techniques but is designed to provide practical techniques that work well for us. Readers should be aware that different methods are employed successfully by others. Many of the techniques presented are modifications of existing techniques, newer techniques and/or areas we feel warrant special attention.

The conditions described will be commonly seen by a busy equine practitioner during the course of a breeding season. Most of the techniques are directed at restoring or improving fertility. Most are elective procedures but require experience in order to make the correct diagnosis and institute the best therapeutic approach. Our experiences have led us to prefer the techniques discussed here to other published alternatives.

Patient Selection

Care should be exercised to select only those candidates that are likely to respond favourably to surgery. The probability of a successful outcome and subsequent production of a live foal must be evaluated with regard to:

a. Severity and nature of the problem
b. Breeding history of the mare
c. Value of the mare and/or offspring (commercial or sentimental)
d. Cost of the procedure
e. Mare age
f. Fertility of the stallion
g. Availability and suitability of assisted breeding techniques such as artificial insemination (AI) or embryo transfer (ET) etc.
h. Long-term predictive value of surgical intervention, i.e., temporary or permanent improvement
i. General health of the candidate
j. Perpetuation of heritable conditions
k. Insurance status and informed client consent
l. Ethical considerations
m. Experience of the veterinarian
n. Quality of on-farm management
o. Previous attempts at repair of the problem

It is futile to perform a sophisticated, expensive surgery, e.g., for vesico-vaginal reflux, if the mare has sustained chronic uterine damage and fibrosis that will render her highly sub-fertile or infertile despite an excellent surgical outcome. Full reproductive evaluation is necessary prior to surgery in any candidate subjected to repeated uterine bacterial insults, e.g., 3rd degree recto-vaginal lacerations that have been present for longer than one year.

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McKinnon, AO –Caudal reproductive tract surgery

Sedation and Local Anaesthesia

Sedation:

All the procedures discussed are performed with the mare standing and utilizing local infiltration and/or epidural anaesthesia. Apart from minor procedures (Caslick's operation - vulvoplasty) we prefer to have mares sedated and restrained in appropriate stocks, with facilities for cross tying and/or tail elevation. Sedation and analgesia are provided most commonly with a combination of detomidine (0.02mg/kg bwt IV) and butorphanol (0.005-0.01 mg/kg bwt IV). Longer surgeries may require the repeated IV use of low doses of xylazine (100 mg). Cost and length of sedation/analgesia are important considerations. In addition, some have other physiological side effects, e.g., increased urinary output with xylazine. Detomidine provides excellent sedation and analgesia (McKinnon et al., 1988); however, sedation may persist for hours after administration.

Analgesia and Anaesthesia:

Epidural anaesthesia can be extremely effective, however variation in response, individual susceptibility and time before appropriate anaesthesia is obtained, occasionally make it less rewarding than tranquilization and local infiltration for some of the procedures discussed here. Our technique for epidural anaesthesia is similar to that described by Turner and McIlwraith (Turner and McIlwraith, 1989). Caudal epidural injection of local anaesthetic to produce anaesthesia of the tail and perineal structures in the conscious standing horse has been further developed by the use of a catheter placed into the epidural space in order to provide long-duration analgesia and anaesthesia. More recently, opioid, alpha-2 adrenergic agonists, ketamine and other analgesic agents have been administered by caudal epidural injection, providing pain relief in both conscious, standing and anesthetized, recumbent horses. (Robinson and Natalini, 2002)

Epidural administration more than 6-9 ml of 2% local anaesthetic to a 500 kg mare can be associated with loss of motor control to the hind limbs and subsequent unexpected recumbency. These mares are difficult to manage and may require support for 2 to 4 hours.
Epidural injection of xylazine for perineal analgesia has been reported to be very efficacious (Fikes et al., 1989; Grubb et al., 1992; LeBlanc et al., 1988; LeBlanc and Caron, 1990). A dose of 0.17 mg/kg (approximately 75-100 mg in an average-sized horse) was as effective as a local anaesthetic agent for anaesthesia and had no or minimal depressive effect on motor nerves in the lumbosacral intumescence. Another advantage was the demarcation of the area of anaesthesia by an area of sweating (dermatome) that was correlated both temporally and topographically with region(s) of anaesthesia (LeBlanc et al 1988). In our experience, the perineal dermatome is not always obvious. In addition, it takes approximately 30-40 minutes for good analgesia to be recognized when performing epidural anaesthesia with xylazine. We prefer the following mixture: xylazine (~0.17mg/kg bwt;), lidocaine (~0.15 mg/kg bwt; ~3mL of 2% lidocaine solution) and ~6 mL of Lactated Ringer's solution to a volume of 10 mL. The volume administered as an epidural in the first intercoccygeal space is titrated based on the size of the mare. Large mares (>600 kg) may receive the full 10-mL dose and smaller mares (450-500 kg) receive between 7-8 mL. With this regime, loss of tail tone has occurred in association with a successful 'block' within 5-10 minutes, and surgery generally has begun by ~20-30 min post administration. Our most used method of epidural anaesthesia is to begin with the infusion 1 mL of local anaesthetic through a 25-gauge needle subcutaneously above the first moveable coccygeal/sacral joint (usually C1 and C2). Next a 38-mm, 18-gauge needle (bevel forward or up and introduced at 45 degrees to the skin) that has a small amount of anaesthetic solution to create a meniscus on the needle hub, is introduced through the local bleb. Recognition of the fluid being aspirated into the

needle infers correct placement and the appropriate amount of solution is injected slowly into the epidural space whilst continually evaluating the ease of administration to ensure the needle tip is still in the appropriate place.

Occasionally epidural anaesthesia is difficult to achieve (e.g., infusion outside the epidural space before proper epidural placement, haemorrhage through the needle and tissues, anatomic abnormalities and fat horses).

Provided local anaesthesia can be obtained by infiltration without adverse effects on wound healing, we prefer to perform surgery without the epidural and provide incremental IV administration of xylazine i.e., 50-100 mg dose to already sedated patients when or if minor surgical discomfort is recognised.

In some instances, we have been able to identify correct epidural placement by injecting the anaesthetic slowly while moving the needle until there is an obvious change in the amount of resistance needed to advance the solution. This situation is usually associated with a mare with abnormal anatomy (i.e., after a traumatic foaling) and lack of movement of fluid in the needle hub.

Vestibule and Vulvar Labia

Pneumovagina

There are three barriers to aspiration of air and contaminants into the caudal reproductive tract:

1) The vulvar labia
2) The vestibular sphincter
3) The cervix

It would appear that the vestibular barrier is more important than previously recognized (Hinrichs et al., 1988); however, when any of these protective barriers are rendered incompetent, contamination and concomitant vaginitis, cervicitis and metritis may result. Pneumovagina can result from faulty perineal conformation, previous injury to perineal tissues or effects of poor body condition. Pneumovagina can also be iatrogenic, i.e., from reproductive examination or breeding.

Recognition of candidates with potential pneumovagina is made by anatomical observation (Pascoe, 1979). Predisposition to the condition is associated with a low pelvis, tilted vulvar labia and a sunken anus. If possible, mares should be examined during oestrus when the reproductive tract is relaxed. Parting the vulvar labia results in loss of integrity of the vestibular sphincter and an audible ingress of air into the vagina in mares predisposed to pneumovagina (Figure 1a and 1b).

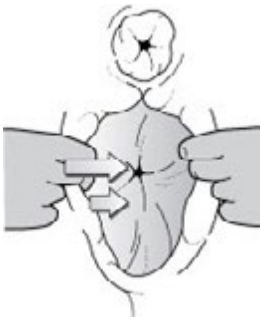


Figure 1a. Opening the vulvar lips in a mare with a good seal against aspiration of are reveals the vestibular sphincter (larger arrow). The approximate location of the urethra is indicated by the small arrow.

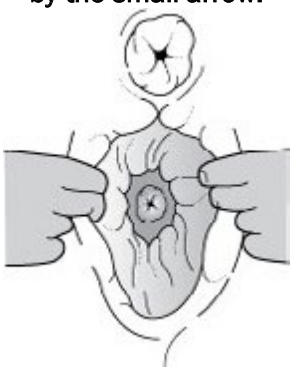


Figure 1b. A mare with a weak vestibular sphincter will aspirate air into the vagina revealing the cervix in the background.

Recognition of foamy exudates from the vulva may often be noted during rectal examination. In addition, ultrasonography has been used to visualize air in the uterus (McKinnon et al., 1987) (see chapter on Ultrasound of Uterine Abnormalities, McKinnon). If in doubt about a mare's potential to aspirate air into the vagina after clinical evaluation, we recommend surgical treatment of all mares that have been barren one or more breeding seasons, unless other causes of reduced fertility are obvious e.g., stallion or farm management.

Caslick's Operation (Vulvoplasty)

The procedure was first described by Dr. E.A. Caslick in 1937 (Caslick, 1937), and involves apposition of the dorsal portions of the vulvar labia. Local anesthesia of vulvar labia, removal of a thin portion of mucosa from the mucocutaneous junction and apposition of the cut edges is simple, quick and effective. Length of apposition is determined by height of pelvis relative to vulvar labia. In general, the join should continue to a point at least 3 to 4 cm below the level of the pelvic brim, and approximately 3 cm should be left unopposed to allow for urination (Figure 2a-e). If this is not possible, other surgical techniques (see below) may be necessary.

Points for consideration:

1. Suture material – We use 0 chromic catgut (Metric 4) on a continuous spool. The benefit of gut is that is degraded by the time of either pregnancy testing (~15 days) or the next breeding cycle (if the mare is not pregnant). Other materials that are non-absorbable or have delayed absorbable properties may damage the stallion's penis if the mare is rebred and serve as an excellent material for faecal accumulation. In addition, the continuing presence of the non-absorbable suture creates the potential for fistula formation.

2. Suture pattern – A simple continuous pattern is quicker and just as effective as a simple interrupted pattern. However, failure to oppose the deepest layers of the exposed tissues may result in fistula formation. Failure to exit/enter in the middle of the cut tissue caused by taking bites too deep will result in wound breakdown. In general, it is best to start at the dorsal vulval commissure to ensure good apposition there, as this is the most common site of fistula formation

3. Method of mucosa removal:
    - Tissue should be removed from the mucosal surface of the vestibule at the mucocutaneous junction and *not* the skin of the vulva lip
    - Removal of too much tissue will result in excess fibrosis and difficulty with the next closure.
    - If multiple procedures are performed in one season, slight debridement with a scalpel blade or scissors is sufficient, providing sutures are carefully placed.
    - Incision with a scalpel blade rather than mucosal removal is recommended for maidens (i.e., race mares). This results in a thinner join that is easily opened at the breeding farm.

4. Post-operative treatment
    - Frequent transrectal palpations within the first few days of placement can result in fistula formation. This may occur when a mare stays in oestrus despite ovulating and is re-presented for further ovarian evaluation after a Caslick's operation.
    - Remove (open) 2-3 weeks prior to anticipated foaling

5. Variations
    - Placement of a breeding stitch (usually doubled heavy gauge suture material or umbilical tape) will save unnecessary replacement and facilitate breeding and some manual vaginal manipulations for uterine treatments. Care is needed with natural service to prevent penile trauma.
    - Intrauterine treatments can still be performed and protection of the vulvoplasty is possible by introduction of an infusion pipette into the vagina using one of two fingers under the ventral border of the Caslick (past the vestibular sphincter) and using transrectal manipulation of the pipette through the cervix similar to, but with a little more difficulty, than in cattle. Alternatively, the pipette can be visually guided through the cervix with the aid of a tubular vaginal speculum.
    -

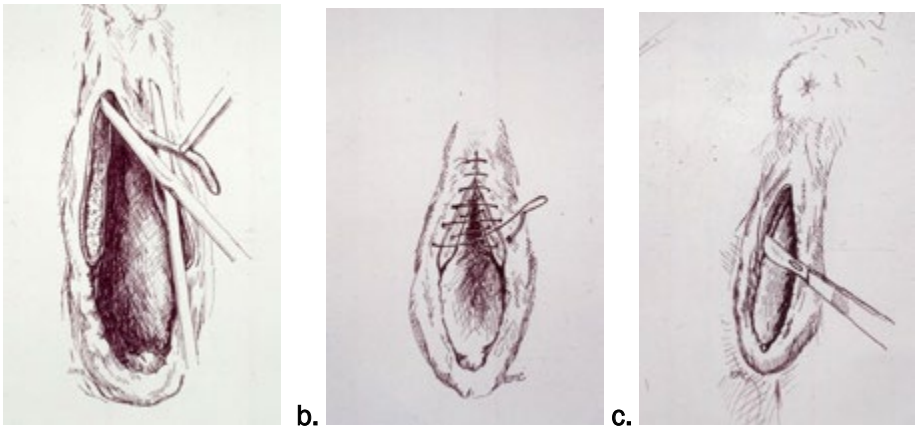


Figure 2 a. Tissue should be removed from the mucosal surface of the vestibule at the mucocutaneous junction and *not* the skin of the vulva lip; b. A simple continuous pattern is quicker and just as effective as a simple interrupted pattern. Failure to exit/enter in the middle of the cut tissue may result in wound breakdown; c Incision with a scalpel blade rather than mucosal removal is recommended for maidens (e.g., race mares)

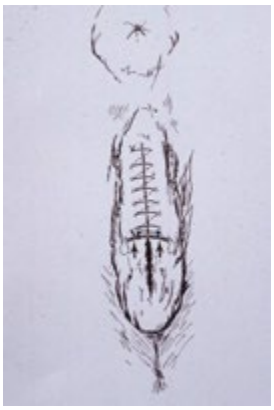
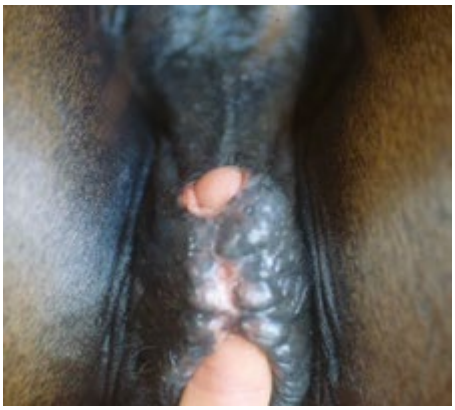
d.  e. 

Figure 2 d. A breeding stitch may save unnecessary Caslick replacement and facilitate breeding and some manual vaginal manipulations for uterine treatments; e. Fistula formation is commonly associated with poor tissue debridement, poor tissue apposition or transrectal palpation within a few days of the procedure

Episioplasty

This procedure is used in cases with more severe anatomic abnormalities where the Caslick's vulvoplasty is ineffective and in mares with extensive or repeated second-degree perineal lacerations. Technically the previous surgery is an episioplasty as well, however we (Trotter and McKinnon, 1988) use the term to describe the more extensive procedure previously referred to as 1) a deep Caslick, 2) the Gadd technique (Gadd, 1975), or 3) perineal body reconstruction, or perineoplasty. The procedure is designed to restore some degree of function to the perineal body.

The surgery is performed on the standing mare using local anaesthesia or, occasionally, epidural anaesthesia. Visualisation is improved by good lateral retraction of the vulvar labia and light sources with good illumination. We prefer to perform all perineal surgeries using a head lamp like those designed for campers. A rechargeable battery and the ability to focus the light and modify the intensity are advantages.

A triangular portion of the dorso-caudal mucosa is removed from both sides of the vestibule. The ventral borders are apposed with a simple continuous suture pattern using an absorbable material (2/0 polydioxanone (PDS) or chromic catgut), and dead space with single interrupted or simple continuous sutures of the same material. The muco-cutaneous junction is closed in a similar fashion to the Caslick's operation. Apposition of the ventral borders of the incision and obliteration of the potential space above, result in an increase in sizeof the perineal body and decreased propensity of the vestibule to expand and generate negative pressure.

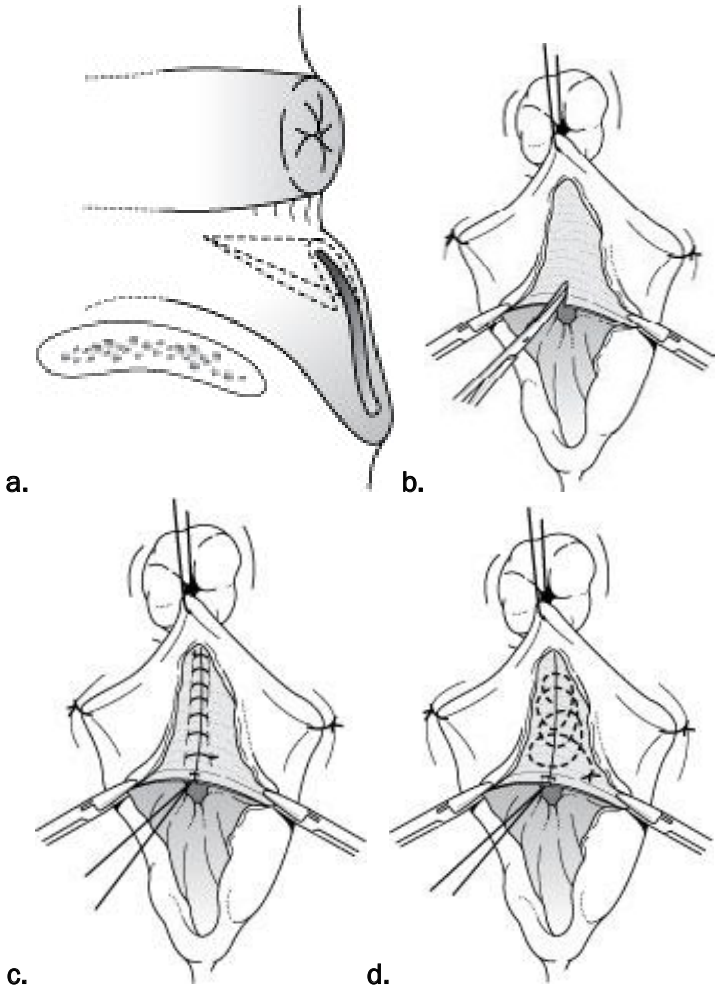


Figure 3 a. A triangular portion of the dorso-caudal mucosa is removed from both sides of the vestibule; b. Excess vestibular mucosa is trimmed; c. The ventral borders are apposed with a simple continuous suture pattern using an absorbable material; d. The dead space is apposed with single interrupted or simple continuous sutures of the same material

Points for consideration:

- The procedure may be most beneficial when combined with a perineal body transection (see on next page).
- Failure to "open" the dorsal vestibule and vulval lips (episiotomy) prior to foaling (2-3 weeks) may result in a minor to severe perineal laceration.
- The surgery usually requires 30-40 minutes and is sometimes associated with significant and annoying obscuring haemorrhage.
- It is also possible to close the perineal body dead space with externally placed vertical mattress sutures with tension spreading devices such as pieces of rubber tubing or buttons.

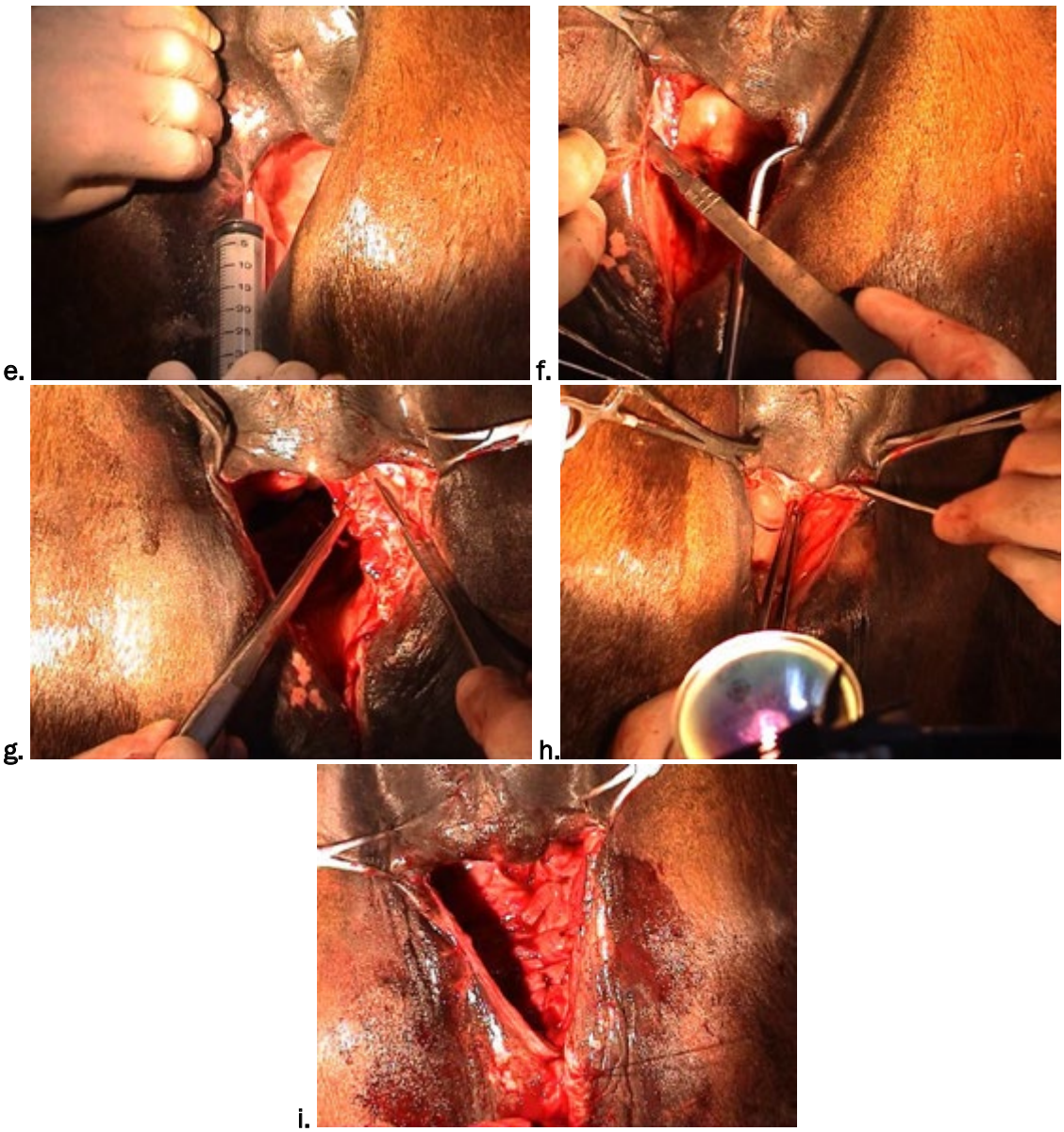


Figure 3 e - i. Large perineal body defect repair illustrated from local anaesthetic infiltration to closure of the ventral perineal body.

Urovagina

Vesicovaginal reflux (also termed urovagina or urine pooling) is a relatively common cause of subfertility/infertility seen predominantly in older multiparous broodmares. The resultant vaginitis, cervicitis, and endometritis compromises fertility by causing degenerative endometrial changes over time, that lead to an increased early embryonic death and increased susceptibility to infection associated with poor uterine defence mechanisms. In addition, urinary osmolarity and pH markedly reduce spermatozoal motility (Griggers et al., 2001) and may prevent spermatozoa from reaching the oviduct.

In 1985, Jay Belden, then of Fossil Creek Farms, Colorado, demonstrated to us his technique for surgical correction of urovagina. After many surgeries between us using Jay's technique, we decided the technique was efficacious and should be reported (McKinnon and Belden, 1988). Prior to this, we had tried the other described techniques such as caudal relocation of the transverse fold described by Monin (Monin, 1972), and the urethral-extension techniques of Brown (Brown et al., 1978) and Shires (Shires and Kaneps, 1986), and had difficulty obtaining consistently good results.

The technique to treat urovagina described below (Figure 4a-k) is based on the original publication of McKinnon and Belden (McKinnon and Belden, 1988). Recently we have reviewed an extended series of cases treated this way (Jalim and McKinnon, 2010b).

Aanes-modified Finochietto retractors (Sontec Instruments, Englewood, Colorado) placed into the vagina are used for exposure and visualisation of the transverse fold and vestibule. The midline caudal border of the transverse vaginal fold is grasped with Allis tissue forceps and retracted caudally toward the surgeon. A horisontal transverse incision to the submucosa is made 2 to 4 cm cranial from the caudal border of the transverse fold with a No. 12 Bard Parker blade (Figure 4a). The incision is continued laterad and then slightly dorsad to the vaginal wall. The vaginal retractors are then repositioned, and a No. 20 Bard Parker blade is used to extend caudad the incision from the lateral border of the transverse folds. This incision is made approximately one half to two thirds of the distance between the vestibular floor and roof and is extended to the vulvar labia. The caudal cut edge of the transverse fold incision and the ventral cut edge of the vestibular wall incision are dissected so that the free tissue flaps are reflected caudad and mediad, respectively. This causes the vestibular mucous membranes of the free tissue flaps to be reflected ventrally and the underlying cut surface to be exposed dorsally. Next, the retractors are repositioned, and an identical incision is made on the opposite vestibular wall, extending from the lateral extremity of the transverse fold caudally toward the surgeon (Figure 4b).

Dissection of the tissue flap from the transverse fold is continued until the caudal cut edge can be reflected approximately 3 to 6 cm toward the surgeon. The dissection of both vestibular wall flaps is continued ventrally until the cut edges can be reflected without tension past the midline. (Figure 4c) The suture pattern (Figure 4d) used to appose the submucosal tissue layer is a continuous modified Connell's, using 2-0 chromic gut or polydioxanone suture (PDS). The final configuration is in the shape of a Y, with the tail of the Y pointing caudally. The first suture line begins cranially and laterally at the junction of the transverse fold and vaginal wall incisions and is ended at the midpoint of the transverse fold reflection (junction of the Y). Cut edges of the transverse fold and vaginal wall are inverted so that denuded tissues are in apposition. The second suture line began on the opposite side, at the junction of the transverse fold and vaginal wall incisions and is continued to the end point of the first suture pattern and then on the midline, toward the surgeon, to the caudal end of the vaginal wall reflections. The result is an extended tunnel from the urethral orifice

(under the transverse fold) to the caudal vestibular vault. It is important to have minimal tension associated with the suture line and have all apposed edges inverted so that dissected or denuded tissues are in apposition. (Figure 4e). An extra suture layer is often over sewn which apart from preventing leakage on the original suture pattern aids in establishing a tunnel of correct size and tension.

The denuded tissues created dorsally by the dissection of the transverse and vaginal folds are allowed to heal by second intention. If necessary, an episioplasty may be performed after surgery.

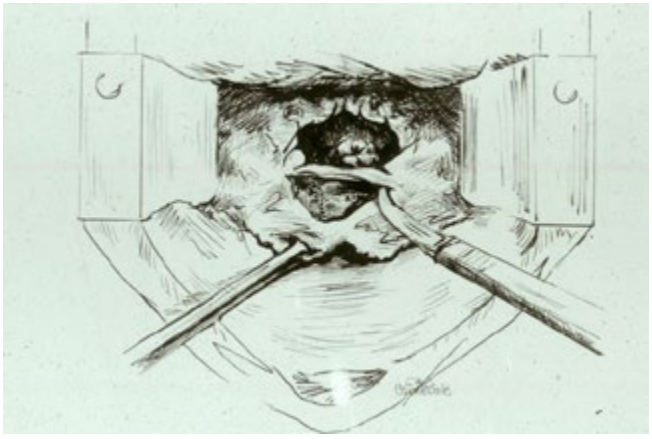
a. 

Figure 4a. A horizontal transverse incision to the submucosa is made 2 to 4 cm cranial from the caudal border of the transverse fold with a No. 12 Bard Parker blade

b. 

Figure 4b. The incision is continued laterally and then slightly dorsally to the vaginal wall and then extended caudally from the lateral border of the transverse folds approximately one half to two thirds of the distance between the vestibular floor and roof to the vulvar labia

c. 

Figure 4c. Dissection of the tissue flaps continues until the cut edges can be reflected without tension and they are then inverted in the suture pattern to form a tunnel

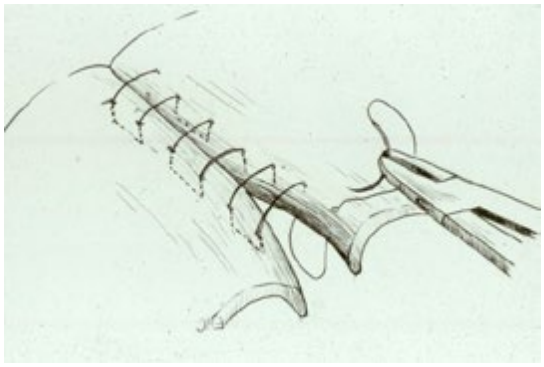
d. 

Figure 4d. The suture pattern used to appose the submucosal tissue layer is a continuous modified Connell's (horizontal mattress)

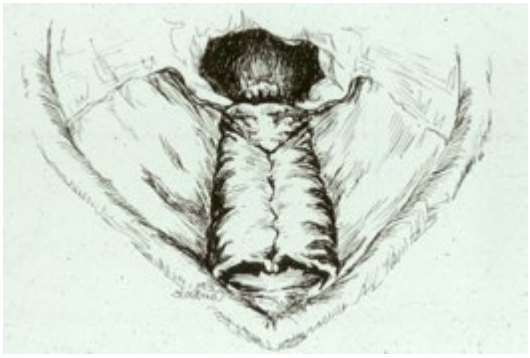
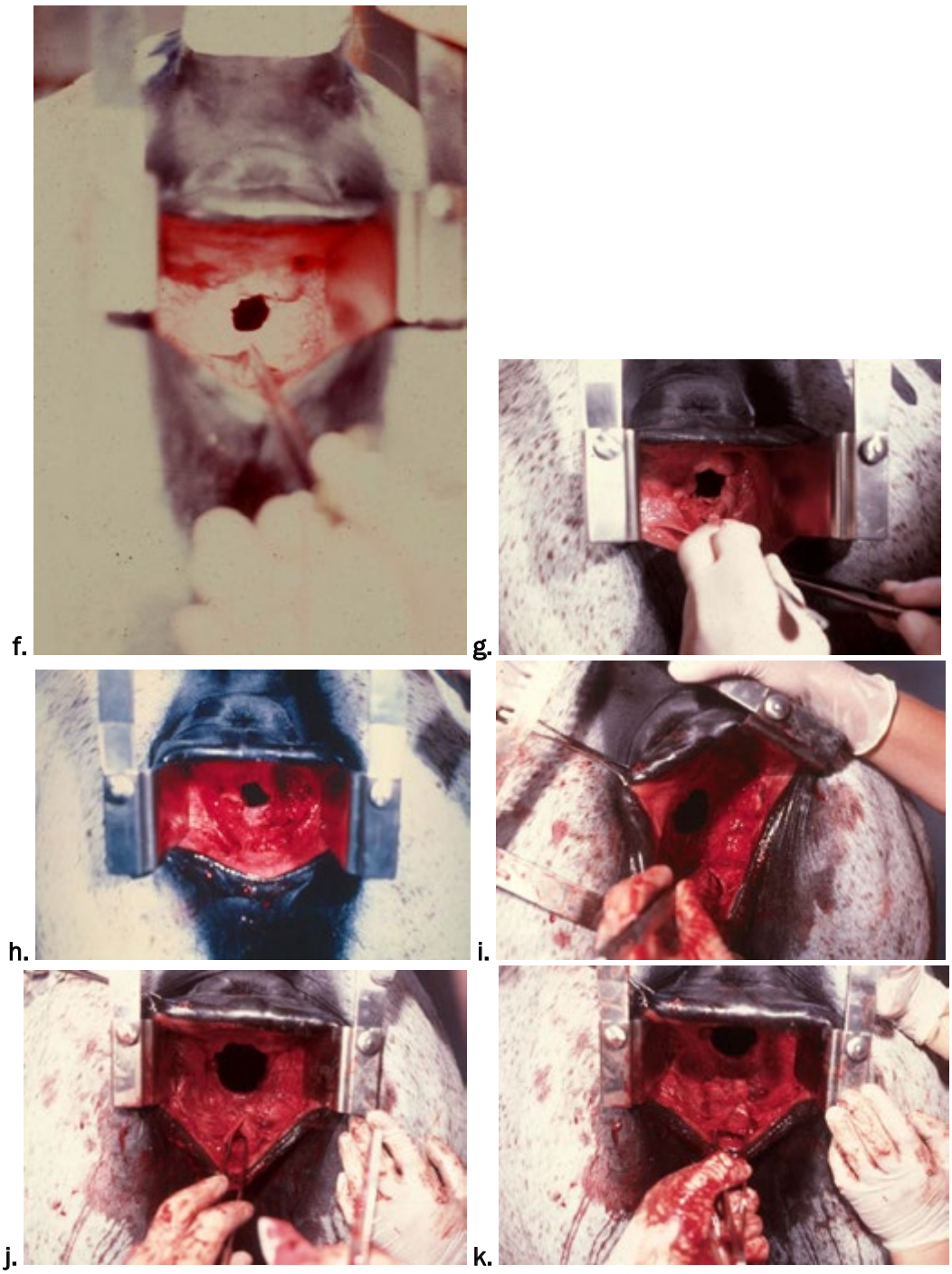
e. 

Figure 4e. The final configuration is in the shape of a Y, with an extended tunnel from the urethral orifice (under the transverse fold) to the caudal vestibular vault



Figures 4 f – k. Surgical illustration of the urethral tunnel: f Forceps grasping the middle of the transverse fold; g. Dissection of the transverse fold; h. Completed dissection of the transverse fold; i. Dissection of the vestibular wall; j. Completion of both sides of the Y pattern; k. completion of the urethral tunnel

Additional points:

- A uterine biopsy should be performed before surgery on all mares that have been barren for longer than one season. This will aid in giving an accurate prognosis for future fertility and is important in cases of financial constraints.

- The technique described is our technique of choice for treatment of all mares with urovagina. Other treatments such as perineal body transection (Pouret, 1982) or caudal relocation of the transverse fold (Monin, 1972) often appear only temporarily effective.

- Episioplasty performed at the same time may result in excessive ablation of vestibule.

- In some mares' urine will pool in the caudal relocation of the urethral tunnel and it will be voided during exercise, commonly resulting in urine staining of the perineum. While this does not affect fertility, it is unsightly and may be treated by incision into the new urethral tunnel to a point 2-4 cm cranial to the vulval lips but not far enough to allow urovagina to reoccur.

- Some mares that pool urine, do so due to nerve injury associated with foaling. This commonly results in full atonic bladders. These mares benefit from sexual rest and time and often will pool urine in the tunnel that is created if surgery is performed. The condition is more common in maidens. We recommend waiting for at least one breeding season before attempting a urethral extension on mares with bladder atony.

- Fistula formation has been noted as a problem (Beard, 1991; Embertson, 2009; McKinnon and Belden, 1988; Woodie, 2006). In the series published from our hospital (Jalim and McKinnon, 2010b) we noted a fistula formation rate of 11.5% (7/61,). In all cases fistulas occurred at the junction of the "Y" shaped incision and were repaired successfully in standing sedated mares. The mean age of mares with fistula formation was 16 years (median 17 years). Six of the 61 cases in this series were referred to our facility for surgery following previous unsuccessful attempts at urovagina correction. Four of the fistulas identified were in this population of mares which had previously undergone an unsuccessful surgery. In addition, these were the majority of mares (3/5, 60%) which had a continuation of urovagina after the initial surgical repair. Of the seven mares in which fistula formation occurred, five were corrected following a single surgery; two mares required two surgeries to correct the fistula.

- Some clinicians choose to manage urovagina by evacuating urine from the vagina pre-breeding; however chronic urovagina could lead to sustained endometritis and other irreversible endometrial changes. Unless the mare is young, has undergone substantial reversible weight loss or is recently post-partum we always elect to treat the condition of urovagina surgically if the economic constraints of the owner are able to be met.

- The surgery has classically been left to the end of the season in barren mares that were identified as having urovagina during the breeding season. A more recent development has been to breed the mares during the same breeding season in the cycle immediately after repair or to breed the mares prior to repair (immediately after identification of urovagina) and then perform the surgery

within 48 hours of breeding. The later group requires evacuation of any urine with a large tampon immediately prior to service. When mares were bred in the same cycle as surgery; the first cycle following surgery, second cycle following surgery or the following breeding season after surgery the seasonal pregnancy rates were 89% (8/9), 63% (10/16), 67% (2/3) and 63% (15/24) respectively. After removing four mares that died of natural causes pre-foaling, the foaling rates were 88% (7/8), 50% (7/14), 0% (0/3), 52% (12/23), respectively. All mares bred in the same cycle or next cycle as surgery were bred once only that season, so the pregnancy rate per cycle of 72% (18/25) was identical to the seasonal pregnancy rate (Jalim and McKinnon, 2010b).

Perineal Body

Surgeries of the perineal body are necessary to correct severe anatomic defects (perineal body transection - PBT) or injuries due to foaling (perineal lacerations and fistulae).

Perineal Body Transection

Perineal body transection (PBT) has been described as beneficial for both pneumovagina and urovagina (Pouret, 1982). Our experiences would suggest the former, but rarely the later.

The primary indication for PBT is in mares with such external reproductive conformation that we are not able to prevent pneumovagina after either Caslick's vulvoplasty or episioplasty, i.e., mares with a severely sunken anus and most or all of the vulvar labia positioned above the brim of the pelvis (Figure 5a and b).

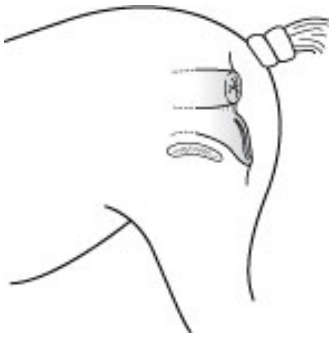


Figure 5 a. Mares with most or all of the vulvar labia positioned above the brim of the pelvis are ideal candidates for PBT

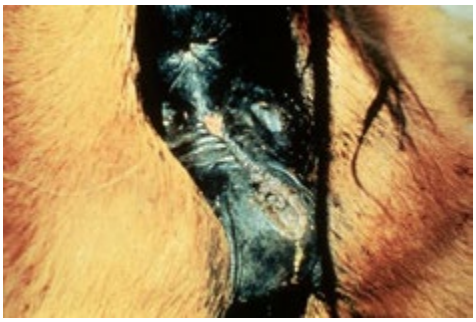


Figure 5 b. Mares with a severely sunken anus and little vulvar lips beneath the pelvis will benefit from a PBT

The procedure is performed with local anaesthesia with or without sedation. Local anaesthesia (40-70ml) is liberally infused into the perineal body and laterally to include the vaginal walls to a cranial depth of 8-14 cm.

Towel clamps positioned just ventral to the anal sphincter and dorsal to the dorsal commissure of the vulva are used to provide retraction and tension while a 4-6 cm-horizontal incision is made midway between the anus and dorsal vulva. The incision is continued for a short distance (2-5cm) ventrally along either side of the vulva. Sharp and blunt dissection are used to completely transect the muscular and ligamentous supporting tissues between the rectum and vestibule (Figures 5c and d).

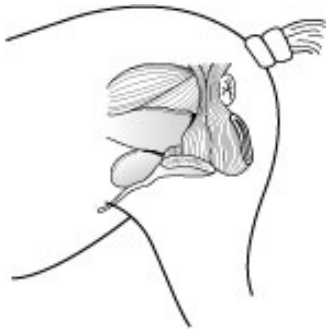


Figure 5 c. The PBT surgery aims to completely transect the muscular and ligamentous supporting tissues between the rectum and vestibule.

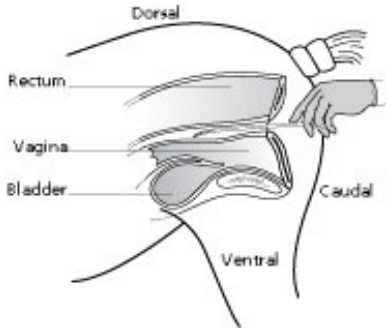
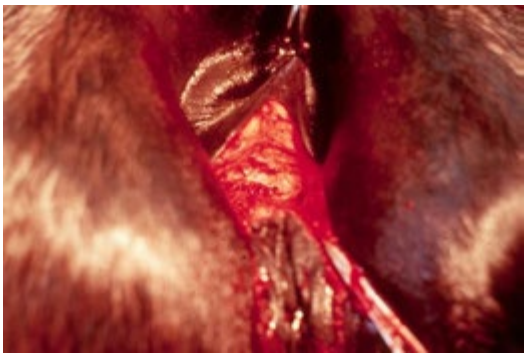


Figure 5 d. The dissection proceeds cranially for 8-14 cm and finishes before entering the peritoneal cavity

Depending on the individual mare, the dissection proceeds cranially for 8-14 cm and finishes before entering the peritoneal cavity. The aim of the surgery is to allow the vulval lips to assume a more horizontal position by freeing them from attachments to the rectum. Generally, the surgery is finished when the desired external conformation is achieved after allowing for wound contraction (Figures 5 e-g).

e.  f. 

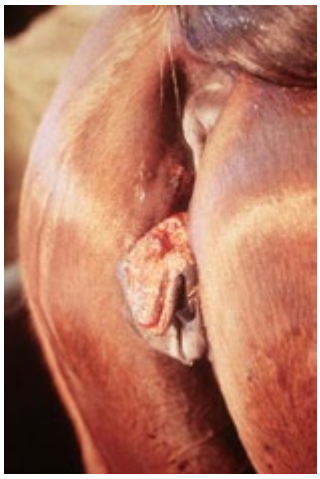
g. 

Figure 5 e. A 4-6 cm-horizontal incision is made midway between the anus and dorsal vulva; f. Sharp and blunt dissection are used to completely transect the muscular and ligamentous supporting tissues between the rectum and vestibule; g. Closure of the skin incision was originally recommended however we commonly leave the incision open

Closure of the skin incision was originally recommended (Pouret, 1982) however we commonly leave the incision open. Closing the skin occasionally results in dehiscence and seems to influence the result after wound contracture occurs. The wound heals by second intention and healing is surprisingly rapid (2-3 weeks).

Points for consideration:

- Placing a hand in the vestibule enables accurate dissection. Penetration of the vagina or rectum should be avoided.
- The wound is unsightly while healing and we recommend careful client communication and/or hospitalization for 1-2 weeks.
- The benefits from corrective surgery are immediate and mares can be bred at the first opportunity providing qualified personal are available. Natural service is generally delayed 2-3 weeks to allow strengthening of the dorsal vestibule and vagina.
- Despite the surgery causing moderate haemorrhage we have not had to take any special precautions or protective measures.
- Placing a towel clamp on the dorsal commissure of the vulva after surgery (with a small weigh such as appropriately sized nut) will aid in the correct anatomical alignment of the vulval lips while the wound is healing

Perineal lacerations

Most perineal injuries occur at the time of foaling and are associated with:

1. a mal-presented or occasionally oversized foetus or
2. extensive, vigorous, inappropriate or sometimes unavoidable manual manipulation during parturition
3. violent expulsive efforts of the mare
4. maiden mares are most predisposed to 3rd degree perineal lacerations and rectovaginal/rectovestibular fistulas.

The injuries are commonly referred to as 1st, 2nd or 3rd degree perineal lacerations (PL).

1. 1st degree PL involves only the mucosa of vestibule and skin of the dorsal commissure of the vulva.
2. 2nd degree PL involve both the mucosa and submucosa of the dorsal vulva, and some of the musculature of the perineal body, in particular the constrictor vulvae muscle. There is no damage to rectal mucosa.

Minor 1st degree PL may require no treatment. Extensive lacerations may require episioplasty or perineal body reconstruction. If tissue damage results in significant oedema, inflammation and infection, then surgical correction is often delayed for 2-4 weeks.

3. 3rd degree PL results in tearing of the dorsal vestibular wall and sometimes vaginal wall and the disruption of the perineal body, anal sphincter and rectal wall. This results in a common opening between the rectum and the vestibule (Aanes, 1988). The constant presence of faeces in the vestibule and occasional unpleasant sound from air movement make repair imperative for breeding and recommended for future riding horses. Occasionally mares will get pregnant and foal with 3rd degree PL but they are uncommon and should not be encouraged unless financial restraints preclude surgery.

Immediate care for the mare at foaling involves protection against tetanus, and can also involve systemic treatment with antibiotics, anti-inflammatory drugs and perhaps faecal softening. The surgical correction is delayed at least 4 weeks to allow second intention wound healing to occur. Immediate repair at the time of foaling can be successful but is generally not recommended (Aanes, 1988). The longer the injury is left untreated the more opportunities for continual contamination of the reproductive tract; however, this is related to functional capabilities of vestibular sphincter. Surprisingly, endometritis induced by 3rd degree PL can often be resolved within 2 weeks following surgical correction without concomitant intrauterine treatments (Schumacher et al., 1992) Most commonly, surgery is performed after weaning if a live foal was delivered. The cervix must be examined prior to surgical correction of PL and if a prolonged time between foaling and repair has occurred a full reproductive evaluation including a uterine biopsy is warranted.

Surgical technique

There are many methods described. They are all are modifications of either a single or two-stage repair. A modification of the single-stage repair (Stickle et al., 1979) is the technique we prefer. Prior to surgery, vigorous efforts are made to modify the consistency of the faeces. Mares are held off feed for at least 24 hours and given a mineral oil drench (2-3 litres) immediately before or after surgery. Following surgery, mares are placed on pasture if available and mineral oil is administered by nasogastric tube daily for 3 days as necessary to maintain a soft faecal consistency.

Mares are restrained standing, sedated and epidural anaesthesia is employed. Faecal material from the rectum is removed as far cranial as possible. Large wads of cotton wool are inserted into the cranial rectum to absorb faecal fluid and prevent faecal contamination of the surgical site. Tissues are cleansed and prepared for aseptic surgery. Towel clamps are inserted into the ventral anal sphincter in a configuration that when apposed represent the ideal surgical apposition point. In addition, towel clamps are placed on the dorsal vulvar commissure and then retracted to provide visualization for surgical access or, alternatively, stay sutures are used to retract the vulvar labia. An incision is made 5-10 mm ventral to the scar tissue line marking the junction between the vestibule/vagina and rectum (Figures 6a and b). Tissues ventral to the incision are dissected to create mobilized vestibular mucosa and submucosa that when apposed from side to side will form the ventral border of the perineal body (Fig 6c). Dissection is continued for a distance of 3-4 cm cranially and 5-8cm ventrally on the vaginal/vestibular wall. Tissues dorsal of the incision are debrided as necessary and rectal submucosa is undermined to allow sufficient mobilization to permit side to side apposition of the ventral border of the terminal rectum with as much caudal retraction of the rectal mucosa and submucosa as can be achieved without undue tension. All tissues are then sutured concurrently and incrementally from craniad to caudad. The rectal and vaginal reflections are apposed by a continuous modified inverting Connell suture. Suture material preferred is No. 1 or 2 PDS for the fibrous layer that will reform as the perineal body, 0 or 1 PDS for the vagina/vestibule and 0 or 2/0 for the rectal mucosa. The first layer sutured is the vaginal/vestibular submucosal layer that will form the ventral portion of the perineal body (Fig 6c). This can be advanced to within 3-5 cm of the vulvar lips before beginning the rectal submucosal layer. When the suture pattern of the rectal submucosa has advanced 3-5 cm the suture material is held with slight tension by an assistant and the resulting dead space in between that will be the *recreated perineal_body,* is obliterated with one of several different methods.

1. Classically this layer is apposed with single multiple-bite purse string sutures either close to the midline of the vagina/vestibule (Figures 6d-f) or involving the lateral margins of vaginal/vestibular walls (Figures 6g and h). The former technique results in dead space laterally that is not properly obliterated, and the later technique creates excessive tensions on lateral tissues. An alternate is to create a similar suture pattern but to use a continuous purse string suture.

2. Another option is to use two concurrent and side by side continuous apposition sutures that overlap on the midline (Figures 6i and j) and progress caudally towards the surgeon. This technique has been in use at the GVEH for quite a while.

3. Finally, we have recently (about 2008) developed an alternate technique that creates the perineal body with less side to side (lateral to medial) tension. The technique aims to create the tension in a transverse orientation similar to the tensions with recto-vaginal fistulae (see below). The suture pattern is a simple continuous pattern apposing dorsal rectal submucosa to dorsal vaginal/vestibular submucosa (ventral wall of the perineal body) from the lateral border of the perineal body, through midline to the opposite side and back again (Fig 6k and l). It is frequently necessary to tie and begin another new suture as the pattern will result in use of as much as 2 or 3 packets of 2 PDS.

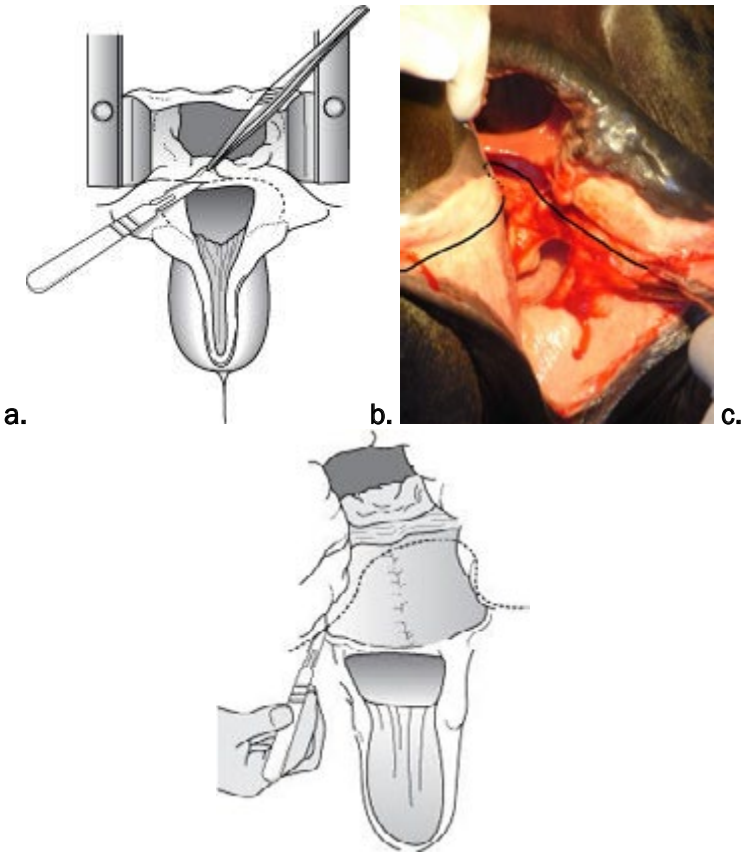
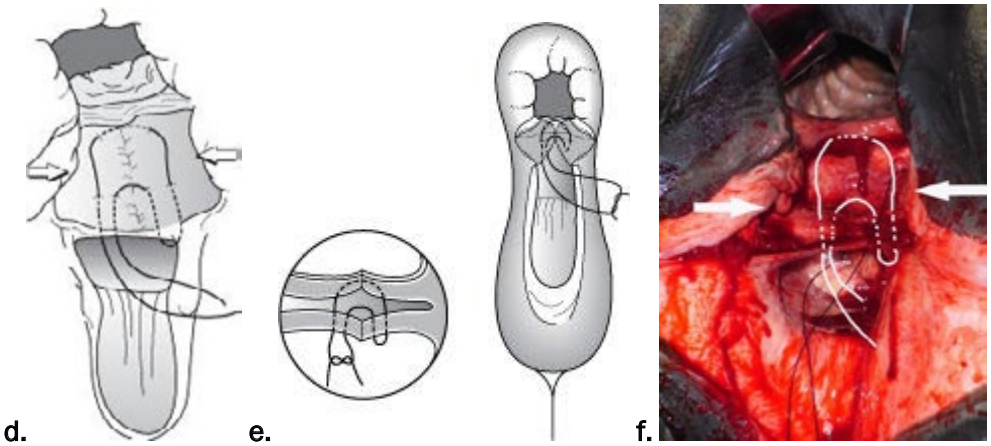
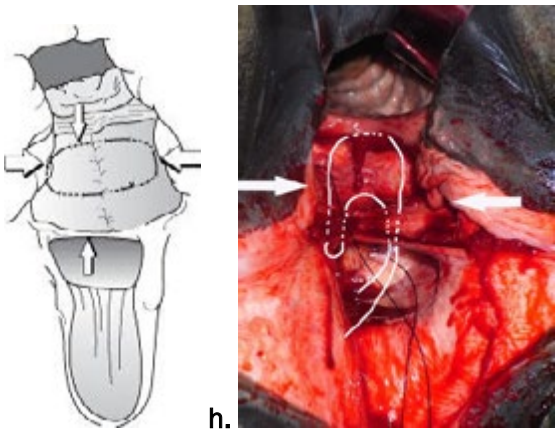


Figure 6 a. An incision is made 5-10 mm ventral to the scar tissue line marking the junction between the vestibule/vagina and rectum; b. The black line represents the approximate dissection plane along the perineal body; c. The tissues mobilised from dissection (dotted line) are joined ventrally to form the ventral border of the perineal body

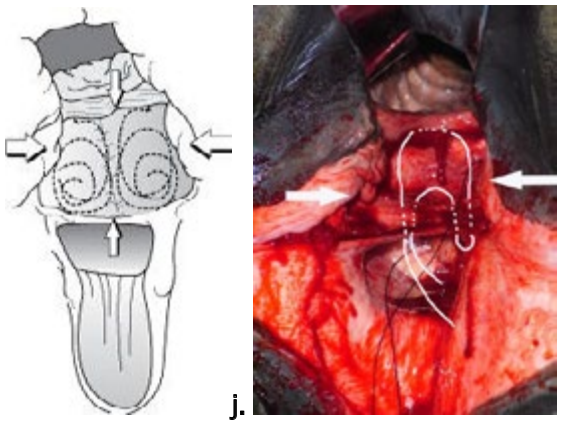


Figures 6 d – f. Schematic and surgical representation of placement of the 6-bite suture to obliterate the space between the rectum and vagina/vestibule. The arrows in 6d and 6f represent the area of poor tissue apposition with this suture pattern.



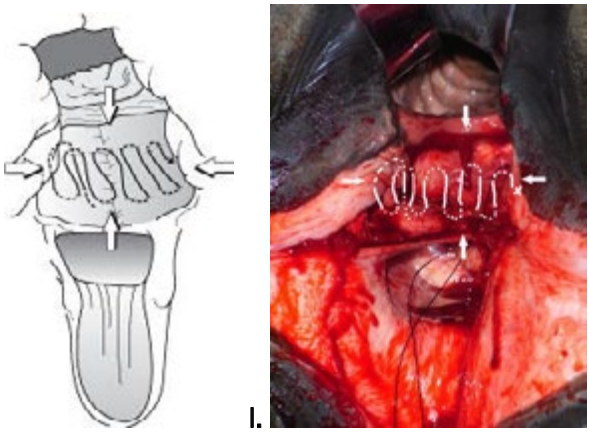
g. h.

Figures 6 g – h. Schematic and surgical representation of placement of a simple-interrupted sutures. The arrows and their relative size represent comparative tension areas which are largest from lateral to medial



i. j.

Figure 6 i – j. Schematic and surgical representation of placement of the two concurrent and side by side continuous apposition sutures that overlap on the midline. The arrows and their relative size represent comparative tension areas which are largest from lateral to medial



k. l.

Figures 6 k – l. Schematic and surgical representation of placement of a simple continuous pattern apposing dorsal rectal submucosa to dorsal vaginal/vestibular submucosa (ventral wall of the perineal body) from the lateral border of the perineal body, through midline to the opposite side and back again. The arrows and their relative size represent comparative tension areas which are now even.

The three areas of apposition are then alternatively progressed caudally, 2 - 4cm each time, diverging at the perineal body, until the ventral vestibular / vaginal submucosa stitch can be tied and vulval lips closed by a Caslick's operation. The horizontal shelf of the internal perineal body created by the technique above is terminated approximately 4-6 cm from the anal sphincter and the caudal portion of the internal perineal body is created by apposition of the remaining tissue from side to side vertically. The apposing suture of the rectal submucosa is terminated at the dorsal perineal body at the level of the defect in the anal sphincter. The anal sphincter edges may or may not be apposed.

Post-operatively, and apart from antimicrobials, anti-inflammatories and protection against tetanus, the management of faecal consistency is most important.

Points for consideration:

1. Techniques are largely dictated by experience. We believe these cases are best treated by referral practices accustomed to performing this surgery.
2. The better the surgeon's anatomical and functional understanding of the perineal body the greater the likelihood of a favourable surgical outcome.
3. Management of faecal consistency with green pasture and mineral oil are important.
4. The use of alternate methods to create less side-to-side tension in the perineal body appears to allow better functional repair of the rectum (increased size) with less danger of dehiscence and a better functioning perineal body.

Fertility is generally good after successful repair, provided no other causes of reduced fertility exist. A slightly higher incidence of perineal trauma is expected with these mares. Attendance at foaling is advised.

Rectovestibular Fistula (RVF)

Rectovestibular fistula (RVF) is a relatively common injury sustained during foaling. It may also arise because of an unsuccessful surgical repair of a third-degree perineal laceration. Fistulae most commonly occur secondary to dystocia and are usually in primiparous mares. They may be caused by the foal's nose or, more commonly, a foot (or feet) being forced through the dorsum of the vestibule or vagina into the rectum. Spontaneous retraction or manual replacement of the foals' head or extremity(s) into the correct position limits the injury to a RVF. If parturition proceeds before correction, the result is usually a third-degree perineal laceration.

It is our impression that improved management of stud farms with early appropriate intervention during dystocias has meant that RVFs now less commonly progress to third degree perineal lacerations.

We have reported on breeding the mares on an induced (prostaglandin) second post-partum oestrous period and then immediately (within 2 days) performing the fistulae repair (Jalim and McKinnon, 2010a). When mares were bred in the same cycle as surgery; the next cycle following surgery or the following breeding season after surgery the pregnancy rates were 5/5, 5/6 and 10/12 respectively. Foaling rates were 4/5, 4/6 and 7/12 respectively. The two mares already pregnant at the time of surgery foaled successfully (Jalim and McKinnon, 2010a). For breeding immediately prior to surgery to be successful, as much faecal material as possible is removed immediately before breeding (either natural or AI). After breeding the uterus is lavaged at least daily until the repair is performed.

Many surgeons treat RVF by converting them to 3rd degree PL and repairing as previously described either standing or under general anaesthesia (Aanes, 1964; Colbern et al., 1985; Hilbert, 1981). For deep (cranial) RVF's a perineal body transection has been utilized (Aanes, 1988). Recently a pedicle flap has been described (Schoenfelder and Sobiraj, 2004).

During the past two decades we have repaired RVF with a trans-rectal approach, which we first described in 1991 (McKinnon et al., 1991). A similar technique was described in the 1996 AAEP proceedings by Dr. Stephen Adams (Adams et al., 1996). In our hospital, Aanes modified Finochietto retractors are inserted into the rectum through the anal sphincter. These retractors make the surgery quite simple (Figures 7a and b).

The edges of the fistula are dissected rostrally and laterally using a No. 10 or No. 12 Bard Parker blade (Figure 7c). The rectal mucosa of the caudal border is inverted into the rectum with Babcock or Vulsellum forceps to allow easier dissection of the caudal border, using a No 12 Bard Parker blade (Figure 7d). The tissue planes are undermined for at least 4 mm commencing in approximately the middle of the fibrous scar that would become the reconstructed perineal body. This initial dissection allows exposure of three clear tissue planes – the rectal mucosa, the perineal body and the vaginal mucosa. These three tissue layers are then closed separately inverting the rectal mucosa into the rectum and inverting the vaginal mucosa into the vestibule. The first layer closed is the perineal body (middle layer) using a simple continuous appositional pattern with size 0 or 1 PDS. Next the rectal mucosa is inverted with a Connell pattern (continuous horizontal mattress) using 0 PDS and following this the rectal mucosa is over sewn with size 2/0 polydioxanone with a simple continuous pattern. Finally, the retractors are repositioned in the vestibule/vagina and the vaginal mucosa is apposed and inverted into the vagina with a modified Connell pattern with size 0 or 1 PDS. All three tissue layers are closed transversely to minimize tension on the suture lines and best align normal anatomy.

When we previously described the surgical correction (McKinnon et al., 1991) it was recommended to alternate the orientation of the closure. Now we recommend that all three layers be closed transversely, not longitudinally.

Restraint and postoperative care are similar to mares with perineal lacerations, however little attention is given to faecal softening.

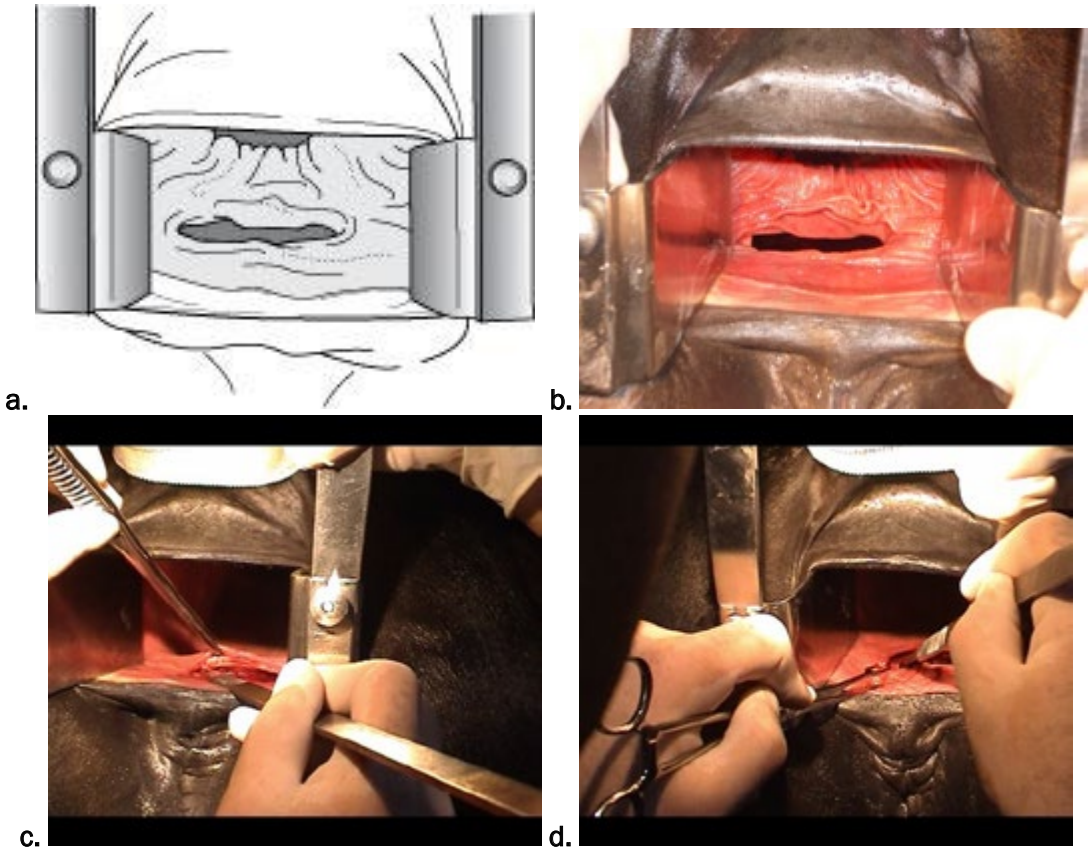


Figure 7a. Aanes modified Finochietto retractors in the rectum; b. Surgical exposure is improved with retractors placed rectally; c. Cranial dissection of the RVF ring with a number 12 Bard Parker 'hook' blade; d. Caudal dissection of the RVF ring is improved with inversion into the rectum with Babcock or Vulsellum forceps

Points for consideration:

- The caudal border of the fistula may be difficult to debride and is most easily rotated towards the surgeon by grasping it with Babcock or Vulsellum forceps (from the rectum) and inverting it caudally (Figure 7d). On occasion it may be helpful to have the surgeon's hand in the vagina while working on this part of the fistula.
- The use of a No 12 blade greatly facilitates dissection of the caudal fistula ring.
- These results above suggest delaying breeding until the following breeding season is not necessary. In addition breeding on the same cycle as surgical repair is a recently reported technique (Jalim and McKinnon, 2010a; McKinnon and Vasey, 2007) that should be considered in an effort to maintain a yearly foaling interval.

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Notes

Recurrent Laryngeal Neuropathy (RLN): Diagnosis, Treatment and Prognosis

Brian Anderson
ba@bvp.com.au
Ballarat Veterinary Practice
Ballarat, Victoria
Australia

What is the Definition and Aetiopathogenesis of RLN?

Recurrent Laryngeal Neuropathy (RLN) is a disease that affects the neuromuscular function of the larynx of horses and has been recognised for centuries. The precise cause of RLN is still unknown despite the essential pathology being well described. A detailed description of RLN is available.[1] Paralysis of the equine larynx can be bilateral or unilateral. Unilateral paralysis is more common and is called laryngeal hemiplegia (LH). Two studies showed that in 6% and in 11% of the LH case series the cause was known and was the result of damage or injury to the left or right recurrent laryngeal nerve by inadvertent perivascular injection of irritant medication, trauma to the neck, or the result of guttural pouch mycosis, thyroid carcinoma or thymic lymphosarcoma. [2,3] In addition, organophosphate poisoning, plant toxicity, B vitamin deficiency, lead poisoning and strangles abscessation of the head and neck have caused or are associated with unilateral and bilateral laryngeal paralysis.[4,5] In the remaining cases, which are the vast majority and predominantly left-sided, the cause is unknown, and the term idiopathic laryngeal hemiplegia (ILH) is used to describe the condition.

Neuropathy of the left recurrent laryngeal nerve and, to a lesser extent the right, is the primary lesion detected in most cases.[6,7,8,9,10] Progressive loss of large, myelinated nerve fibres in the distal part of the left recurrent laryngeal nerve results in atrophy of the intrinsic laryngeal muscles innervated on this side. Although adductor muscles of the arytenoid cartilage are affected earlier and more profoundly, it is atrophy of the *cricoarytenoideus dorsalis* muscle (CAD; also known as the dorsal cricoarytenoid muscle or DCAM) that results in significant clinical disease.[11] This muscle is the only abductor of the arytenoid cartilage and vocal fold, and deficient function results in a significant reduction in airflow to the lungs in exercising horses. Histopathological examination of horses with LH and individuals with endoscopic evidence of CAD dysfunction shows that a repetitive, progressive denervation/reinnervation process occurs.[6,12,13,14] Attempts at repair, including collateral axonal sprouting and reinnervation of previously denervated muscle (demonstrated by fibre type grouping) are evident. When axonal loss and muscle atrophy is extensive and repair processes insufficient, paralysis of the left side of the larynx results. Recurrent Laryngeal Neuropathy or RLN is preferred instead of LH because it describes a range of lesions and associated clinical disease which can vary from mild to severe. Laryngeal hemiplegia is, therefore, the end stage of neuropathy of the recurrent laryngeal nerve.

Is RLN a bilateral mononeuropathy or a polyneuropathy, inherited or acquired?

Why is this important? Discovering the precise cause of RLN is critical to being able to either prevent or treat the condition. For example, in humans and other species, most distal axonopathies are polyneuropathies involving more than one nerve, and the causes are genetic, metabolic, or toxic. In contrast, a traumatic aetiology is often involved with mononeuropathies.[15] Currently RLN is considered a bilateral mononeuropathy, which would set the disease apart from distal axonopathies in other species.[16] However, recent research using immunohistochemical methods found evidence of pathology in the distal and deep

branch of the radial nerve in horses with significant RLN pointing to the condition being a polyneuropathy.[15]

What is the Prevalence of RLN?

An accurate figure of the prevalence of RLN and LH is difficult and depends on the criteria and methods used to determine the presence of the condition. Endoscopic surveys on mainly Thoroughbred horses estimate RLN and LH to be present in some 2.6-8.3% of adult horses.[17] In yearling Thoroughbreds, including those at public auction sales, significant RLN and LH is reported to be 0.14%-0.65%.[18-20] Neurogenic atrophy of the left intrinsic laryngeal muscles has been identified in clinically normal horses in 30% of mixed breeds and 80% of Thoroughbreds, indicating a high level of subclinical cases.[6,13]

What are the risk factors for RLN?

Histopathological, clinical, and endoscopic surveys have indicated that significant RLN is more common in males than in females, but conclusive evidence is lacking.[2,13,21] In general, RLN affects heavier, taller breeds such as Draft breeds, Warmbloods and Thoroughbreds, and is rarely described in ponies. Laryngeal hemiplegia was identified in 35% of 183 Belgian, Percheron and Clydesdale horses. [22,23] Approximately 95% of horses affected with LH are taller than 160 cm.[2,21,24] There is no doubt that RLN in horses has a genetic component, given the higher prevalence of the condition in offspring of RLN-affected than unaffected stallions, and heritability has been estimated at between 8 and 40%.[25,26,27] Several studies have shown a correlation between RLN risk and body size, particularly height at the withers.[25,28,29] Recently, a site (locus) on gene ECA15, linked to growth traits, was found to increase the risk of RLN in male American Belgian Draft horses.[28] Identification of risk genes would allow the implementation of preventative or treatment strategies.

What is the age of onset of RLN?

Histological changes typical of RLN have been recorded in male draft horse foals two-weeks and six-months old, lending weight to the opinion that the disease is hereditary and/or has a congenital aetiology.[30,31] In one survey of predominantly Thoroughbred horses, the histopathological changes typical of RLN increased dramatically in prevalence and severity in horses one to two years of age.[13] Despite these findings, the age at which clinical signs of RLN occur can be variable and appear to relate to the start of breed-related work. In Thoroughbreds, particularly those intended for flat racing, the clinical signs of RLN commonly occur in one- to three-year-old horses. RLN is often diagnosed in unbroken yearling Thoroughbreds examined at the time of sale. The peak incidence of diagnosis of RLN in these animals, however, is when training and racing begins between 2 and 3 years of age. In hunters and national hunt racehorses not broken until aged four to five years, the median age for diagnosis of RLN was 6 years.[2] In summary, RLN can affect animals at almost any age but the most commonly noted time for clinical disease is between one and six years.

Diagnosis of RLN

RLN is suspected when there is a history of exercise intolerance and an abnormal respiratory noise evident at exercise. Palpation of the larynx is performed to detect evidence of atrophy of the CAD. Confirmation of RLN is achieved following endoscopic examination of the larynx at rest or if necessary, during exercise on a treadmill or overground.[1] The diagnosis of severe RLN is not difficult, but the detection of mildly affected horses and determining the best

treatment approach or predicting the rate of progression and clinical effect the disease process will have in any one horse is more difficult.

Respiratory noise and RLN

In horses afflicted with RLN, an abnormal audible noise is heard during inspiration. Coupled with the normal expiratory noise, these two sounds have been compared to that heard when sawing wood. Although the quality of the inspiratory sound has been described in a variety of ways, two different sounds are commonly heard. The first is a finer, higher pitched sound which is described as "whistle" and the other is more course/harsh, lower pitched sound and usually louder referred to as a "roar". Hence, the description "whistlers" or "roarers". The precise source of the abnormal inspiratory noise within the larynx has not been identified. Air turbulence caused by air moving across an incompletely abducted or collapsed arytenoid cartilage and an open laryngeal ventricle or collapsed vocal fold are involved.[33] Recently the association of owner-reported noise with findings during dynamic respiratory endoscopy (DRE) in Thoroughbred racehorses was reported.[34] When 85 horses underwent DRE due to a reported abnormal noise by owners, 82% were found to have an obstruction. Characteristic owner reported noise patterns (i.e., gurgling for palate dysfunction and whistling/roaring for RLN) showed only moderate to low sensitivity for specific conditions, i.e., quite a few misdiagnoses (false positives). This was due to the number of combined obstructions, e.g., RLN combined with axial deviation of the aryepiglottic folds or dorsal displacement of the soft palate with various other soft tissue collapse. However, if owners did not report a whistling or roaring noise as being present, the negative predictive value was 97% indicating laryngeal dysfunction was unlikely. The researchers concluded that DRE should be performed in horses with abnormal respiratory noise to rule out complex conditions of the URT.

External examination and palpation of the larynx

All horses suspected of suffering from RLN should have the larynx palpated as well as the sites where incisions are made for laryngeal surgery (laryngotomy, laryngoplasty, nerve graft) and both jugular veins. Significant atrophy of the intrinsic laryngeal muscles, predominantly the CAD, results in a palpably more obvious, more pointed, and firmer muscular process on the left side. The technique has been well described.[35] Laryngeal palpation is useful in detecting hemiplegic horses when an endoscope is unavailable and supports a diagnosis of RLN where laryngeal endoscopy is equivocal, but an inspiratory noise is heard at exercise. However, in recent years a greater amount of information has been provided by percutaneous ultrasound examination of the larynx, and this procedure should now form part of all examinations for the presence of RLN.

Percutaneous ultrasound of the equine larynx for diagnosis of RLN

RLN is characterised histologically as neurogenic atrophy of the intrinsic laryngeal muscles. The ultrasonic appearance of denervated and atrophied muscle is an increase in echogenicity, i.e. it looks whiter (Figures 1 and 2).

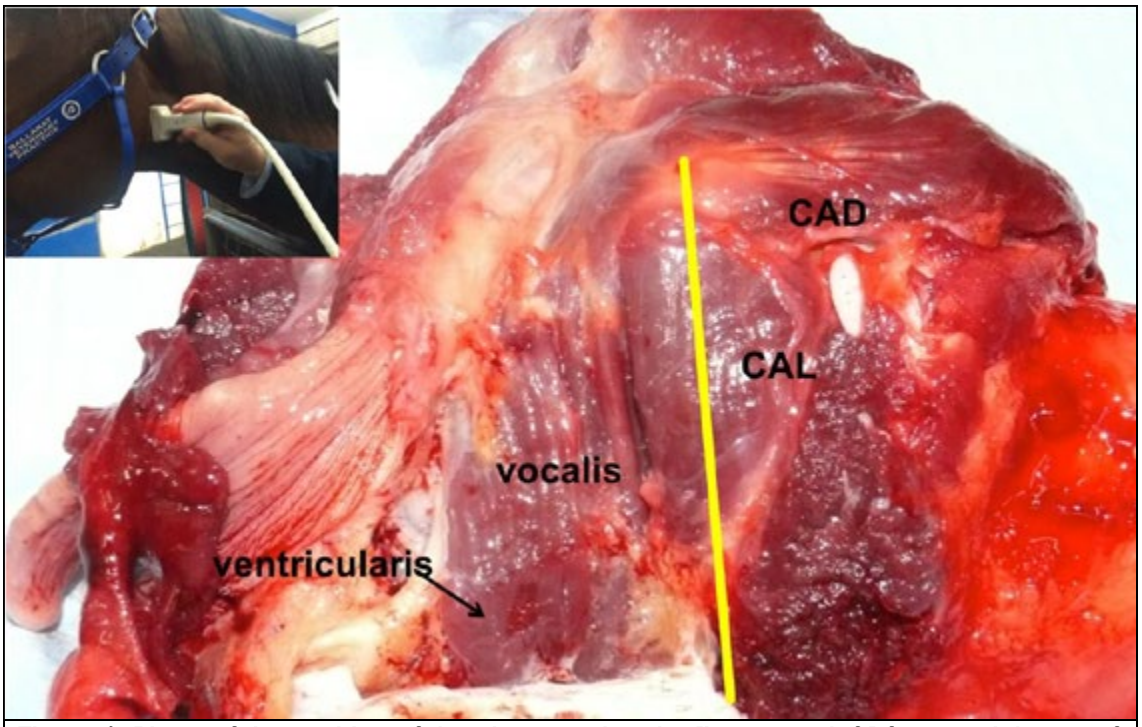


Figure 1. Photo of the position of the ultrasound probe [insert top left] for an axial scan of the larynx, and the anatomical structures imaged in the ultrasound image in Figure 2. The yellow line is the plane of tissue imaged. The thyroid cartilage has been removed. Rostral is to the left. Image courtesy of Dr Ian Fulton.

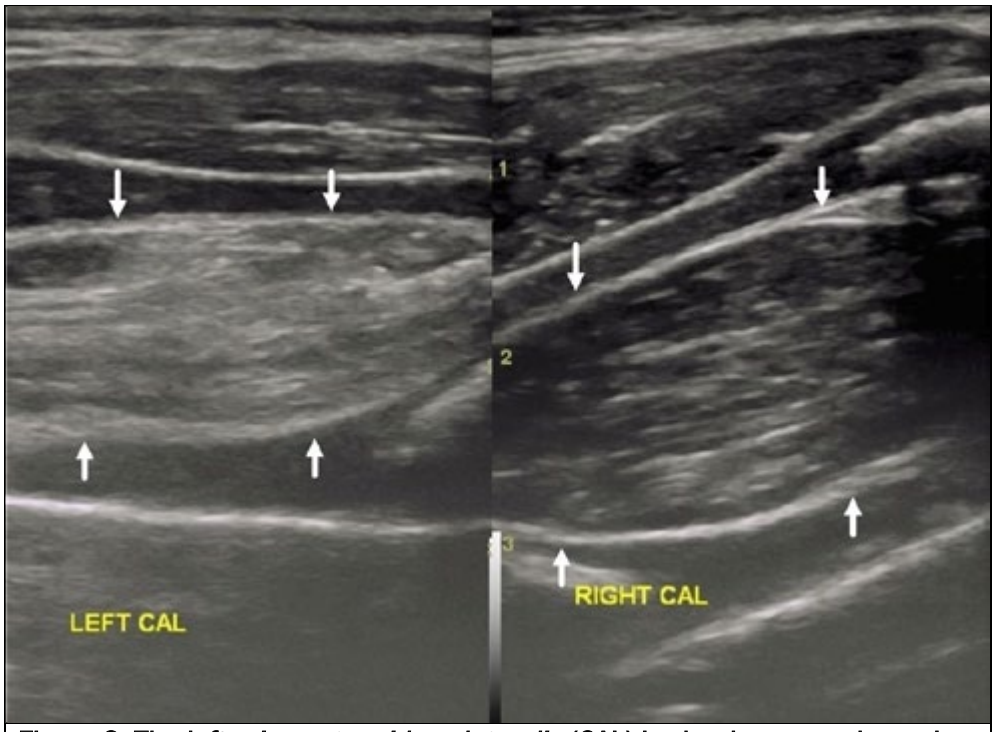


Figure 2. The left *cricoarytenoideus lateralis* (CAL) is clearly more echogenic compared to the right CAL [between sets of arrows]. In 90% of horses with this finding, dynamic airway collapse occurs during exercising respiratory endoscopy. Image courtesy of Dr Ian Fulton.

In horses that had a right-sided neurectomy of the recurrent laryngeal nerve, the increase in echogenicity is a result of reduced minimum muscle fibre diameter, increased muscle fibre density and increased muscle collagen content.[36] Early ultrasound changes occur at four weeks, becoming more obvious at eight weeks after neurectomy. The advantage of percutaneous ultrasound of the larynx is that it is non-invasive, easy to perform and is quite sensitive for determining if neurogenic atrophy of the intrinsic muscles is present on the left side compared to the right side. The technique has been described in detail.[37] Ultrasound of the larynx is recommended for all cases of suspect RLN and prior to surgical intervention. The benefit is in determining that significant RLN is present in cases where laryngeal movement grade is equivocal for RLN, e.g., 2.ii or 3.i. Garrett *et al.* showed that the presence of increased echogenicity in the CAL was a good predictor of arytenoid cartilage collapse under exercise conditions.[38] Laryngeal ultrasonography had a sensitivity of 90% and specificity of 98%, whereas resting URT endoscopy had a sensitivity of 80% and specificity of 81% for abnormal laryngeal function during treadmill exercise. In addition, ultrasound evaluation can differentiate severe RLN from chondritis or 4-BAD syndrome being the cause of incomplete arytenoid abduction and is of great use to determine the cause of right-sided laryngeal dysfunction. The use of transoesophageal ultrasound and computer tomographic (CT) assessment of the CAD are other techniques that could be used in the future to detect signs of early muscle atrophy before a complete functional deficit is observed endoscopically at rest or exercise.[39]

Endoscopic diagnosis

Endoscopy of the larynx is essential to confirm the presence and determine the severity of RLN. An endoscopic examination of the pharynx and larynx is indicated in the following situations:

- As part of the pre-purchase examination.
- Owner/trainer/jockey/driver or rider reports an unusual or excessive respiratory noise during exercise.
- As part of the examination of horses which are performing poorly.

What are we looking for?

Essentially the examiner is looking for abnormal laryngeal movements caused by RLN. Normal and abnormal laryngeal movements have been reviewed in detail (Figures 3,4 and Table 1).[1] RLN causes functional deficits in the adductor muscles of the larynx, but most importantly the only abductor, the CAD. It is generally considered that if resting asymmetry of the larynx is present, i.e., the left arytenoid cartilage is positioned closer to the midline than the right for prolonged periods, and that full abduction is never achieved, then significant and clinical RLN is present. Several grading systems for laryngeal movements have been reported. It is important to remember that although a correlation exists between resting endoscopic laryngeal movement grade and underlying RLN this is not an exact fit and grades for laryngeal movement should not be interchanged with grades for RLN.[40] The reason for this is that the function of the larynx and the movement of the arytenoid cartilages is not determined only by underlying neuropathology, but is influenced by other intra-horse variables such as level of excitement or fatigue and the effects of drugs (e.g., sedatives). Anatomical and other physiological effects as well as temporal variability can all affect laryngeal movements.[41,42] Currently, the best studied and validated grading system of laryngeal movements is the Havemeyer Classification (Table 2).[43] The Lane classification (Table 3) has been used for many years in New Zealand and Australia, and while it has proven

to be a useful and a practical method of grading laryngeal movements, it is not validated.[44] Both systems are presented below.

For those horses with resting asymmetry, for which full abduction is achieved but not maintained (compared to the position of the right arytenoid cartilage), the presence of clinically significant RLN is more difficult to determine. Age, occupation, presence of abnormal respiratory noise and performance history are all important in the interpretation and significance of endoscopic findings. Laryngeal ultrasound may be useful, but a definitive diagnosis is best made using exercising URT videoendoscopy on a high-speed treadmill or using overground endoscopy (OGE). Each modality has advantages and disadvantages. With the use of a treadmill, standardised exercise tests are easier to do, and adjustment and positioning of the endoscope possible mid-test. A higher diagnostic rate for IDDSP is reported for treadmill use, especially in racehorses but is lower in sport horses. OGE has the practical advantages of horses exercising in their own environments and under competition-like conditions without the need to travel and no requirement for acclimation to the treadmill. OGE in sport horses is preferred as head and neck position and rider interaction have been shown to influence upper airway function. Figures 4 and 5 show the progressive collapse of the left arytenoid cartilage in cases of severe RLN during high-speed exercise due to loss of abductor function.

	A. Normal, quiet breathing.
	B. Normal, full adduction.
	C. Normal, full abduction.
	D. Left sided asymmetry during quiet breathing.
	E. Partial RLN, incomplete left sided abduction.

Figure 3. Sketches of freeze-frame videoendoscopic images of the larynx show positioning of the corniculate cartilages during different phases of breathing.

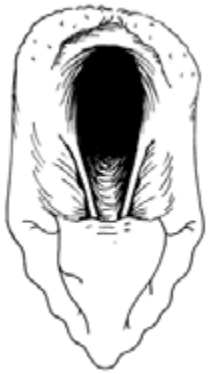
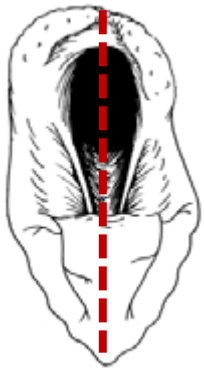
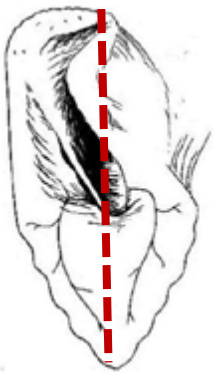
Grade	Appearance	Definition
A		Full abduction of the arytenoid cartilages during inspiration.
B		Partial abduction of the affected arytenoid cartilages (between full abduction and the resting position).
C		Abduction less than resting position.
D		Abduction less than resting position with collapse into the contralateral half of the rima glottidis during inspiration.[46]

Figure 4: Grading system of laryngeal function as assessed in the horse during exercise.[45] Description generally refers to the left arytenoid cartilage in reference to the right. However, this grading system can apply to the right side (i.e., right grade B). The addition of a grade D was to differentiate complete obstruction of the *rima glottidis* compared to marked restriction but not complete obstruction.[46]

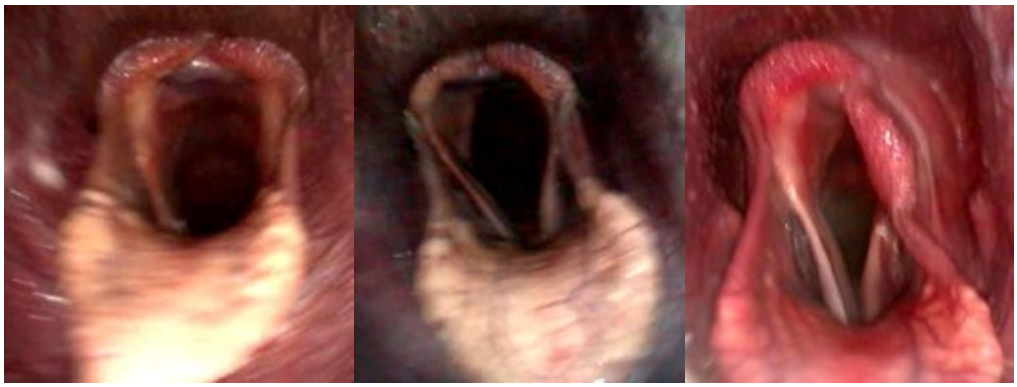


Figure 5: Dynamic URT videoendoscopic images of Grade A, B, C and D from left to right and below.

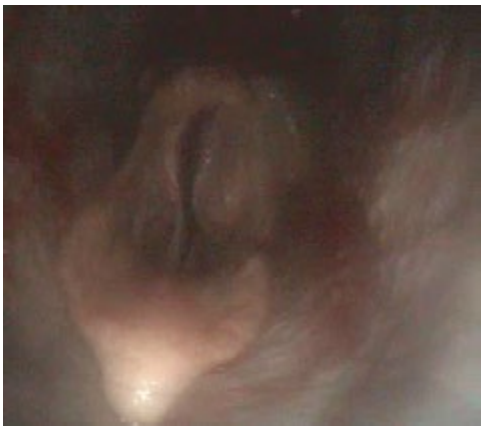


Table 1. Definitions of terminology used to describe endoscopically observed laryngeal movements.[43]	
Abduction	Movement of the corniculate process of the arytenoid cartilage away from the midline of the *rima glottidis*.
Adduction	Movement of the corniculate process of the arytenoid cartilage toward the midline of the *rima glottidis.*
Full abduction	Most of the corniculate process lies horizontally (90° to the midline of the *rima glottidis*).
Asymmetry	A difference in position of the right and left corniculate processes of the arytenoid cartilages relative to the midline of the *rima glottidis*.
Asynchrony	Movement of the corniculate processes of the arytenoid cartilages at different times. This can include twitching, shivering and delayed or biphasic movement of one arytenoid cartilage.

Table 2. Havemeyer System for laryngeal movements.[43,†]			
Grade	**Description**	**Sub-grade**	
1	All arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.	-	
2	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetric at times, but full arytenoid cartilage abduction can be achieved and maintained.	(i)	Transient asynchrony, flutter or delayed movements are seen.
		(ii)	There is asymmetry of the *rima glottidis* much of the time due to reduced mobility of the affected arytenoid and vocal fold but there are occasions, typically after swallowing or nasal occlusion when full symmetrical abduction is achieved and maintained.
3	Arytenoid cartilage movements are asynchronous and/or asymmetric. Full arytenoid cartilage abduction cannot be achieved ***or if it can, it is not**** maintained.	(i)	There is asymmetry of the *rima glottidis* much of the time due to reduced mobility of the arytenoid cartilage and vocal fold but there are occasions, typically after swallowing or nasal occlusion when full symmetrical abduction is achieved but not maintained.
		(ii)	Obvious arytenoid abductor muscle deficit and arytenoid cartilage asymmetry. Full abduction is never achieved.
		(iii)	Marked but not total arytenoid abductor muscle deficit and arytenoid cartilage asymmetry with little arytenoid cartilage movement. Full abduction is never achieved.
4	Complete immobility of the arytenoid cartilage and vocal fold.	-	
† Description generally refers to the left arytenoid cartilage in reference to the right. However, this grading system can apply to the right side [e.g., right grade 3(i)]. *** Author edit.**			

Table 3: Lane Grading System for laryngeal movements.[44]	
Grade	**Description**
1	All LM by the left and right arytenoid cartilages (adduction and abduction) are synchronous and symmetrical.
2	All major movements of the arytenoid cartilages are symmetrical with a full range of adduction and abduction. Transient asynchrony, flutter or delayed abduction may be seen especially by the left arytenoid cartilage.
3	Although the left arytenoid is still capable of full abduction, activity is generally reduced on the left compared with the right with periods of prolonged asymmetry, particularly during quiet movements. Full bilateral abduction can be stimulated transiently by partial asphyxiation using nostril occlusion or after swallowing but is not sustained.
4	There is consistent asymmetry of the rima glottidis at rest: the left arytenoid cartilage is not capable of full abduction, but some residual movements are present.
5	True hemiplegia: active movement is absent on the left side with the arytenoid cartilage resting on or near the midline.

In older horses with an exercise history of abnormal respiratory tract noise and/or poor performance, the relationship between resting laryngeal grade and exercising laryngeal grade in sport horses and horses used for racing was recently evaluated. A meta-analysis of twelve published studies involving 1827 horses was performed.[47] Grading systems with 4, 5 and 7 subgrades were used in these studies. Normal resting endoscopy was defined as grade 1 or 2 on a 4-point or 7-point scale (North American and Havemeyer Classification) or 1, 2 or 3 on a 5-point scale (Lane Classification), and normal dynamic endoscopy as a dynamic laryngeal grade A. The aim was to determine the sensitivity and specificity of resting laryngeal endoscopy to predict the presence of laryngeal collapse at exercise. The sensitivity of resting endoscopy was 74% and the specificity 95%. The positive predictive value was 86% and the negative predictive value 91%. What this study confirms is that resting laryngeal endoscopy is a good predictor of laryngeal collapse at exercise in older horses with a history of abnormal respiratory noise and/or performance when full arytenoid abduction is not achieved. If laryngeal ultrasound is included in the examination, the accuracy is even better. In these studies, the amount of data available for evaluation on horses that can fully abduct both arytenoid cartilages but in which the left cartilage is not abducted for as long compared to the right (grade 3i on the Havemeyer scale) is limited. In three studies there were 45 horses classified as grade 3i, 38 as grade 3ii and 2 as grade 3iii. Within grade 3i, 23 (51%) were classified during exercise as grade A, 10 (22%) as grade B and 12 (27%) as grade C. Despite the accuracy of resting endoscopy in these studies dynamic laryngeal endoscopy is needed for those horses with a resting LM grade of 3i to be sure of a diagnosis. In addition, other dynamic upper airway obstructions can also be detected.[48]

Progression of RLN

Evidence for progression of RLN based on endoscopic and clinical studies are conflicting. In young Thoroughbred and Standardbred horses (1-2 years old at the start of the study), endoscopic progression of LM from normal to that indicative of RLN was 12% over 16 months and to full LH was 5%.[41] Dixon *et al.* reported on endoscopic and/or clinical progression of RLN in older national hunt and sport horses (predominantly Thoroughbred).[49] Fifty-two of the 351 horses examined (15%) showed evidence of progression of the degree of laryngeal dysfunction over a median period of 12 months (range 1.5-48 months), with the onset of progression occurring at median age of 7 years. In another study, 11% of Thoroughbred horses (average age of 2.4yrs) examined with overground endoscopy on two occasions, 7-8 months apart, developed worse laryngeal exercise grades indicative of progression of RLN.[50] In summary, it appears that in most horses, laryngeal function remains constant over time, but in some horses, laryngeal function can deteriorate quickly over a few weeks or more slowly over some years irrespective of initial endoscopic and clinical examination findings. In Australasia, experience indicates that in Thoroughbred horses bred and trained for racing, clinically significant RLN develops and progresses most often between 1-4 years of age.

Treatment of RLN

For a balanced and comprehensive review of the surgical management of recurrent laryngeal neuropathy, readers are directed to the article by Cramp and Barakzai (2012).[51]

Therapy can be divided into the following areas:

1. Conservative
2. Surgical
    - Operations on the laryngeal ventricles and vocal cords
    - Operations on the arytenoid cartilage
    - Laryngeal reinnervation procedures

The aim of treating RLN is to improve, or hopefully reverse, the inspiratory obstruction that is caused by defective abductor function. Depending on the athletic activity required, a secondary aim is to reduce the abnormal respiratory noise present and most importantly try to avoid significant contamination of the lower airways with feed material, saliva, and respiratory irritants.

Conservative Therapy

Doing nothing can be very beneficial to the horse. For horses performing athletic activities for which respiratory demand is low, such as living at pasture, pleasure riding and low-level equestrian pursuits, no treatment is required. Because of the complications associated with laryngeal surgery, most particularly prosthetic laryngoplasty (PL), it is wise to choose surgical candidates carefully and with full disclosure and informed consent. This particularly relates to young fillies who may have significant breeding value. For potential racehorses and those intended for elite equestrian endeavours, careful consideration should be given to surgical therapy. If the ability of the horse is unknown or was substandard prior to RLN becoming significant, or if there is no history of siblings or related horses performing well, then retirement is entirely reasonable. Similarly, in competition horses that develop significant RLN, retirement to less strenuous activities is a reasonable management option.[51] Either way, in the words of the great Dr Brian Goulden, owners or trainers might sensibly consider a “whole horse transplant”!

Surgical Therapy

The choice of surgical therapy depends on several factors including the presenting complaint (abnormal respiratory noise, poor performance or both) age, sex and occupation of the horse, severity of RLN and tolerance for potential complications.

Operations on the Laryngeal Ventricles and Vocal Cords

The literature in this area is confusing. Some definitions will help:

Ventriculectomy (VC) – involves the removal of the mucosal lining of the lateral laryngeal ventricles in the hope of creating a fibrous adhesion between the vocal fold, thyroid, and arytenoid cartilages.[51] One or both ventricles can be operated on. The most common approaches are via a ventral laryngotomy under general anaesthesia using a burr and sharp dissection (it can also be done standing under sedation), or during standing sedation with a laser.

Ventriculocordectomy (VeC) – involves a ventriculectomy **plus** excision of the vocal cord(s). A ventriculocordectomy can be unilateral or bilateral (more on this below) and performed as for a ventriculectomy above.[51]

For some owners and for some horses performing low level athletic pursuits, the inspiratory noise associated with RLN – a whistle or a harsh noise (a roar) - is objectionable. Resolution of the noise can be achieved by removal of the laryngeal ventricles +/- vocal cords. Current opinion is that bilateral ventriculocordectomy is the treatment of choice to most effectively reduce abnormal inspiratory noise caused by RLN. There are two further advantages: removing the vocal cord will prevent vocal cord collapse and this may have a modest effect on reducing airway obstruction (up to 30%) and improving airway mechanics in cases of severe RLN and in cases of vocal cord collapse in the absence of RLN.[51,52] Typically, a 4–6-week rest period allows for healing.

Operations on the arytenoid cartilage

Prosthetic Laryngoplasty (PL)

The most common operation for treating severe RLN is the prosthetic laryngoplasty (tie-back). This is almost always combined with a unilateral or bilateral ventriculocordectomy (with some variations). The aim is to permanently abduct and stabilise the arytenoid cartilage (almost always the left) to provide sufficient airflow for athletic activity, reduce abnormal inspiratory noise and avoid the complications of aspiration of food, water and saliva that causes chronic cough and lower airway disease. One end of a prosthesis (usually 2 sutures of various types are used) placed through the caudal and dorsal aspect of the cricoid cartilage is then passed through the muscular process and when the two ends are tied the arytenoid cartilage becomes abducted and the *rima glottidis* dilated (Figure 6). The operation has been shown to be effective in reversing the hypercapnia and hypoxaemia observed in RLN affected horses after galloping. Similarly, the decreased inspiratory airflow and increased inspiratory resistance that is evident following experimentally induced laryngeal hemiplegia can be reversed following prosthetic laryngoplasty.[51]

The reported success rates of laryngoplasty depend on the intended use of the horse and the different criteria used to measure success. Reported success rates of the procedure when performed on horses **not intended** for racing can be as high as 70-92%. Using objective measures of performance, the success rates reported for **racehorses** vary between 38-80%.[51,52] In our hands, approximately 60% of operated horses can have improved race performance. The most important aspect of the procedure is the many complications that can occur. These complications include but are not limited to:

1. dysphagia with aspiration of food, water and saliva into the trachea and subsequent coughing and nasal regurgitation of feed, water, and saliva
2. chondritis
3. intermittent or persistent dorsal displacement of the soft palate
4. lower airways inflammation or infection (pneumonia)
5. immediate or progressive loss of arytenoid abduction
6. wound infection and continued respiratory noise
7. poor performance.

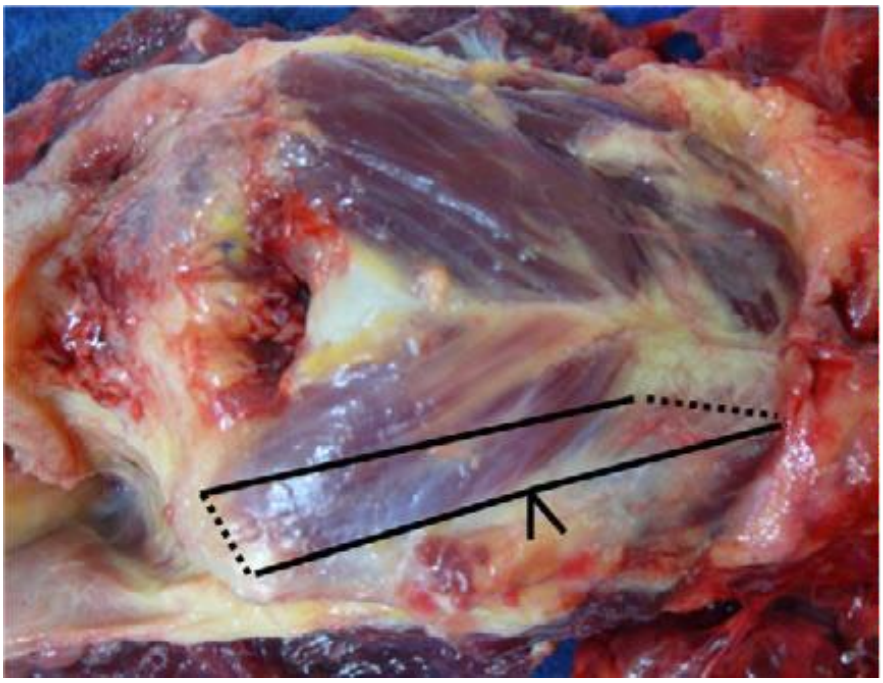


Figure 6. Post-mortem picture of the larynx (rostral to left) showing schematically the position (∧) of the prosthesis through the cricoid cartilage (right dotted line) and muscular process of the arytenoid cartilage (left dotted line).

Partial Arytenoidectomy

Although partial arytenoidectomy (PA) is mostly indicated for treating advanced arytenoid chondritis, it can also be used as a primary procedure for RLN, or a secondary procedure following a failed tie-back or following complications from a tie-back e.g., chondritis. The muscular process is left in *situ.* We prefer a modification of the technique as described by Radcliffe *et al.* (2006) and suture the abaxial corniculate mucosa and part of the aryepiglottic fold over the deficit left after the body of the arytenoid has been removed.[53] PA can also be done in the standing sedated patient under endoscopic control via a small stab incision through the cricothyroid membrane without mucosal suturing. We do not routinely see post-operative dysphagia and coughing, although this can occur. Collapse of unsupported abaxial mucosa, IDDSP and inspiratory noise remains in many horses. PL is preferred over PA for the treatment of RLN as airway ventilation at maximal exercise and racing performance appears to be better.[51] Clinical studies indicate up to 82% of horses can return to racing after PA, and approximately two thirds can earn prize money.

Laryngeal Reinnervation Procedures

The techniques of PL and PA are invasive and create anatomical distortion, damage neuromuscular structures and can significantly alter normal swallowing mechanisms. Complications are common. Arytenoid cartilage position shows a typical loss in abduction of 1-2 grades within days to weeks. Given the variable degree of lower airway insult and other consequences such as an increase in exercise induced pulmonary haemorrhage (EIPH), athletic performance declines over time either due to resumption of airflow restriction or secondary disease.[54] A more physiological method of ameliorating the effects of RLN would be desirable. Laryngeal reinnervation procedures aim to replace the innervation of the CAD muscle with a nerve/s innervating accessory muscles of respiration. Typically branches of C1-C2 which innervate the *omohyoideus* muscle are used because this muscle is active on inspiration and the nerve is in close proximity to the larynx. The most common method to

date has been the nerve muscle pedicle graft (NMPG) performed under general anaesthesia.[52,55-58] Small blocks of *omohyoideus* muscle (3-5 mm) up to 5 in number attached to branches of the C1-C2 nerve are implanted into the CAD. Free nerve endings can also be implanted. Typically, a bilateral ventriculectomy and a minimal left vocal cordectomy is also performed. Reinnervation of the CAD muscle is frequently evident at 4 months following surgery. Ultrasound guided stimulation of the C1 nerve where it exits the ipsilateral foramen of the atlas at this time will determine if arytenoid abduction due to reinnervation is present. Full training can then commence. However, it can take between 6 and 12 months for reinnervation to be successful. Results of the technique are as follows:

- In 71 unraced horses the average time to first start was 13.5 months. Of these 51 raced and on average had 14.6 starts and 63% of these horses won at least one race.
- In 94 raced horses the average time to first start was 8 months. Sixty percent of horses won at least one start and 52% won more prize money in total after surgery. Thirty-nine percent earned more money per start after surgery and 45% had an improved performance ranking.

Clinical experience indicates that the best candidates are those without significant CAD muscle atrophy. Recently two changes to the technique have occurred. A modified first or second cervical nerve transplantation technique using tunnelling and direct implantation of the donor nerve into the *cricoarytenoideus dorsalis* muscle has replaced the NMPG and the procedure is now performed in the standing horse.[59] The results of this technique in combination with ipsilateral laser ventriculocordectomy have been reported on seventeen client-owned horses which included 7 French Warmbloods, 6 Thoroughbreds, 2 Anglo-Arabs, one Dutch Warmblood and one Standardbred; age range: 2–11 years; use: 7 show jumping, 7 racing and 3 eventing. Reinnervation was confirmed by nerve stimulation and subsequent arytenoid abduction observed in 11 out of 12 cases between 4 and 12 months post-operatively. Fourteen horses had exercising endoscopy before and after surgery. Nine horses had an improved exercising RLN grade, four horses had the same exercising grade, and one horse had a worse exercising grade after surgery. Arytenoid stability was subjectively evaluated and before surgery 13 of 16 horses had an unstable left arytenoid cartilage, but after surgery only 1 of 9 had an unstable arytenoid cartilage. It is important to note this is a mixed sample of horses with only 7 racehorses and objective performance outcomes were not measured. A study comparing performance outcomes between PL and nerve reinnervation is not available. The authors noted that it is possible that the neural input from C1/C2, or the amount of muscle reinnervated was insufficient to restore complete abduction of the arytenoid. It is our own experience that despite successful reinnervation of the CAD with marked abduction on nerve stimulation, arytenoid collapse during strenuous exercise can still occur. Without doubt, reinnervation techniques hold the greatest promise for better outcomes for athletic animals. Current research is focused on using nerves innervating muscles that are better in phase with inspiration and improving muscle strength through electrical stimulation while the CAD becomes reinnervated.

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Notes

Endoscopy of the upper respiratory tract in Thoroughbred horses at public auction sales

Brian Anderson
ba@bvp.com.au
Ballarat Veterinary Practice
Ballarat, Victoria
Australia

Introduction

Normal form and function of the upper respiratory tract (URT) is critical for successful athletic performance in Thoroughbred racehorses. It is, therefore, common practice to endoscopically evaluate the URT of horses sold as yearlings or two-year-olds at public auction sales in Australasia and around the world to determine whether pre-existing conditions are present, which would reduce the chance of a successful athletic career. Traditionally only a post-sale endoscopic examination was performed in Australasia, but in recent years it has become standard practice for pre-sale URT videoendoscopic examinations to be placed in a repository during a sale and be available online for registered veterinarians to examine for potential purchasers.

What is the current situation?

- Endoscopy is pre-sale and post-sale.
- Pre-sale examination before arrival or at sales complex (NZ) or after arrival (Australia) but within 7 or fewer days – unregulated, but examiners should follow pre-sale EVA and NZEVA endoscopy protocols.
- Videoendoscopic examination for lodging in the repository for veterinary evaluation.
- Post-sale is under regulation by conditions of sale. A well-regulated system exists in NZ, and very experienced practices are involved in Australia. Horses are returnable if they fail conditions of sale. Arbitration via the sales company and selection of a panel (NZ).
- All post-sale evaluation of laryngeal movements is graded using the Lane system 1-5. Grades 4 and 5 fail and horse is returnable.
- All pre-sale is available on video, and the viewer can grade on whatever system they desire but are advised to use the Lane System because this is the post-sale system and the most used in Australasia.
- I advise buyers to always do a post-sale scope as some protection afforded by the sales company.

What are the problems?

1. Suboptimal videoendoscopic examinations, questionable scoping practices, and hyperstimulation of the larynx.
2. Disagreement between veterinarians on grading of laryngeal movements, intra- and interobserver variability.
3. Current grading systems (Lane and Havemeyer) not fit for purpose?
4. Variability in day-to-day or even within-day laryngeal movements for individual horses – what does this mean?
5. The predictive value of a single endoscopic examination of laryngeal movements in a yearling or two-year old and future race performance is unclear.
6. The rate and degree of progression of recurrent laryngeal neuropathy (RLN) in racing Thoroughbreds is unknown. Is the laryngeal movement grade identified at the

yearling or two-year-old year important, i.e., is a grade 1 horse less likely to progress to clinical RLN than a grade 3 horse?

7. Different results in studies evaluating the relationship between laryngeal movement grade when a yearling/two-year-old and race performance between studies performed in Australia, UK, and USA – likely due to differences in interpretation and grading.
8. What is the relationship between resting laryngeal movement grade and underlying neuromuscular pathology in yearling and two-year-old Thoroughbreds?
9. Buyers perceive a linear scale with respect to laryngeal movements and relate this to degree of laryngeal disease and likely rate of progression. Thus, it is perceived that a grade 1 is better than 2, is better than 3 etc., and a grade 3 is close to a 4 and therefore more diseased and likely to become a 4 vs a grade 1. Veterinarians have not done enough to dispel this. Anecdotal evidence by some buyers is that grade 3 horses make worse racehorses and are more likely to "go in the wind" and they prefer not to buy them.
10. Gold standard is laryngeal function during fast exercise - examined using treadmill or overground scope (not available for yearlings -some attempt has been made). This could be available for two-year-olds but would vendors agree to this? Perhaps happening in the USA?
11. Some vendors feel aggrieved about the variation in opinion over laryngeal movement grades by veterinarians and the loss of sales, especially with respect to horses being graded as a 3.
12. Purchasers are ignorant of laryngeal movement grading in horses and what it means. They only want a perfect throat and are not buying horses that would be normal due to fears of throat disease.

What do we know?

1. Subclinical RLN is common in the Thoroughbred breed.
2. Estimates of progression from subclinical to clinical is up to 15%.
3. There is good correlation between laryngeal movements and severity of RLN at the low and high ends of scale, but there is poor correlation in the middle. For example, grades 1 or 2 are considered to have little pathology and grades 4 and 5 have marked pathology but grade 3 unknown.
4. Some effects on future performance are clear for grade 4 horses and for some parameters in some studies for grade 3 horses, i.e., less money at 2 and 3 vs. 4 years of age, with no effect on starts.[1,2]
5. Anderson *et al.* 2018[3] showed no difference in race outcome when comparing grade 1 and 2 horses vs. grade 3 horses, but this finding is not universally accepted. Ahern et al[4] also did not show any statistically significant difference in race outcome when comparing grade 1, 2 and 3 horses using multiple grading schemes.
6. Assessment of endoscopic videos of laryngeal movement has poor interobserver reliability centred around if (1) full arytenoid abduction is achieved and if it is (2) for how long it is obtained. Intraobserver reliability is better but still relatively poor for a diagnostic test. The conclusion is that agreement between veterinarians is poor.

How do we solve the problems?

1. Education of vets, buyers and vendors on what RLN is, including cause, pathology, diagnosis, clinical effects and progression. This is a dynamic disease, most likely inherited, with clinical effects most observed in the 2–3-year-old period but can occur later.
2. More longitudinal data that relates laryngeal function with future race performance.
3. It is important to appreciate that examination and grading of the laryngeal movements of young unbroken horses may in fact be inaccurate/unreliable as a predictor of future race performance. The reason for this, in my opinion, is that laryngeal function is affected by several factors at any given time in a young horse.

While the underlying degree of neuromuscular pathology, and therefore RLN, is a prime reason for defective laryngeal function and future poor race performance, other factors such as excitement, fatigue, drugs, time of day, use of a twitch, head position and level of stimulation could all make laryngeal movements look defective, or better than what would be seen under exercise conditions.

To answer some of these questions would require:

A prospective study using an agreed laryngeal grading system that involved endoscopic examination using an approved protocol performed in a repeatable manner. The grading system would have to have a high inter- and intra-observer repeatability/reliability and all users would need to be trained in its use. Preferably horses would be examined on several occasions (statistical analysis may determine how many times would be needed). Repeat examinations over time using the same methods would allow us to determine the rate and change of grading/disease. Entire race careers would be examined and all horses that developed laryngeal disease would be identified. All horses that were operated on and the results of operation would be recorded. All race data, including race distance, race quality, racing jurisdiction and multiple measures of race performance would be collected. Data on sire and dam influence would also be evaluated (controversial). The study may be a matched cohort study, with each horse compared to each other horse from the same sale/sale year and laryngeal grades compared between each other. The outcome of such a study would tell us:

- Prevalence of each grade
- Change in grade over time
- Incidence of clinically significant laryngeal disease
- Outcome of surgical treatment
- Effect of race distance, surface, class, and jurisdiction i.e., Australia vs Hong Kong
- Relationship of laryngeal grade as a yearling/two-year-old and future race performance.
- The outcomes would allow purchasers to make decisions on risk for purchase.
- Vendors could identify horses unlikely to sell
- Disagreements over grading would be less if a grading system (possibly we have one already) and the interpretation of grading was agreed by all and reinforced by education.

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BJ Ahern, K de Klerk, LR Hogg, SA Vallance, FR Bertin and SH Franklin

Recognising the problem broodmare: diagnosis and treatment tips

Chelsie A Burden
Goulburn Valley Equine Hospital
905b Goulburn Valley HWY, Congupna VIC 3633

In many disciplines, the broodmare is expected to have normal oestrous cycles, be breed and conceive in an acceptable timeframe to produce a healthy foal each breeding season. When the mare shows inappropriate cyclicity, is unable to be bred, does not conceive despite multiple breeding attempts, or has pregnancy loss she becomes categorised as a problem broodmare. While some mares need simple changes in breeding management, there are a vast number of reasons why inappropriate cyclicity, infertility or pregnancy loss may occur. A systematic and complete clinical evaluation of these problem broodmares is crucial for diagnosis and treatment of causative pathologic conditions and to further develop an effective breeding management plan.

A complete reproductive evaluation should include general health history of the mare, history of past reproductive performance and previous known pathologies, a general physical examination, perineal examination, transrectal palpation and ultrasonography, and vaginal examination with assessment of the cervix. Further diagnostic procedures routinely performed may include endometrial culture, cytology, and biopsy. Hysteroscopic examination, hormone assays, endocrine or cytogenetic testing maybe necessary to identify specific abnormalities. Once aetiology has been identified, treatment and management options can be determined.

Recognising the problem broodmare

History

One of the most important components of working up the problem breeder is reviewing the breeding history when available. A thorough history should include age, previous breeding attempts, pregnancy loss, foaling trauma, dystocia, retained foetal membranes, abnormalities in the reproductive cycle, diagnostics and therapy previously performed along with other relevant health information such as lameness, dental or endocrine disorders. A detailed history will help guide the breeding soundness examination as well as highlight potential concerns for discussion with the client or breeder.

Physical examination

A physical examination is recommended for all mares presenting for veterinary care with attention to body conditions score, musculoskeletal soundness, dental and endocrine health. Mares that are severely underweight or overweight, suffering from chronic lameness issues or have untreated metabolic syndrome or PPID can all present with abnormal cyclicity.[1] While a complete breeding soundness examination is still warranted, treatment of clinical abnormalities is a large component of the subsequent reproductive management plan.

Perineal Evaluation

Abnormal perineal conformation is one of the most common findings in problem broodmares in the author's experience. There are three anatomical barriers in the caudal reproductive tract that protect the uterine environment. These include the vulva, vestibulovaginal fold (hymen) and the cervix.[1,2] Good conformation of the vulva and the perineal body (muscular tissue between the vulva and anus) are imperative for reproductive efficacy. The vulva lips need to have good tone with tight closure supported by the perineal body. The normal anatomic arrangement is that the vulva should be vertical and below, not caudal to, the anus with the majority of length below the level of the ischial tuberosities or pelvic brim.[3,4] The vestibulovaginal seal should be maintained when the vulvar lips are parted. A breakdown of this seal will allow aspiration of air into the vagina. A complete cervix consisting of an internal

and external os connected by a long tubular body that is tight under the influence of progesterone works to further prevent contamination and infection if the uterus. Age and parity have a significant influence on vulvar and perineal body conformation.[2] Many mares with uterine pathology, a history of placentitis or pregnancy loss do not have an adequate perineal anatomy. A sunken anus with tilted vulva resulting in pneumovagina can be easily recognized by visual examination of the perineal region.[4] This is often a result of a weakened perineal body. An incompetent vulvar seal can be obvious with air aspiration noted during exercise or reproductive procedures; however, mares with seemingly normal conformation may show more subtle signs of an abnormal vulvar seal. The observation of air within the vaginal cavity when performing transrectal ultrasonography or faecal debris on the sleeve following transvaginal procedures are both suggestive of a weak vaginal seal.

An incompetent vulvar seal may lead to reproductive failures. In the author's opinion re-establishing good perineal and vulvar conformation is the most important preventative treatment for endometritis causing infertility. Surgical intervention such as a Caslick's or Gadd or Pouret procedure maybe required.[4,5] Both techniques are relatively quick and simple to perform in the standing horse under local anaesthetic. While the Caslick procedure is routinely performed, Papa *et al.* described a modified Pouret's technique that the author finds useful in performing perineal body reconstructions.[2] Another consideration in mares with a history of poor perineal conformation and intrauterine fluid is the uteropexy. While the aim of the surgery is to lift the uterine body and horns, perineal conformation has also been documented to improve after uteropexy.[6]

Diagnostics

Transrectal Palpation and Ultrasonography

Transrectal palpation and ultrasonography is a routine component of breeding management. The technique is well described and is of paramount importance in the initial diagnosis, monitoring and management of the mare presenting with pathology of the reproductive tract.[7,8] A single examination may identify anatomical abnormalities and gross pathology, but often serial examinations are necessary to fully appreciate pathology related to the reproductive tract and cycle. Good record keeping is critical for retrospective assessment of the breeding cycle. Each ovary, the uterine horns, body, cervix, and vaginal cavity should be evaluated even on routine examinations. Any structure of interest and notable pathology should be palpated and imaged completely and in multiple orientations if possible to better define the abnormality.[9]

Ovarian structures should be evaluated for size, consistency, and presence of ovarian structures such as follicles and corpora lutea. Ovarian motility and/or mare response to palpation should be noted along with presence of parovarian cysts, epithelial inclusion cysts or other notable structures such as haemorrhagic follicles or suspect ovarian tumours.[7,8]

The uterus should be palpated for tone and consistency along with position in relation the pelvis. Ventral positioning over the pelvic brim may be problematic for mares with endometritis and difficulty clearing fluid. Ultrasonography is further utilised to record the presence of oedema relative to the stage of the cycle (ovarian findings) and presence of luminal fluid. For record keeping, oedema is scored for 0 to 4, with 0 being uniform echogenicity of the endometrial folds and 4 being significant or pathologic with excessive oedema present.[10] The intrauterine location, amount and character of luminal fluid are evaluated and recorded. Fluid is not normally present in the uterine lumen outside of oestrus. Normal uterine fluid is anechoic and small in volume.[11] Fluid of increased echogenicity, volume or fluid noted in dioestrus is abnormal and further diagnostic techniques are warranted to determine the cause of fluid production and retention. Other ultrasonographic findings that maybe of interest on uterine examination include the presence of cysts, mass-like structures and hyperechoic structures. Hyperechoic structures are associated with conditions including intrauterine air (gas echoes), urine sedimentation, scaring or fibrosis, foreign objects, or mineralised material such as with retained endometrial cups.

Transrectal ultrasonography of the cervix can identify anatomic defects that are not easily palpable.[12] The linear longitudinal fibres of the cervical lumen can be easily imaged in dioestrus and is often combined with digital examination in mares suspect of cervical pathology. Disruption of the linear pattern, pockets of air or fluid, along with general changes in echotexture may suggest pathology that can be further categorised by repeat examination.[12]

Ultrasonography of the vaginal cavity is very useful in identifying pathology prior to any vulvar or vaginal manipulations or in mares with such poor perineal conformation that air is introduced into the caudal reproductive tract during transrectal palpation. In the normal mare, the vagina is collapsed and can be difficult to image above the bladder. Abnormalities often identified on transrectal vaginal ultrasonography include fluid and air that suggest pathology and abnormal perineal conformation that should be further investigated.

Vaginal Examination

It is critical that the perineal region is cleansed prior to any vaginal procedures. Vaginal examination can be performed using a speculum to visualize abnormalities of the external cervical os and vaginal cavity. The author prefers to perform a digital vaginal examination to evaluate the cervix for patency, fibrosis, and adhesion formation. A digital examination also allows for characterisation of vaginal mucous, fluid or debris noted on the sleeve following the procedure.

Urine Pooling

Vaginal examination can be diagnostic for vesicovaginal reflux or urine pooling. Urine pooling can be noted at all stages of the oestrus cycle in some mares or more obviously during oestrus with relaxation of the reproductive tract.[13] If the mare is in dioestrus, the cervix is closed and the urine remains in the vagina producing a vaginitis and cervicitis. However, if the mare is in oestrus, the open cervix allows the urine to enter the uterus leading to endometritis, with intrauterine fluid often seen on ultrasonography.

Treatment can be divided into medical and surgical. In the author's experience, mares that exhibit minimal pooling mainly during oestrus, can be managed using lavage, prostaglandin (prior to ovulation) and oxytocin therapy to establish uterine contractility and tone. In more severe cases, surgical intervention such as urethral extension maybe necessary.[13,14] Endometrial biopsy is recommended prior to surgical intervention to determine the severity of endometrial fibrotic and inflammatory changes cause by chronic endometritis from the urine contamination.[15] Mares with advanced inflammation and fibrosis may have a low chance of carrying a foal to term despite surgical correction of urine pooling.

Cervical abnormalities

Transvaginal examination is important to determine the anatomical integrity of the cervix along with function at different stages of the oestrous cycle. Closure and structure should be evaluated manually during dioestrus for deviations and tears and estrus for proper relaxation and dilation.[10,12] Lacerations and adhesions of the cervix can have a serious impact on a mare's fertility if they are not diagnosed, treated, and managed appropriately. The treatment of choice depends on the severity and extent of the lesion. A mare that presents with minimal damage to the cervical os or an incomplete defect may be medically managed with progesterone supplementation or given the opportunity to avoid pregnancy complications by utilisation of embryo transfer or other ART procedures. Surgical correction of cervical defects should be cautioned recognition of post-surgical complications. These include resultant incomplete cervical dilation, intraluminal fluid retention, pyometra and recurrent laceration during foal that would necessitate repair.[15] When ART procedures are not an alternative option, it is recommended to proceed with surgical correction when a large component (>33 to 50%) of the cervix is compromised.[15,16]

Another commonly diagnosed cervical abnormality is failure to dilate under the influence of oestrogen during oestrus. Gentle manual massage of the cervix maybe utilised to enhance cervical relaxation. Placement of a synthetic Prostaglandin E_1 (Misoprostol®) or Buscopan® (N-Butyl Scopolamine) on the cervix, can be used to further enhance cervical relaxation.[17] A wedge resection may be indicated if intra-luminal fluid or a pyometra persists during which a dorso-lateral wedge is removed from the cervix allowing complete drainage.[18] This procedure should be limited to non-breeding mares or mares available for ART procedures.

Uterine Culture and Cytology

A uterine sample for culture and cytology is easily performed following vaginal examination with the mare in oestrus. While a double-guarded swab is routinely used for pre-breeding cultures in the Thoroughbred industry, the author prefers uterine low volume lavage for culture and cytology in the problem breeder with suspect endometritis.[3] While not routinely performed by the author, bacteriological and cytological results obtained by endometrial biopsy are considered the "gold standard" in the diagnosis of endometritis.[19]

Bacterial culture and sensitivity profiles should be used to guide treatment options. While many commercial laboratories provide both culture and PCR analysis, in-house microbiology can be beneficial to the clinician. Early results can be obtained and subtle growth, such as small colony *Streptococcus sp*. can be identified and treated appropriately. Our lab uses a combination of horse blood agar (HBA) or MacConkey agar and Brilliance Urinary Tract Infection (UTI) Agar (chromogenic agar) for routine and diagnostic uterine culture.[20,21]

Cytology may correspond with culture results and identify bacteria and inflammatory cells associated with bacterial endometritis. Cytological evaluation of any uterine sample should not only include the presence, type, and quantity of inflammatory cells, but also identify endometrial cells, red blood cells, bacteria, amount of mucous and proteinaceous materials, debris, fungal elements or yeast and anything else that is seen that would provide diagnostic potential such as urine crystals or sperm cells. The author finds cytology especially beneficial in cases of fungal or yeast infection, identification of eosinophilic inflammation that may also be associated with urine pooling or chronic pneumouterus.[22] Furthermore, identification of inflammation on cytology without growth can indicate the need for treatment focused on non-antimicrobial therapy including immune modulation.

bActivate

Endometritis in horses caused by *Streptococcus equi* subspecies *zooepidemicus* may be underdiagnosed due to a reservoir of dormant bacteria residing within the endometrium, and not as an active or ascending infection. A relatively new product, bActivate, is used as a uterine infusion capable of inducing growth of dormant *S. zooepidemicus*, which subsequently allowed detection by standard diagnostics.[23,24]

Uterine Biopsy

Endometrial biopsy sample collection should be considered in barren mares, repeat or problem breeders, and mares that have undergone pregnancy loss. The equine uterine biopsy technique and diagnostic potential has been well described with a general guide of fertility based around the Kenney-Doig grading system.[25] Category I has little or no pathologic changes with an 80-90% foaling rate. Category IIA has either: slight to moderate diffuse superficial inflammatory changes; infrequent fibrotic changes involving individual glands (<3 layers of fibrosis) or mild fibrotic nesting; lymphatic lacunae. Mares with a category IIA endometrium would be expected to carry a foal to term 50-80% of the time. Category IIB is any mare barren for 2 years or more or/with widespread diffuse, moderately severe inflammation; or more extensive fibrotic changes of individual glands with increased gland nesting. These changes lead to a 10-50% expected carry to term foaling rate. Category III has widespread, diffuse, severe inflammation with extensive fibrosis and nesting. While this predicted foaling rates provide beneficial information to the owner, the classification system

often skims over information relevant to treatment such as lymphatic stasis and vascular abnormalities that might be present in the endometrium. In our lab, endometrial biopsy samples are collected, shipped to a local laboratory for cut and mount and returned to be interpreted in-house. Epithelial cell structure, presence of oedema and inflammatory cells, glandular density and evidence of glandular distension or dilation are noted along with severity of fibrosis around glands, lymphatics and vascular structures. In addition to routine hematoxylin-eosin staining, specific histological stains can be utilised to further interpret inflammatory chronicity and degree of fibrosis. The lymphocyte T specific CD3 marker can be used as a complementary tool for the diagnosis of lymphocytic, chronic inflammation. Masson's trichrome enhances the ability to detect the incidence and severity of stromal fibrous connective tissue. The use of the trichrome stain highlights increased distribution of stromal fibrous connective tissue and the assessment of connective tissue in oestrous samples, where oedema is increased.[26-28]

Hysteroscopy

While not part of the routine breeding soundness evaluation, hysteroscopy is a valuable diagnostic tool in mares suspected to have uterine adhesions, foreign material within the uterus, persistent endometrial cups, fungal plaques or pathology of the oviductal papillae. The technique is described along with therapeutic uses such as endometrial cyst ablation and oviductal hydrotubation.[29]

Treatment: Endometritis

By identifying the underlying clinical or subclinical pathologies within the uterus, specific management and treatment is possible. In cases of endometritis, understanding and determining the underlying pathology present on culture, cytology or biopsy allows the identification of the cause of subfertility in the problem mare. Appropriate treatment recommendations should be determined by the results. Treatment options may include: antimicrobials (based on sensitivity results), anti-fungal medications, anti-inflammatories, ecbolic agents, uterine lavage, mucolytics, buffered chelators, immunomodulators, as well as medications such as aspirin or pentoxifylline, in mares with poor vascular perfusion and blocked lymphatics.[30]

Additional Therapy Considerations

Oviductal Prostaglandin E (Misoprostol) Treatment

In mares with suspect oviductal blockage due to unexplained fertility, Misoprostol therapy is a relatively safe and fast procedure compared to hydrotubation or laparoscopic therapy. Misoprostol is part of the prostaglandin E hormones, which promotes oviduct dilatation and motility. Treatment is performed by deep uterine horn application onto the oviductal papillae, using one Cytotec tablet (200 mcg of Misoprostol,) diluted in 3 mL of sterile water per horn. The procedure can be performed during the dioestrus period with mares were bred in the next cycle or in early oestrus.[31] Some mares with underlying pathology may show evidence of an inflammatory response following misoprostol therapy. As inflammation is not a sequela of treatment in the normal mare, further diagnostics should be performed on mares showing evidence of intrauterine oedema or fluid production post-treatment.[32] In the author's experience, mares with reduced uterine clearance may benefit from lavage 12 to 24 hours post misoprostol infusion.

Mycobacterium cell wall fraction (MCWF)

MCWF is an immunomodulator that has been shown to reduce bacterial growth and alter aspects of the immune response to breeding based on the stimulation of the cellular and humoral immune responses.[33,34] Following treatment with MCWE at the time of AI, one study noted uterine immunological changes in susceptible mares resulted in an endometrial immune environment similar to that found in normal mares.[35] Additionally, treatment with MCWF increased the expression of endometrial proinflammatory cytokines, in addition to decreasing the magnitude of bacterial growth in postpartum mares.[34] The author

recommends the use of MCWF (Settle®) in mares susceptible to post-mating induced endometritis.

Platelet-rich plasma (PRP)
Intrauterine infusion of platelet-rich plasma (PRP) may aid in modulation of uterine inflammation. A recent study reviewed the use of PRP in modulating inflammation and concluded intrauterine infusion may be beneficial in modulating endometrial inflammation in mares presenting with subfertility of multiple aetiologies, as well as clinically normal mares being inseminated with thawed frozen semen.[36]

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Notes

The differential diagnosis of abnormal respiratory noise in performance horses

Dr Brian Anderson
ba@bvp.com.au
Ballarat Veterinary Practice
Ballarat, Victoria
Australia

Trainers and owners commonly present horses for evaluation because of abnormal respiratory noise during exercise. Almost all abnormal respiratory noise during exercise is due to static or functional abnormalities of the upper respiratory tract (URT) and while it would be ideal if a characteristic noise was specific for each abnormality, this is not the case. While some abnormal respiratory noises are relatively specific for the underlying URT disorder the differential diagnosis of abnormal respiratory noise requires a careful history and physical examination and some or all of the following ancillary diagnostic aids – ultrasonography, radiology, resting endoscopy, exercising endoscopy with sound recording, listening to the horse exercise and audio and/or videorecording of the horse during exercise. Because abnormal respiratory noise is frequently linked to poor performance and easily identified by jockeys, drivers, or riders it is important that any treatment considered should have the aim of reducing or resolving the noise. In horses exercising at high speed (racehorses, eventers), the resolution of abnormal respiratory noise usually coincides with improved performance. In horses performing low level pursuits (e.g., show horses), abnormal respiratory noise is objectionable and diminishing the noise will be deemed a favourable result.

Normal respiratory sounds at rest and exercise

During quiet breathing in a resting horse respiratory sounds are inaudible. As airflow increases during exercise (>60 fold) vibrations in the tissues of the respiratory tract and changes in air pressure result in audible sounds. With increasing speed, sound intensity increases and because the amplitude of expiratory sound is approximately 2.5 times that of inspiratory sound, the only sound heard in normal horses exercising at a canter or a slow gallop is on expiration and has been described as a "blowing" sound. However, in unfit or overweight horses an inspiratory sound may be audible.[1] Horses described as "high blowers" produce a harsh sound on expiration of variable pitch that is associated with turbulence in the nasal vestibule caused by vibration of both the true and false nostrils.[2] The noise is not associated with respiratory impairment and usually disappears with faster exercise.

Abnormal respiratory noise at exercise

Abnormal respiratory sounds at exercise are referred to as noise and caused by static or dynamic obstructions to normal airflow. Normal airflow in the respiratory passages is largely laminar[1] and any obstruction to airflow results in turbulence causing an abnormal sound and a reduction in airflow. Abnormal noise can be present immediately on the start of exercise (usually due to a static obstruction), be intermittent or only heard at maximal exercise intensity or on pulling up.[3] In general, a louder sound represents increased turbulence due to worsening of a dynamic obstruction. Changes in pitch are thought to occur with a moving obstruction e.g., displacement of the soft palate. In exercising horses, the frequency range of vibrations is between 0.02-8kHZ[4] which is well within the range of human hearing (0.02-20kHz) therefore simply listening to a horse exercise is a valuable step. The matching of any abnormal respiratory noise to inspiration or expiration or both is important because certain conditions cause abnormal noise at different times of the breathing cycle. For example,

intermittent dorsal displacement of the soft palate (IDDSP) causes abnormal noise predominantly on expiration and arytenoid cartilage collapse due to RLN results in noise on inspiration. Breathing and locomotion are coupled in exercising horses such that there is one breath per stride (except in trotters and pacers who can vary locomotor-respiratory coupling - LRC).[5] Expiration occurs when the forefeet hit the ground, while inspiration occurs when the forefeet are being elevated. An irregular sequence of sounds can occur when the regular breathing pattern is interrupted e.g., during swallowing, when breathing is suspended for one stride. The frequency of swallowing has been shown to decrease with increasing speed, but increased swallowing has been related to soft palate dysfunction. A change in LRC may signal an upper or lower respiratory tract disorder is present. Finally, abnormal respiratory noise may be exacerbated by head position. When there is increased poll flexion particularly at lower speed gaits abnormal noise may be more apparent due to increased obstruction of airflow.[1]

Obtaining a history about abnormal respiratory noise

A complete history is vital to determine the likely cause of abnormal respiratory noise at exercise. Careful questioning can be illuminating, and some key questions include:

- How long has noise been present? Has it been slowly getting worse or has it been sudden in onset. Was the noise present before a spell or appeared after a spell.
- Was URT endoscopy normal at purchase?
- Has the horse had any URT surgery, recent respiratory tract infections or thrombophlebitis?
- What does the noise sound like? Describe the noise. Does it occur during breathing in or out or both? Has there been a change in performance?
- Does the noise start immediately or only towards the end of exercise or when pulling up? Is it continuous or intermittent, does it get louder as exercise continues, does it stop when exercise stops?
- Does it vary according to head position, does the horse slow down or stop, change lead, or throw the head in the air. Does the horse tire or drop out at the end of a race? What is the duration of post exercise recovery?
- Does anything improve the noise i.e., head position, tongue tie, dropped nose band/figure of 8 nose band?

Recently the association of owner-reported noise with findings during dynamic respiratory endoscopy (DRE) in Thoroughbred racehorses was reported.[6] When 85 horses underwent DRE due to a reported abnormal noise by owners 82% were found to have an obstruction. Characteristic owner reported noise patterns (i.e., gurgling for palate dysfunction and whistling/roaring for RLN) showed only moderate to low sensitivity for specific conditions i.e., quite a few misdiagnoses (false positives). This was due to the number of combined obstructions e.g., RLN combined with axial deviation of the aryepiglottic folds or dorsal displacement of the soft palate with various other soft tissue collapse. However, if a whistling or roaring noise was not reported by owners as being present then negative predictive value was 97% indicating laryngeal dysfunction unlikely. In summary, if an owner/trainer reports an abnormal respiratory noise then an airway obstruction of some sort is likely. However, a specific diagnosis frequently requires DRE due to many horses having multiple causes of airway obstruction and the low sensitivity for the diagnosis of specific conditions using owner reported noise patterns alone.

Listening to and recording abnormal respiratory noise at exercise

Prior to the availability of treadmill and overground DRE listening to horse's work was used to characterise abnormal respiratory noise. For racehorses (Thoroughbreds and

Standardbreds) strenuous exercise over 1600-2000 m on the racetrack is performed and the clinician is positioned close to the finish line to watch, listen, and record respiratory sounds being made. For sport horses, yearlings or horses not fit enough to gallop, lunging or riding at the canter may be enough to elicit abnormal respiratory noises.[7] A 15-m diameter circle is sufficient, and horses should be exercised hard enough to achieve 1 : 1 stride and respiration coupling. The horse should be worked in both directions for approximately 5 min.[1] The examiner should be positioned close to the horse, on the perimeter of the circle. Recording of respiratory sound has been made using a radio stethoscope attached to the trachea, unidirectional microphones within a facemask, or a flexible wand attached to the halter and incorporated into overground videoendoscopic systems. Other options include mobile phone recordings and GoPro's. Listening to these recordings can be very informative. Spectral analysis of respiratory sounds was used in the early 2000's to attempt to identify distinctive noise patterns or "voiceprints" that could be useful in the diagnosis of specific URT obstructions.[8] High frequency bands of sound (formants) during inspiration were found in horses with RLN and low frequency formants during expiration were associated with dorsal displacement of the soft palate. This technique has been used to assess the effectiveness of surgical procedures used to treat RLN. However, well described "voiceprints" have not been established for other common URT obstructions or in horses with complex URT obstructions.

Abnormal noise associated with various dynamic URT obstructions

Abnormal noise associated with the alar fold and false nostril (nasal diverticulum)

The literature is confusing on this subject. Various terms (redundant alar folds, flaccidity of the alar folds, alar fold stenosis, nasal flutter) have been used to describe a condition where the alar fold collapses into the nasal passage and the nasal diverticulum fills with air on inspiration during exercise. The noise is described as continuous, muffled rattling or vibrating or buzzing emanating from the area of the nostrils on inspiration and expiration but is most prominent on expiration.[2] The noise can be stopped by temporarily suturing the alar folds on either side to close the nasal diverticula or manually compressing the alar folds during heavy breathing. Strand et al[9] also describe nasal flutter as a distinct entity in standardbreds where there is no filling of the nasal diverticulum but severe collapse of the dorsolateral margins of the nares on inspiration causing an abnormal vibrating noise. The noise stops when the nostrils are held open. This is one condition where dynamic endoscopy may be normal and watching and listening to an affected horse work is needed to make a diagnosis.

Recurrent Laryngeal Neuropathy (RLN)

In horses affected with RLN, in addition to the normal sound heard on expiration an audible noise is usually heard during inspiration. Therefore, two noises are noted, and this biphasic sound has been compared to that heard when "sawing wood". Although the quality of the inspiratory sound has been described in a variety of ways, two different sounds are commonly heard. The first is a finer, higher pitched sound which is described as a "whistle", often heard early on in exercise or throughout exercise in less severely affected cases. The other is a coarse/harsh, lower pitched sound that is usually louder and is referred to as a "roar", hence the description "whistlers" or "roarers". The precise source of the abnormal inspiratory noise within the larynx has not been identified but air turbulence caused by air moving across an incompletely abducted or collapsed arytenoid cartilage and an open laryngeal ventricle or collapsed vocal fold are involved.[10]

Vocal cord collapse (VCC)

- Usually in conjunction with arytenoid cartilage collapse but can be a single entity.

- Collapse of one or both vocal folds cause a “whistling noise”.

Dynamic laryngeal collapse associated with poll flexion[9]

- Bilateral arytenoid and vocal fold collapse associated with head and neck flexion.
- Norwegian cold-blooded trotters, standardbreds and some sport horses.
- Loud inspiratory noise when the poll is flexed and instantly resolved when given a free rein.

Arytenoid chondropathy (static abnormality)

- Disease of one or both arytenoid cartilages resulting in abnormal enlargement is referred to as arytenoid chondropathy.
- In mild forms affecting only one side the abnormal inspiratory noise heard mimics that heard with RLN.
- Progressive enlargement of one or both cartilages can result in loud stridor (high pitched squeaking sound due to breathing through a narrow space) with nostril flaring and severe dyspnoea necessitating emergency tracheotomy.

Laryngeal dysplasia (4-BAD syndrome)

- In this condition abnormal development of structures derived from the 4th and to a lesser extent the 6th branchial arch result in a range of laryngeal cartilage and muscle anomalies.
- Abnormal inspiratory noise like RLN but varies with the underlying pathology and degree of soft tissue collapse during exercise.

Nasopharyngeal Collapse[5]

Dorsal displacement of the soft palate (DDSP)

- Gurgling/vibrating respiratory noise on expiration of low frequency (20-90 Hz) usually when horses are fatigued but can be heard at rest in horses with persistent DDSP.
- 30% of horses can be “silent displacers” and seldom is any noise reported in sport horses with DDSP.
- Vibration of the displaced palate on inspiration also occurs particularly when the mouth is open.

Palatal instability

- This is defined as dorsoventral movements of the caudal portion of the soft palate with flattening of the epiglottis against the dorsal surface of the soft palate. Considered the forerunner to DDSP.
- Soft/low grade inspiratory noise.

Rostral soft palate collapse (billowing)

- Low grade inspiratory noise.

Pharyngeal wall collapse

- Can involve the dorsal and/or lateral pharyngeal walls or can be circumferential
- Abnormal inspiratory noise like arytenoid cartilage collapse.
- In severe cases horses are described as “making a racket”.

- Highest prevalence is in sport horses and associated with head/neck flexion
- Progressive collapse can occur with fatigue but in severely affected animals the noise is immediate even at low speed.

Ventro-medial luxation of the apex of the corniculate process of the arytenoid cartilage (VLAC)

- In this condition collapse of the apex of one or both corniculate processes of the arytenoid cartilage occurs but the ventral part of the corniculate process remains abducted.
- Often in conjunction with VCC and axial deviation of the aryepiglottic folds (ADAF).
- Inspiratory noise of variable intensity and pitch – similar to RLN.

Axial deviation of the aryepiglottic folds (ADAF)

- The aryepiglottic folds deviate axially into the *rima glottidis* during inspiration resulting in an inspiratory noise described as a whistle.
- Graded mild, moderate, or severe.

Epiglottic entrapment

- Can be an incidental finding with no reported abnormal respiratory noise.
- Can cause a loud vibrant noise heard on inspiration and expiration. On expiration the entrapped tissue can balloon with air resulting in expiratory obstruction.
- Affected horses often described as "thick winded".
- Can cause DDSP and therefore an expiratory gurgle or vibration may be heard.

Epiglottic retroversion

- Rare URT obstruction which results when the epiglottis becomes retroverted dorsally and caudally to obscure the rima glottidis.
- Intermittent harsh inspiratory or "grunting noise" is described.

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Notes

Ultrasonography of uterine abnormalities

A.O. McKinnon and PM McCue

With ultrasonography, the uterus can be examined non-invasively to evaluate normal reproductive anatomy, detect pathologic changes, and monitor therapeutic regimen(s). The most common forms of uterine pathology detected by ultrasonography in the non-pregnant mare are accumulations of intrauterine fluid, inflammatory changes, uterine cysts and air. Less commonly, foreign bodies, foetal remnants, debris, abscessation, persistent endometrial cups, and neoplastic conditions are observed. Applications of ultrasonography to peri-parturient conditions have demonstrated its usefulness in examining mares with problems such as placentitis and post-partum haemorrhage. Each year, we see pregnant mares presented for either reproductive evaluation or breeding, that have mistakenly been diagnosed as not pregnant during the previous breeding season. The reasons for this are varied, but commonly this occurs when the only pregnancy test is completed at day 13-15 post breeding and the mare is not examined again. Ultrasonographical evaluation of the uterus will identify the pregnancy and will help avoid an inappropriate internal evaluation and iatrogenic abortion.

Inflammation of the uterus

Inflammatory changes in the mares' uterus are recognised by the presence of uterine fluid (see below) and/or markedly increased oedema of the endometrium (endometrial folds). During the normal oestrus cycle, endometrial oedema is detected as a normal development and follows the pattern of oestrogen production and thus similar to oestrogen, uterine oedema peaks approximately 24 hours prior to ovulation (Hayes et al., 1985; McKinnon et al., 1987b). Oedema of the endometrium is graded from 1 to 3 as a normal development during the oestrus cycle(McKinnon et al., 1987b). Typically oedema is minimal or absent close to ovulation, and when multiple frequent examinations are performed, a change from grade 3 to grade 1 or no oedema is quite useful to predict impending ovulation(McKinnon et al., 1987b; Samper, 1997). When oedema is detected in mares that are in dioestrus or pregnant, or when oedema is marked (> grade 3) it is a sign of inflammation (Figures 1a-d). Some uterine oedema of the dorsal surface of the uterus in pregnant mares is normal between days 17 and 30, and is associated with increased tone, fixation and local production of oestrogens from the conceptus(Griffin et al., 1993). Marked oedema (grade 4) can mask the presence of smaller amounts of intrauterine fluid. On occasion, systemic administration of 20-30mg of dexamethasone phosphate can result in a spectacular reduction of oedema from grade 4 to grade 1 or even to non-detectable within an hour.

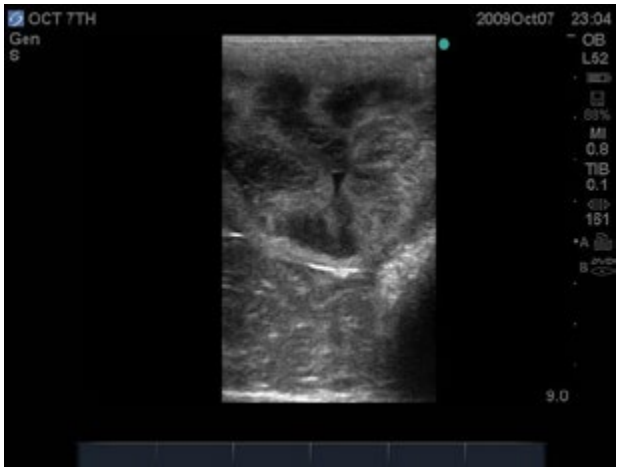

Figure 1a. Grade 4 oedema (endometrial folds) of the uterine horn with a small amount of grade 4 fluid

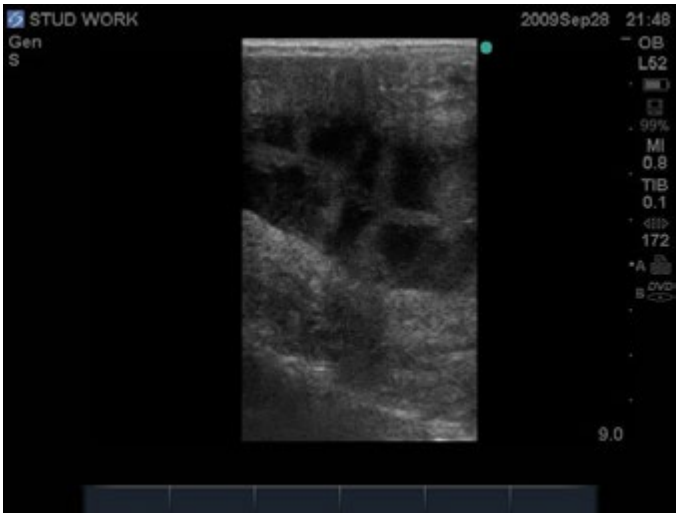

Figure 1b. Grade 4 oedema of the uterine body

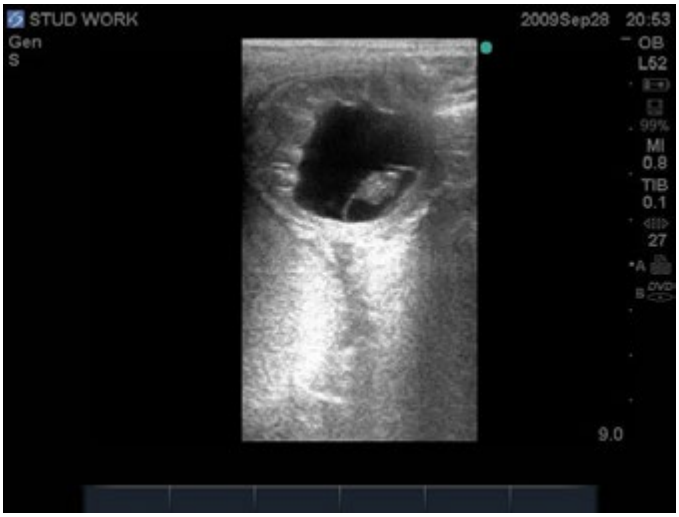

Figure 1c. Oedema of the uterus can be normal in pregnant mares between fixation and 30 days and is usually on visible dorsally. After this time the rapid expansion of the fluid compartments of the pregnancy are associated with progressive thinning of the uterus and loss of any folding.

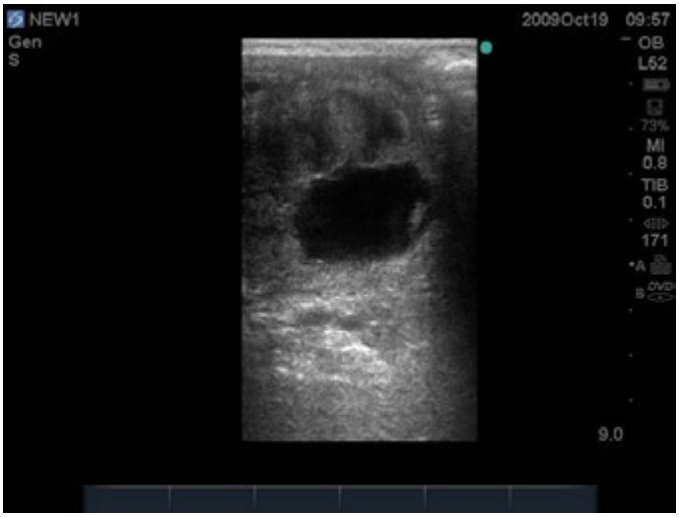

Figure 1d. Oedema of this pregnant uterus is more pronounced than normal and involves both dorsal and ventral uterine surfaces and is likely an abnormality.

Uterine fluid

Ultrasonography is extremely valuable for estimating quantity and quality of fluid in the uterine lumen. Palpation of the uterus *per rectum* is only accurate when quantity of intrauterine fluid is large (> 100 ml) and(or) when uterine tonicity changes. Confirmation of smaller volumes of intrauterine fluid, without invasive techniques such as lavage and cytological analysis, was difficult until direct, non-invasive visualisation was made possible with ultrasonography. Volumes of fluid within the uterine lumen are estimated (graded as very small, small, medium, large or excessive) with ultrasonography and quality is graded from 1 to 4 according to degree of echogenicity(McKinnon et al., 1988)(Figures 2a-d). Degree of echogenicity is related to the amount of debris or white blood cell infiltration into the fluid. Grade 1 fluid has large numbers of neutrophils, and grade 4 has very few neutrophils when echogenicity is related to inflammation. Some other forms of uterine fluid detected are urine, blood, mucus, biofilm and a transudate associated with uterine oedema (Figures 2e-l).

Pyometra refers to an accumulation of purulent material within the lumen of the uterus and typically is identified by ultrasonography as a grossly enlarged uterus, filled with echogenic fluid and a thickened uterine wall (Figures 2m). The degree of uterine thickening may be masked in instances of excessive fluid accumulation and distension but is appreciated when the uterus is drained. Large accumulations of fluid may be associated with physical obstruction or functional defects of the cervix. An intermittent vaginal discharge may be evident if the cervix is patent. Affected mares are usually not systemically ill and clinical signs such as septicaemia, depression or anorexia are absent. Haematologic changes are minimal when present. Endometrial damage due to chronic inflammation may result in failure of prostaglandin secretion and persistence of the corpus luteum. Diagnosis is based on palpation and ultrasonography of the reproductive tract *per rectum*, and speculum examination, as well as culture and cytology if the cervix is patent. The prognosis for future fertility is poor and, in most instances, treatment is aimed at salvaging the mare for non-breeding purposes.

Urine in the uterus may result from vesico-vaginal reflux and subsequent flow forward through an open cervix and may also occur from contamination by a stallion that has urospermia. Urospermia results in an immediate decline in spermatozoal motility(Griggers et al., 2001) and thus impact fertility. In addition, urine in the uterus (Figures 2e) creates a chemical endometritis, and if it persists will interfere with maintenance of pregnancy. Urine in the mares' uterus is identified by hyperechoic accumulations most commonly at the corpus corneal junction that do not swirl easily when balloted with the probe. The echogenicity of the urine is thought to be from a combination of crystals and mucus. Urine in the uterus should be removed with voluminous lavage.

Observations on the quality and quantity of uterine fluid have been used to assess efficacy of various therapeutic procedures on individual animals treated for naturally occurring endometritis(Causey, 2006; McKinnon and Voss, 1993; Pycock, 1994; Pycock and Newcombe, 1996). Experiments(Adams et al., 1987; Ginther et al., 1985; Knutti et al., 2000; Malschitzky et al., 2002; Malschitzky et al., 2003a; Malschitzky et al., 2003b; McKinnon et al., 1987a; McKinnon et al., 1988; Nikolakopoulos and Watson, 1999; Ozgen et al., 2002; Pycock, 1994; Pycock and Newcombe, 1996; Schilela et al., 2001; Troedsson, 1997; Watson et al., 2001) have been conducted to determine the relationship of intrauterine fluid to fertility. Some areas where recognition of intrauterine fluid may be of particular value are 1) evaluation of the post-partum mare, 2) diagnosis and treatment of persistent mating induced endometritis, 3) diagnosis and treatment of infectious endometritis and 4) effects on pregnancy rate and early embryonic death (EED).

Ultrasonography can also be valuable as a diagnostic aid during a therapeutic uterine lavage procedure when fluid recovery is difficult (Figure 2n). A majority of cases involve older mares in which the most dependent part of the uterus is located cranial and ventral to the pelvic brim. The location of the fluid pocket is noted, the cuff of the lavage catheter is deflated, and the catheter is advanced into the pool of accumulated fluid. In some instances, it may be necessary to infuse approximately 500 mls of lavage fluid (i.e. Lactated Ringer's solution) to distend the uterus prior to advancing the catheter.

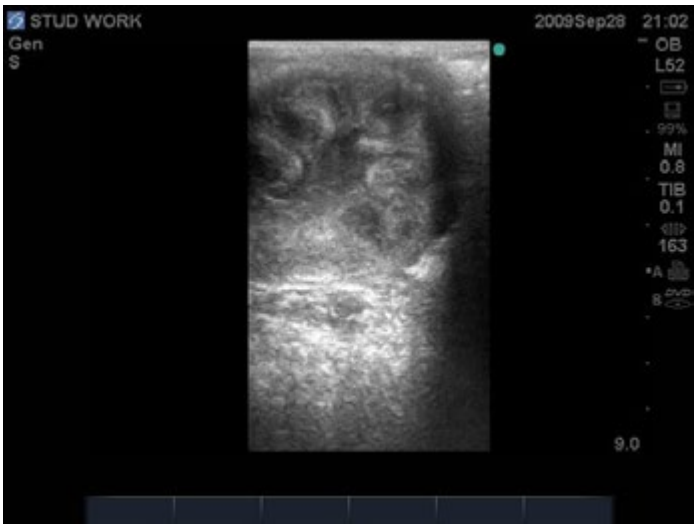

Figure 2a. Grade 1 uterine fluid

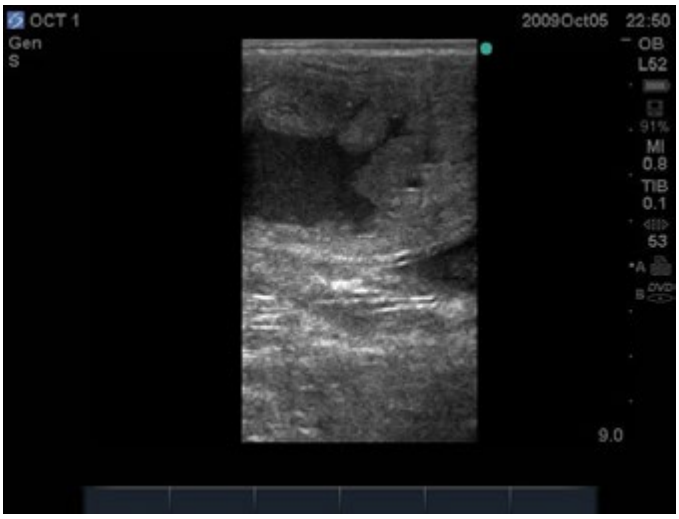

Figure 2b. Grade 2 uterine fluid

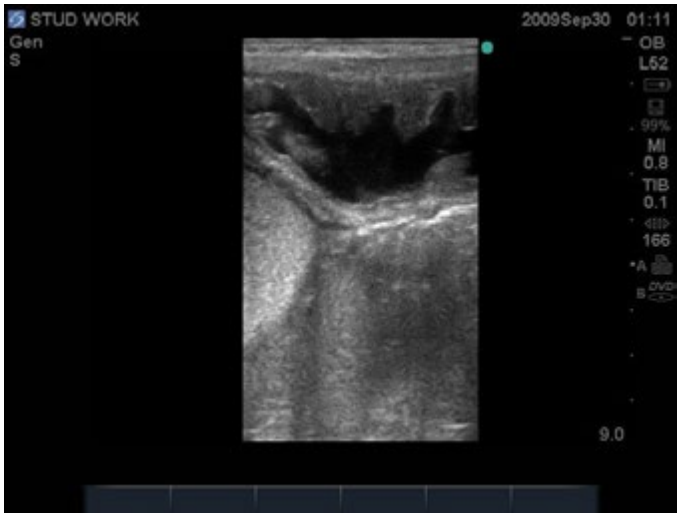

Figure 2c. Grade 3 uterine fluid

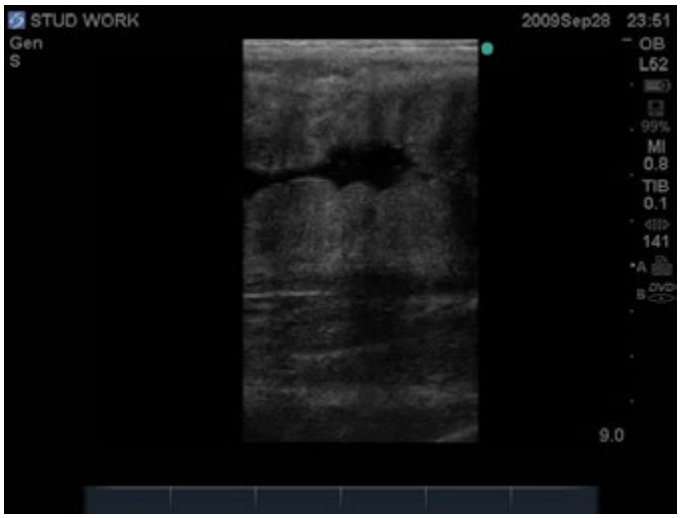

Figure 2d. Grade 4 uterine fluid

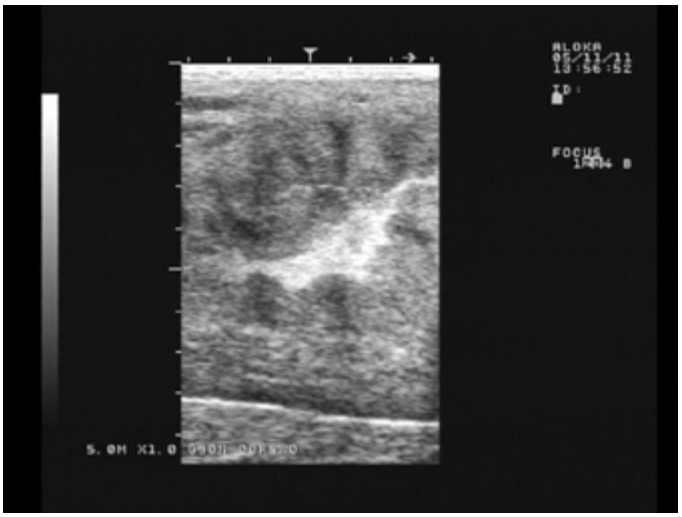

Figure 2e. Urine in the uterus

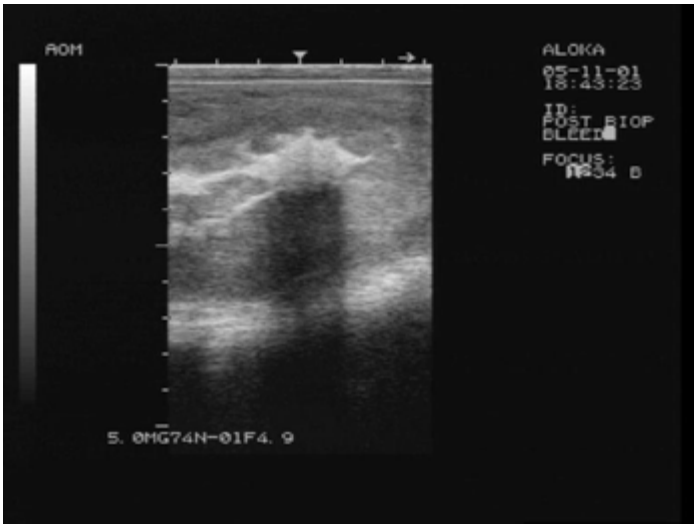

Figure 2f. Blood in the uterus after a uterine biopsy

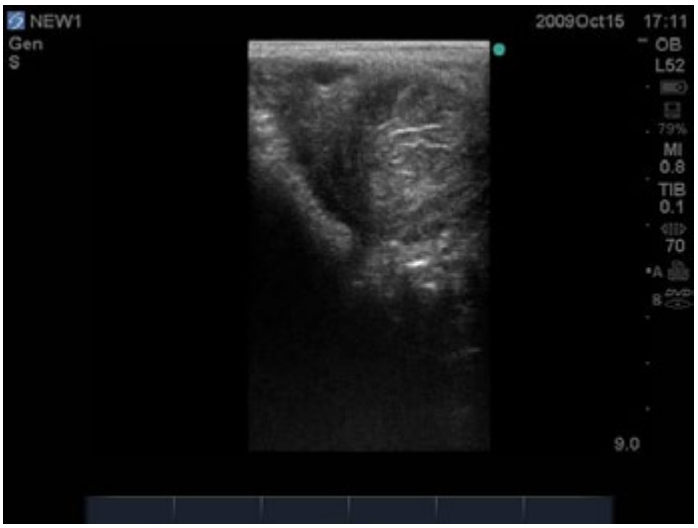

Figure 2g. Biofilm layer in uterine horn

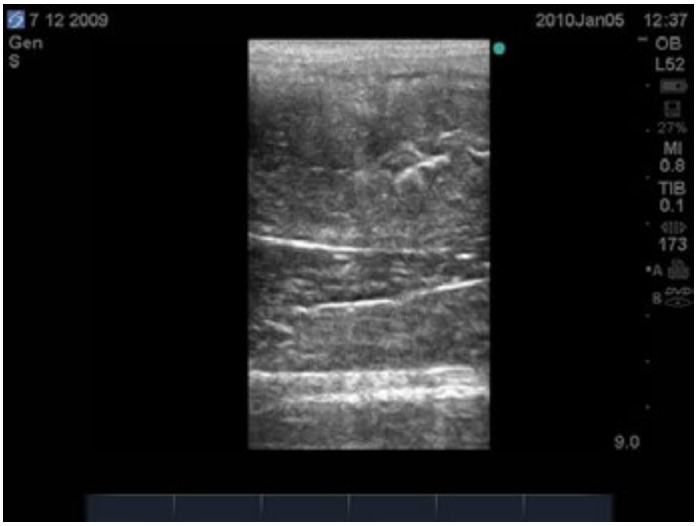

Figure 2h. Biofilm layer in uterine body

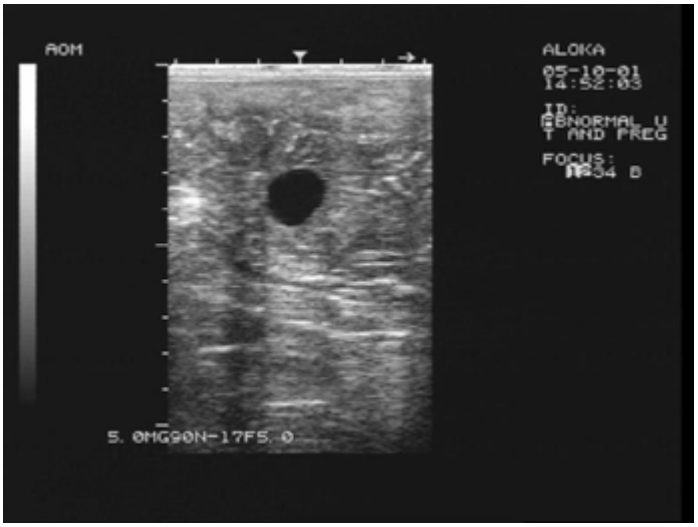

Figure 2i. Biofilm layer in a pregnant uterus

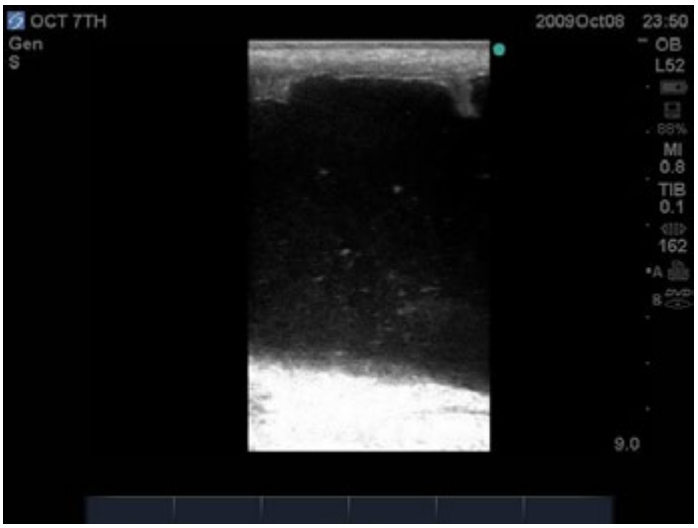

Figure 2j. Mucous that had very little segmented neutrophils on high power

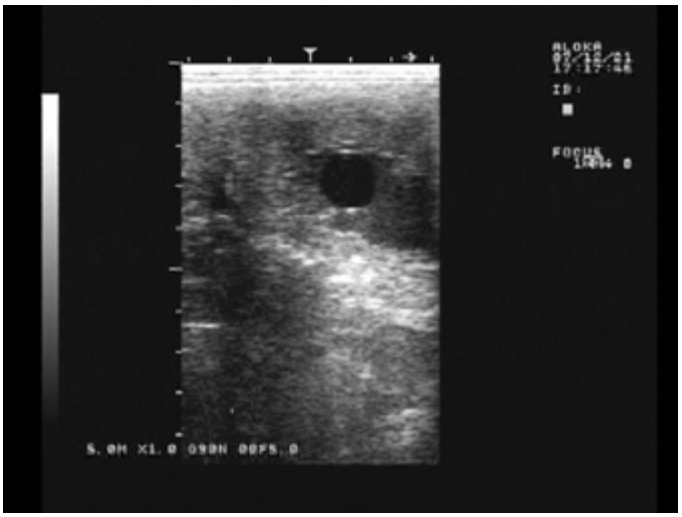

Figure 2k. Pregnancy surrounded by grade 2 uterine fluid

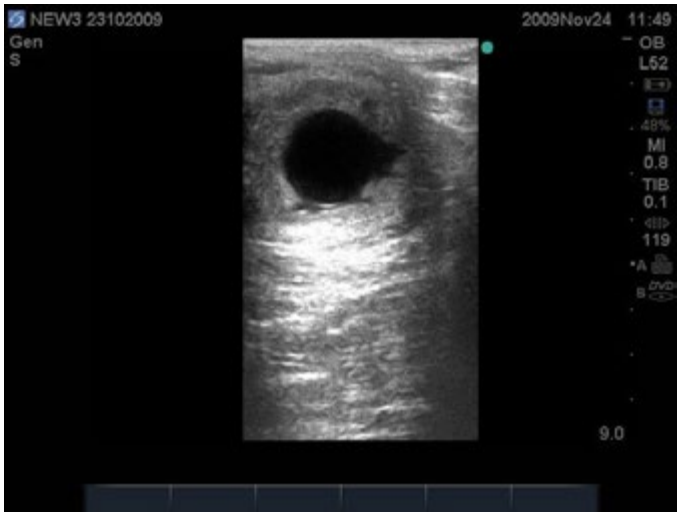

Figure 2l. Pregnancy with a small amount of grade 4 uterine fluid surrounding it

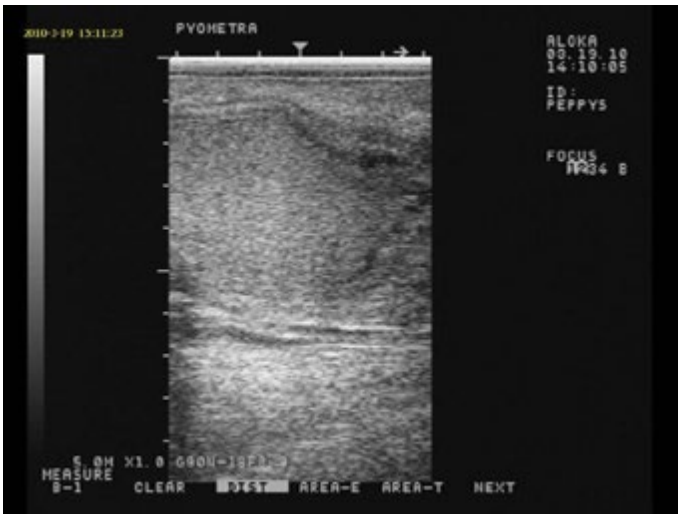

Figure 2m Pyometra can be detected by ultrasonography as a grossly enlarged uterus, filled with echogenic fluid and a thickened uterine wall

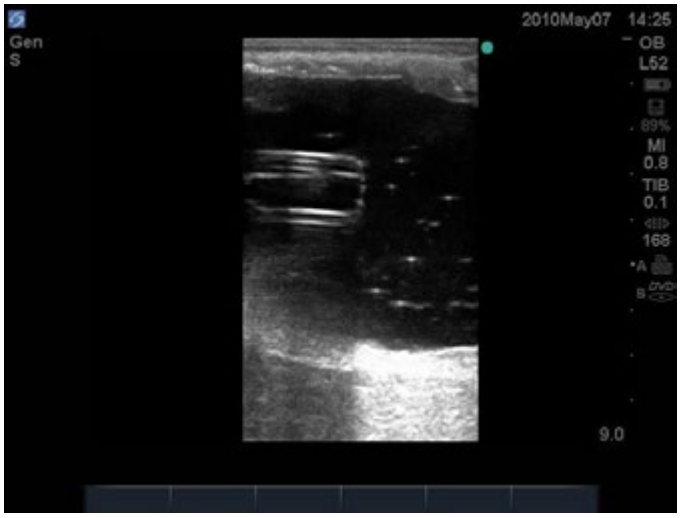

Figure 2n. Catheter tip visible in fluid in the uterus

Ultrasonographic studies of the uterus after parturition

In the equine industry, economic incentives influence breeders to attempt a foaling interval of 12 months or less. This commonly necessitates breeding of mares during the first post-partum oestrus. However, fertility has been reported to be lower in mares bred during the first post-partum ovulatory period compared with mares bred during subsequent cycles(Ishii et al., 2001; Lowis and Hyland, 1991; Merkt and Gunzel, 1979; Sullivan et al., 1975; Woods et al., 1987), and early embryonic death has been reported higher for mares bred at this time(Lieux, 1980; Lowis and Hyland, 1991; Merkt and Gunzel, 1979; Platt, 1973). This decreased fertility may be due to failure of elimination of microbes during uterine involution, introduction of microbes at breeding, or inadequate endometrial repair relative to when the embryo enters the uterus. In addition, presence of uterine fluid during oestrus and dioestrus has been shown to reduce fertility of mares(Adams et al., 1987; McKinnon et al., 1987a; McKinnon et al., 1988).

A study (McKinnon et al., 1988) was conducted to evaluate two hypotheses: 1) uterine involution and fluid accumulation could be effectively monitored with ultrasonography and used to predict fertility of mares bred during the first post-partum ovulatory cycle, and 2) delaying ovulation with a progestin would result in improved pregnancy rates in mares bred during the first post-partum ovulatory period. The previously gravid horn was larger than the non-gravid horn for a mean of 21 days (range 15 to 25) after parturition. Uterine involution was most obvious at the corpus cornual junction. When the results of three ultrasonographic scans performed over a 5-day period were similar, the uterus was considered to be involuted. On the average, uterine involution was completed by day 23 (range 13 to 29). Quantity and quality of uterine fluid were not affected by progestin treatment. Number of mares with detectable uterine fluid decreased after day 5 post-partum. Uterine fluid generally decreased in quantity and improved in quality between days 3 and day 15. Fewer ($P < 0.005$) mares became pregnant when uterine fluid was present during the first post-partum ovulatory period (3 of 9, 33%), compared to when no fluid was detected (26 of 31, 84%). Mares with uterine fluid during breeding did not have appreciably larger uterine dimensions, compared with those mares not having fluid. There was no relationship between uterine size on day of ovulation and pregnancy rate. Ovulation was delayed, and pregnancy rates improved in progestin-treated mares. More ($P < 0.05$) mares became pregnant (23/28, 82%) when they ovulated after day 15, in the first post-partum ovulatory period, than mares that ovulated before day 15 (6/12, 50%). This study demonstrated that ultrasonography was useful in detecting mares with post-partum uterine fluid and can be used to aid in determining whether a mare should be bred and/or treated during the first post-partum ovulatory period(McKinnon et al., 1988).

Uterine fluid may be spermicidal during oestrus and an excellent medium to support bacterial proliferation at any time. In addition, fluid may indicate poor uterine involution. When fluid is present during dioestrus, it may be associated with infectious endometritis and be associated with premature luteolysis or early embryonic death(Adams et al., 1987). The quantity of uterine fluid during the first post-partum ovulatory period appeared to be related to stage of uterine involution, and was reduced or eliminated by delaying the ovulatory period with progestins(McKinnon et al., 1988). Progestin treatment not only allowed time for elimination of uterine fluid before the first post-partum ovulation, but it also significantly delayed the first post-partum ovulation. Progestagen treatment has been demonstrated to delay onset of the first post-partum ovulatory period without affecting the rate of uterine involution(Loy et al., 1982; McKinnon et al., 1988; Pope et al., 1979; Sexton and Bristol, 1985). Long-term progestin administration to normal, cycling mares has not been shown to adversely affect fertility (Squires et al., 1983). However, treatment with progestins will affect uterine defense mechanisms (Evans et al., 1986; Winter, 1982), and thus care is recommended before prolonged progestin treatment is administered to post-partum mares or mares susceptible to infection. Since there were decreased pregnancy rates associated with uterine fluid, and increased pregnancy rates

as ovulation was delayed, it was suggested both techniques could be used to manipulate breeding strategies and improve pregnancy rates from normal mares bred during the first post-partum ovulatory period(McKinnon et al., 1988).

Further studies on post-partum intrauterine fluid have demonstrated that mares that have uterine fluid accumulation during foal-heat also present a larger incidence of post-breeding fluid accumulation, and that treatments of mares with oxytocin did not decrease the incidence of post-breeding fluid accumulation or improve pregnancy rates. Uterine flushes performed 36-48 h after breeding in mares with uterine fluid after foal-heat ovulation also did not improve pregnancy rates (Schilela et al., 2001). In another study, early embryonic death rates were significantly greater (p=0.015) in mares with intrauterine fluid accumulation during foal-heat (30.5%) than in mares without fluid accumulation (11.1%)(Malschitzky et al., 2003a).

A study from Australia clearly demonstrated the generally held contention that fertility is poorer and embryonic death greater when mares are bred on foal heat compared to the next cycle that was induced by prostaglandin(Lowis and Hyland, 1991). First cycle pregnancy rates were 47.9% compared with 55.2% and overall pregnancy rates at the end of the breeding season were 83.3% and 89.7% respectively. Pregnancy losses before day 45 were 10% and 3.9% and after day 45 were 9.3% and 3.6% respectively(Lowis and Hyland, 1991).

Results of clinical, microbiological and hormonal examinations performed during the puerperal period provide useful information when deciding whether to use the foal heat for breeding or to initiate a therapy during this period of time(Glatzel and Belz, 1995). Interpretation of results of lavage or other post-partum treatments(Blanchard et al., 1989; McCue and Hughes, 1990) should be viewed with caution when all mares are treated in different groups because most mares do not need treatment in the immediate post-partum period and our aims should be to identify those that do need treatment.

Foal heat breeding strategies

Mares can be considered for a foal heat breeding if they had a normal foaling (i.e., no dystocia), foetal membranes were not retained, and there was no prolonged discharge of fluid (lochia) from the uterus.

1) All mares are examined at day 2-3 post foaling. By that time, problems that may have occurred during foaling will be identified. We believe that this exam is critical if we are to prevent mares from becoming “problem broodmares”. Each year we identify mares that if inappropriately treated could become long term disasters. Approximately 5% of mares will have placental tags present (despite excellent staff management) and another 5% will have problems with metritis and uterine damage. Many (15%) will have delayed involution suggested by increased size of the uterus and volume of fluids present. Such mares are treated aggressively with large volume uterine lavage and ecbolics, antibiotics and anti-inflammatories and monitored closely.

2) Post-partum mares may be treated with an infusion of antibiotics in lactated Ringer’s solution (LRS) 2-3 days after foaling. This may help some individual mares and may prevent iatrogenic contamination.

3) Mares with no abnormalities identified are scheduled to be re-examined on Day 9-10 post foaling. Mares will not be bred any earlier than Day 10. On Day 9-10 if the mare has ovulated, she is scheduled for $PGF_{2\alpha}$ administration in 6 days. If no uterine fluid is detected and uterine tone is good the mare may be scheduled to be bred. If there is a question, the mare is treated and or re-examined according to follicle size and presence or absence of uterine fluid. It would be rare for a foal heat service to take

precedence over a non foal heat service with a stallion with a busy book (>150 mares per season with natural service).

4) Breeding mares on foal heat with intra-luminal fluid accumulations is avoided. These mares are treated and recycled.
5) Lastly there are two more guidelines that we adhere to. Firstly, we only breed mares on foal heat that are young and reproductively healthy (i.e., <12 YO and with their first, second or third foal) and secondly, mares that have been confined without exercise (i.e., lunging) due to foal problems such as angular limb deformities, are not bred on foal heat regardless.

Diagnosis of endometritis

There are numerous techniques available to diagnose endometritis. However, no technique is completely reliable. The common, currently accepted techniques are: 1) rectal palpation, 2) vaginal-speculum examination, 3) bacterial culture of uterine contents, 4) cytological examination of uterine contents, 5) endometrial biopsy and 6) ultrasonography.

A study was conducted (McKinnon et al., 1987a) to examine the efficacy of individual diagnostic techniques to predict endometritis. The experimental model involved sixty intact mares that were treated with progesterone for 31 days, and 50 were inoculated with a broth of *Streptococcus zooepidemicus.* Reproductive evaluations were performed the day progesterone treatment began, 13 days after progesterone treatment began, and two and seven days after various therapeutic regimens. Thus, for the 60 experimental mares, there were 240 individual examinations for endometritis. The following criteria were used to assess degree of endometritis: 1) ultrasonographic detection of intraluminal fluid accumulation, 2) vaginal speculum examination, 3) cytological examination of uterine contents, 4) culture of uterine contents and 5) acute and chronic inflammatory changes detected by endometrial biopsy. Each individual parameter was assigned a score from 0 to 3. The total index score of 0 to 18 calculated from summation of each component of reproductive evaluation was used as a standard to determine if the mare had endometritis. To determine the efficacy of each individual diagnostic test, a predictive value for each test (for each score >0) was calculated against a positive diagnosis of endometritis obtained from the total index score (at two different levels diagnosis.

Results from this experimental model indicated support for two conclusions: 1) bacterial culture was not as accurate in predicting endometritis as other diagnostic tests, and 2) ultrasonographic detection of any uterine fluid accumulation was an accurate indicator of endometritis(McKinnon et al., 1987a).

This study had several limitations: 1) The model of endometritis was progesterone-dependent and may not accurately reflect the naturally occurring condition of endometritis. A progesterone-dependent model may result in increased bacterial proliferation, decreased neutrophil numbers and function and decreased drainage of uterine contents. 2) Without an independent standard to determine endometritis (i.e., used to compare the individual tests against a positive or negative diagnosis of endometritis), the predictive value in this study may have more accurately reflected each individual component's influence on the total index score. Despite these drawbacks, the study demonstrated the usefulness of ultrasound to diagnose endometritis. The application of ultrasonography becomes even more apparent when considering that it is non-invasive. All 10 uninoculated control mares developed endometritis most likely induced in progesterone-treated mares from repeated invasion into the uterus to collect data during the study. It can also be concluded that despite accepted hygienic techniques, invasion of the uterus in progesterone-dominated mares resulted in endometritis. Other authors have made similar associations(Hinrichs et al., 1992).

Breeding the problem mare

Prebreeding assessment

Ultrasonography is fundamental to any problem mare breeding program(LeBlanc, 2008a; LeBlanc, 2008b; McKinnon and Voss, 1993). The primary goal of the pre-breeding assessment period is to determine if a mare has a uterine environment capable of supporting spermatozoa long enough so they can reach the oviduct in a condition capable of initiating fertilisation. It only takes a few hours for spermatozoa to pass through the utero-tubular junction (UTJ) and remain relatively free from toxic products in the uterus. However, fluid inflammatory products, when mixed with spermatozoa, cause an immediate decline in spermatozoal motility that is proportional to the amount of inflammatory products. Also, addition of uterine fluid of grades 1 to 2 to spermatozoa prior to breeding mares by AI resulted in a decreased embryo recovery ($P < 0.05$)(Squires et al., 1989). Addition of an extender to the uterine fluid prior to addition of spermatozoa was reported to arrest the decline in spermatozoal motility "in vitro".(Squires et al., 1989). However, it appears that for best fertility, removal of inflammatory products from the uterus prior to introduction of spermatozoa is the most logical approach. To meet these objectives, mares are examined in early estrous. Ultrasonographic identification of uterine fluid and culture and sensitivity results are used to determine whether uterine lavage, ecbolic agents such as oxytocin and/or cloprostenol (Combs et al., 1996; Leblanc et al., 1994; LeBlanc, 1997; McKinnon and Voss, 1993; Pycock, 1994; Pycock and Newcombe, 1996), local antibiotics or a combination of all of the above are necessary to obtain a uterus free from inflammatory products at the time of breeding. It is an inappropriate use of time and finances to breed mares destined to return to oestrus, thus mares with abnormal uterine fluid accumulations detected at the time of breeding may have to be recycled as soon as $PGF_{2\alpha}$ can cause luteolysis.
Lavage and retrieval of lactated Ringer's solution from the uterus of mares immediately prior to breeding has been shown not to be detrimental(Vanderwall and Woods, 2003) and should be considered in cases where considerable uterine fluid is detected prior to breeding and recycling the mare is not an option.

Breeding management

If the uterine environment is prepared properly at the time of breeding, then our goal is to prevent contamination at, or immediately after breeding. Clearly, breeding contamination is more easily controlled with AI than with natural service. However, both techniques result in introduction of bacteria. Research in this area was originally reported by Kenney and colleagues in 1975(Kenney et al., 1975). They demonstrated that hygienically collected semen from "non-infected" stallions, contained numerous types of aerobic bacteria and fungi. The total number of aerobic microorganisms in each of eight ejaculations from five stallions collected ranged from 0.09 to 36 million. However, addition of raw semen to nonfat dry milk seminal extenders containing either penicillin-streptomycin (1,500 IU/ml and 1500 μg/ml respectively) or gentamicin sulfate (1 mg/ml) resulted in no growth on any of the subcultures from treated samples, including zero time which was after about 5 minutes of exposure. There was heavy growth in all subcultures from raw semen(Kenney et al., 1975). Aerobic bacteria commonly isolated from the urethra, semen and prepuce of stallions are *E. coli* and other coliforms, *Pseudomonas aeruginosa*, beta haemolytic and non-haemolytic streptococci (*S. zooepidemicus*), Klebsiella sp., haemolytic and non-haemolytic staphylococci, *Proteus* sp., and Corynebacterium. Further experimentation has demonstrated effective elimination of bacteria without affecting motility using seminal extenders containing either penicillin-gentamicin or polymyxin B sulfate (1,000 IU/ml). Thus, it appears that addition of raw semen to appropriate antibiotic-containing seminal extenders is one method of ensuring minimal contamination at the time of breeding(Arriola and Foote, 1982; Blanchard et al., 1987; Danek et al., 1994; Padilla and Foote, 1991; Timoney et al., 1979; Vaillancourt et al., 1993). It is important to remember that some antibiotics affect spermatozoal motility at high concentrations and may

adversely affect fertility(Jasko et al., 1993; Pickett et al., 1987). Currently we favour the use of ticarcillin, timentin, or amikacin at 1 mg/ml in extender preparations.

Artificial insemination

If possible, mares are inseminated without disturbing reproductive surgeries such as the Caslick procedure. The perineum is diligently cleaned and dried as previously described and 500 x 10^6 progressively motile spermatozoa (PMS) mixed with appropriate antibiotic-containing extender are inseminated. Proper technique to ensure cleanliness of the stallion and collection equipment is important. To ensure the antibiotics have had adequate time to eliminate bacterial growth, it is best to allow at least 15 minutes at 37^{0}C prior to insemination. If a longer interval is required, extended semen may be cooled to 20^{0}C and stored for up to 12 hours(Francl et al., 1987) and often considerably longer at 4^{0}C(Douglas-Hamilton et al., 1984). To reduce contaminating organisms to an absolute minimum, a method was devised(Kenney et al., 1975) to "wash" spermatozoa by dilution with an antibiotic-containing extender, followed by centrifugation (300 G) to produce a "soft" pellet, decantation of the supernatant and resuspension of the resulting pellet in fresh, warm extender. This technique has the added advantage of removing much of the seminal plasma which reduces motility after prolonged incubation with minimal damage to spermatozoa(Pickett et al., 1975), however it is time-consuming and may not always be necessary.

Natural service

Mares and stallions to be bred by natural service should be well cleaned. It is wise to avoid strong disinfectants that may cause overgrowth of potentially pathogenic bacteria after prolonged use. A technique of minimizing contamination by prebreeding infusion of 100 to 300 ml of antibiotic-enriched seminal extender has been described. This technique has advantages; however, caution should be advised. For maximum reproductive efficiency, 500 x 10^6 PMS should be deposited into the reproductive tract. However, lower spermatozoal numbers are quite effective in highly fertile stallions. This information was derived from AI with small volumes of semen or semen plus extender. Recent information has indicated that spermatozoal concentration or volume of inseminate may be important factors in fertility. When mares were bred with 250 x 10^6 PMS in 100 ml of extender, embryo recovery rate was significantly depressed (13.6%, $P < 0.001$) compared to mares bred with these same spermatozoal numbers from the same ejaculates in 10 ml of extender (70.6%)(Squires et al., 1989). This finding becomes important when mares are bred by natural service to stallions that, due to frequent breedings, may have low spermatozoal numbers in normal ejaculate volumes (30 to 150 ml) and thus spermatozoal concentrations lower than the threshold for normal fertility.

Our approach for mares bred by natural service is to use ultrasonographic detection of quality and quantity of fluid combined with culture and sensitivity results to determine optimum treatments. For instance, if mares have a large volume of fluid detected, then voluminous lavage of the uterus with a physiological solution such as Lactated Ringer's Solution (LRS)Dulbecco's phosphate buffered saline that is not expected to be detrimental to spermatozoal survival is instituted immediately prior to breeding. Increased temperature (41-45^{0}C) of infused fluids or oxytocin may aid in evacuation of uterine contents. The aim is to clear the uterus of inflammatory products and enable spermatozoa to have a relatively safe passage into the oviduct before further inflammatory products are released. Lavage and retrieval of LRS from the uterus of mares immediately prior to breeding has been shown not to be detrimental(Vanderwall and Woods, 2003).

If small quantities of uterine fluid are detected, then intrauterine antibiotic solutions are infused prebreeding (> 12 hours). If uterine fluid is detected at the time of scheduled breeding, depending on the type and volume of fluid, the mare is either recycled, a small

volume of antibiotic containing extender (< 50 ml) is infused immediately prebreeding or oxytocin is administered intravenously and the mare re-examined in 1-2 hours(LeBlanc et al., 1994; Neuwirth et al., 1995). When organizing timing of breeding the problem mare, much effort is directed toward trying to breed only once, just prior to ovulation (< 12 hours). Induction of ovulation with human chorionic gonadotropin (hCG) or deslorelin acetate is routine (see chapter Induction of Ovulation-McKinnon and McCue). Mares are bred regardless of the number of preovulatory follicles and multiple ovulations are actively encouraged. There are few effective, commercially available drugs to increase the number of ovulations per cycle. Recombinant follicle stimulating hormone (eFSH) (see chapter superovulation Squires), although effective, is very expensive for equine use and is no longer commercially available. Immunization against an inhibin subunit has been demonstrated to be effective in increasing ovulation rates in mares(McCue et al., 1992; McKinnon et al., 1992). Conception rates are proportional to ovulation rates(Squires et al., 1987), thus treatment by immunization against inhibin should improve pregnancy rates in normal and subfertile mares and in mares bred to subfertile stallions. With intensive reproductive management, multiple pregnancies when diagnosed early present little difficulty in reduction to a singleton (see chapter on Management of twins -McKinnon).

Post breeding management

The aim of therapies in the immediate period after breeding is to: a) reduce infection and inflammation to create a uterine environment capable of supporting pregnancy, and b) prevent further contamination. Spermatozoa are safely in the oviduct within 4 hours of breeding and are protected from inflammatory products in the uterus and/ or uterine treatments by the utero-tubular junction. The embryo will not be transported from the oviduct through the UTJ until around 5.5 to 6 days after ovulation, however because most intrauterine therapies have an attendant degree of inflammatory response, and to allow time for foreign material to be expelled or absorbed, no treatments are administered after day 4 post- ovulation. The cervix begins to exhibit increasing tone and improves as a barrier to infection within 2 days of ovulation, although it remains more relaxed when inflammation of the reproductive tract is present. In addition, the corpus luteum remains resistant to $PGF_{2\alpha}$ released from local inflammatory responses until at least day 5 after ovulation. Approximately 12-24 hours after breeding, the uterine response to breeding and contamination is assessed by ultrasonography. If fluid is absent, then either no treatments are administered or treatments are administered as indicated daily for 2 additional days. If small amounts of fluid are detected, the same treatment is applied after first lavaging the uterus with LRS. Saline is quite an irritant, especially with low pH(Pascoe et al., 1989). Usually, 1-2 L are necessary to remove inflammatory products. When large amounts of fluid are detected, the fluid is recultured and removed by voluminous lavage until returning fluid is free from debris. Oxytocin may be added (20 to 40 IU/L) to flushing solutions and/or administered intravenously. Oxytocin increases myometrial contractions in estrogen dominated reproductive tracts(Jones et al., 1991; Liu et al., 1991) and aids in expulsion of material. Administration of oxytocin causes uterine contractions for 30 to 45 minutes. Alternatively, cloprostenol may be administered to mares that do not evacuate their uterine fluid in response to oxytocin. Uterine contractions following cloprostenol administration last for 2 to 4 hours(LeBlanc, 2008a). From the preceding discussion it should be clear how fundamental ultrasonography is to good management decisions when breeding mares.

Effect of intrauterine fluid on pregnancy rate and early embryonic death

Mares with intrauterine fluid detected prior to ovulation have reduced pregnancy rates. Mares with intrauterine fluid detected after ovulation have reduced pregnancy rates and increased early embryonic death (EED).

A study was designed to determine the influence of intrauterine fluid on pregnancy rate and early embryonic death(McKinnon et al., 1987a). It was concluded from this study that: 1) presence of small quantities of intrauterine fluid during oestrus in cycling mares did not affect pregnancy rates at either day 11 or 50; 2) intrauterine fluid, detected 1 or 2 days after ovulation, did not affect day-11 pregnancy rates, but was associated with a significant increase in EED and reduced day-50 pregnancy rates; and 3) presence of intrauterine fluid during dioestrus was associated with a significant decrease in day-50 pregnancy rates. Another study revealed the incidence of intrauterine fluid during dioestrus (12/43,28%) was associated with the presence of an inflammatory process as indicated by a high biopsy score, reduced progesterone concentrations, and a shorter inter-ovulatory interval(Adams et al., 1987). Mares with intrauterine fluid during dioestrus had a lower pregnancy rate at Day 11 and a higher embryonic loss rate by Day 20 than did mares without fluid. The progesterone profile and length of inter-ovulatory interval for mares with uterine inflammation suggested that embryonic loss in this herd was due to uterine-induced luteolysis rather than primary luteal inadequacy(Adams et al., 1987).
The effects on pregnancy rate of three different treatments to remove intrauterine fluid were assessed in 1267 mares(Pycock and Newcombe, 1996). The mares were mated and allocated, in strict rotation, to four treatment groups: 1) untreated controls, 2) intrauterine infusion of broad spectrum antibiotics, 3) intravenous injection of oxytocin, 4) intravenous injection of oxytocin followed by intrauterine antibiotics, The pregnancy status of the mares was determined 13 to 15 days and 27 to 30 days after ovulation by ultrasonography, The pregnancy rate of group 4 (72 per cent) was higher than that of group 2 (64 per cent, P<0 . 01) or group 3 (63 per cent, P<0 . 01). The pregnancy rates of groups 2 and 3 were higher than that of group 1 (56 per cent, P<0.01). The treatment with antibiotics and oxytocin appeared to have an additive beneficial effect which suggested two different modes of action of the combination treatment, namely antibacterial activity and fluid drainage. In the untreated mares more fluid accumulated in the uterine lumen after mating, and this was the most likely reason for their lower pregnancy rate(Pycock and Newcombe, 1996).

In our breeding programs, the amount and quality of fluid detected with ultrasonography is used to determine how to treat the mare. Large volumes of poor quality (grade 1 or 2) fluid are treated with voluminous saline lavage, whilst small volumes of grade 4 fluid quite often are treated with local antibiotics and oxytocin and/or cloprostenol. When fluid is detected after ovulation then efforts are directed at reducing fluid and inflammation, such as intravenous non-steroidal anti-inflammatory agents, intrauterine antibiotics, immune stimulants(Rohrbach et al., 2007) and systemic oxytocin. Cloprostenol is not used after ovulation due to the potential of modification of CL function(Brendemuehl, 2002; Gunthle et al., 2000; Nie et al., 2003a; Nie et al., 2003b).

Uterine Cysts

Prior to ultrasonography, uterine cysts were most commonly diagnosed from post-mortem examination and occasionally by rectal palpation(Kenney and Ganjam, 1975). Subsequently they have been diagnosed by hysteroscopy and ultrasonography(McKinnon et al., 1987c). Cysts in the uterus are fluid-filled and apparently have two origins. Endometrial cysts arise from endometrial glands and are usually < 10 mm in diameter. The second form of uterine cysts are lymphatic in origin (Figures 3a and b) and generally are larger than endometrial cysts. They are common in older mares(McKinnon et al., 1987c; Stanton et al., 2004; Tannus and Thun, 1995), and have been associated with

both normal and abnormal uterine biopsies(Kenney and Ganjam, 1975). Size of uterine cysts may be indicative of origin. Little data has been reported on growth rate of uterine cysts. Despite the occasional large cysts, it is unlikely that they grow at a similar rate as the early embryonic vesicle (days 10 to 20). When visualised with ultrasonography, cysts are commonly rounded, sometimes with irregular borders, and occasionally are multiple or compartmentalised (lymphatic lacunae) (Figures 3c-f). Most cysts are luminal however occasionally transmural and sub-serosal cysts are detected. Movement of the early equine conceptus (days 10 to 16), presence of specular reflection, spherical appearance and growth rate of the embryo should aid in its differentiation from uterine cysts. The relationship between infertility and uterine cysts is axiomatic as cysts increase in number as mares age(Tannus and Thun, 1995). Cysts may impede movement of the early conceptus, restricting the reported ability of the vesicle to prevent luteolysis after day 10(McDowell et al., 1988). Later in pregnancy, contact between the cyst wall and yolk sac or allantois may prevent absorption of nutrients (Fig3f). This may be more important when considering the recognition that large uterine cysts are more commonly located at the junction of the uterine horn and body(Tannus and Thun, 1995), which is the most common site of vesicle fixation(Ginther, 1983). Finally, cysts are commonly indicative of uterine disease. They may reflect senility or be associated with endometrial fibrosis. It has been reported that there is an association between number of uterine cysts, age of mare(Ginther, 1983), and endometrial biopsy score(Adams et al., 1987). The number of treatments proposed for uterine cysts probably reflects inability of any individual treatment to consistently be useful. Rupture of the fluid-filled structures has been attempted via uterine-biopsy forceps, surgery, fine needle aspiration and puncture via hysteroscopy. Electro-coagulative removal of cysts has also been described. Endometrial curettage and repeated lavage with warm saline (40 to 45^{0}C) have also been advocated. Although there are no reports on respective efficiency of these treatments, endometrial curettage and saline lavage are frequently applied to treat the primary problem, which would appear to be lymphatic blockage. It was concluded from one study that: 1) uterine cysts, when detected by ultrasonography, were lymphatic in origin; 2) uterine cysts did not change rapidly in size or shape, although they were more difficult to detect during oestrus; 3) treatment with infra-red radiation was not effective; 4) there was no consistent location for uterine cysts; and 5) uterine cysts were commonly associated with chronic, infiltrative, lymphocytic endometritis(McKinnon et al., 1987a).

Another study on 259 normal fertile Thoroughbred mares (Tannus and Thun, 1995) revealed the incidence of uterine cysts was 22.4%. Of the 95 cysts observed during the trial, 87.4% were in the middle and posterior segments of both uterine horns. The size of all cysts ranged between 3 and 48 mm. When all mares were assigned to three age groups, A < 7 years (n = 116), B 7-14 years (n = 117) and C > 14 years (n = 26), a significant ($P < 0.01$) increase in the number of cysts was observed with advancing age (4.3%, 29.1% and 73.1%, respectively). The seasonal pregnancy rates at Days 14 and 40 were significantly ($P < 0.01$) lower in mares with cysts (77.6% and 71.4%) compared to mares without cysts (91.5% and 88.0%). This suggested to the authors that the presence of uterine cysts plays an important role in the reduction of fertility of Thoroughbred mares(Tannus and Thun, 1995).

From our experience cysts are more commonly noted in older mares and are also more commonly recognised in the immediate postpartum period and at the corpus cornual junction. Uterine cysts are more commonly pedunculated and luminal; however, transmural and non-pedunculated cysts are also seen. Our preferred treatment for uterine cysts when we believe treatment is necessary, has been to dilate the mare's cervix whilst in heat and ablating the cyst manually. This performed by placing the cyst in between two fingers and tearing or crushing the cyst of the uterine wall. Non-pedunculated cysts may be more difficult to destroy this way, however they seldom appear to cause many problems. Recently, a simple improvised snare technique has been described to remove

pedunculated uterine cysts(DeLuca et al., 2009). The snare consists of two insemination pipettes fixed together with a long portion of thick suture material as the snare. The suture material is passed over the cyst and fixed at the pedunculated base of the cyst. A sawing motion is used to transect the stalk (Figures 3g and h).

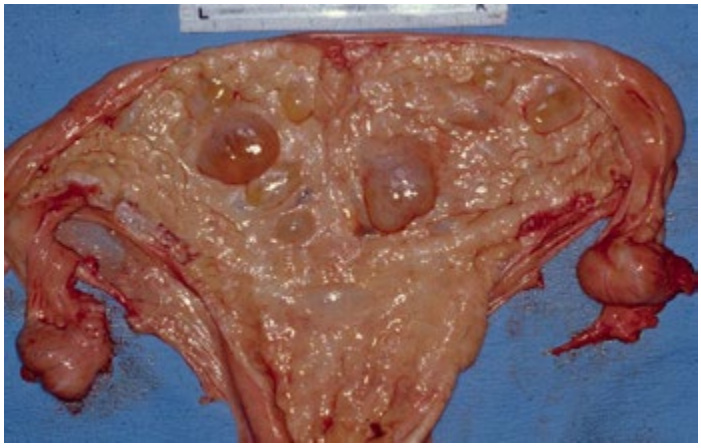

Figure 3a. Uterine cyst on the surface of the endometrium

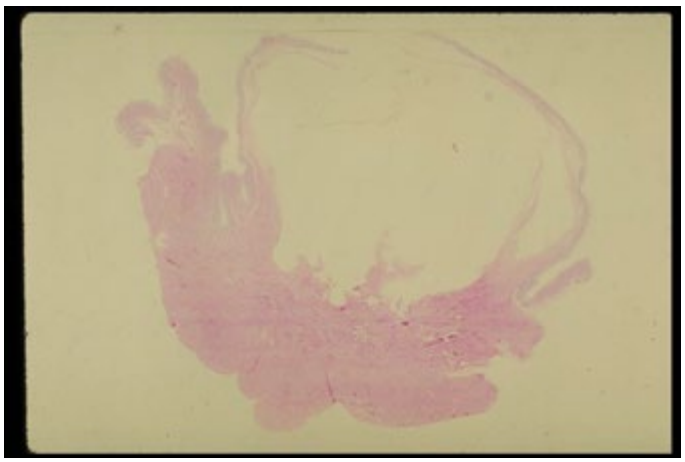

Figure 3b. Histology of a uterine lymphatic cyst (x 2)

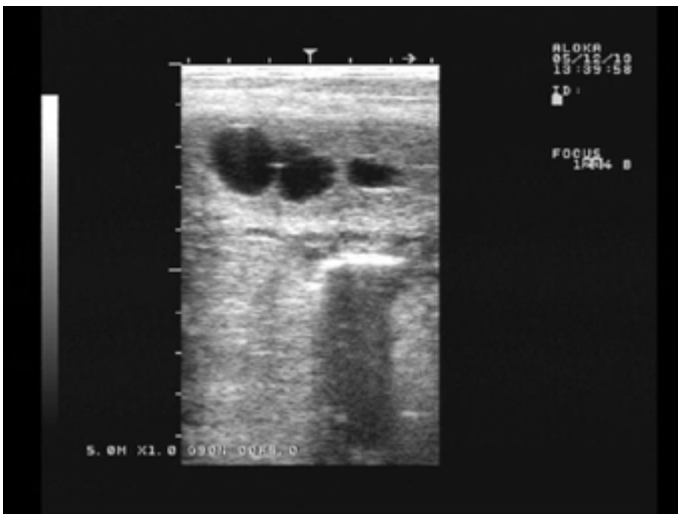

Figure 3c. Day 12 pregnancy in front of the cervix with two cysts of similar size immediately cranial

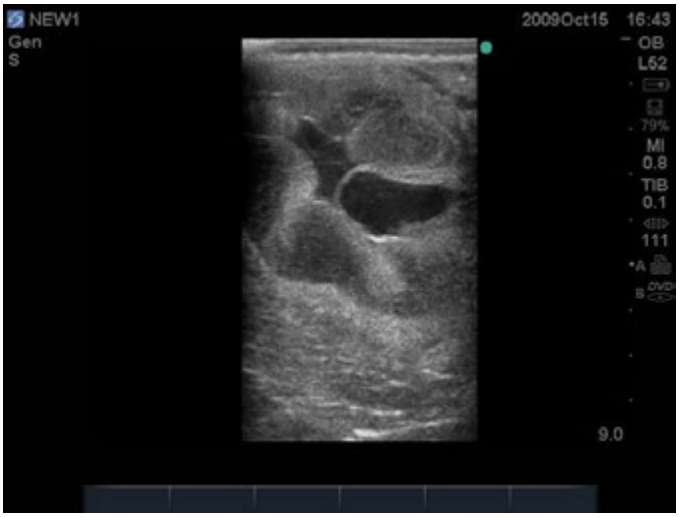

Figure 3d. Single cyst with inflammatory folds and fluid

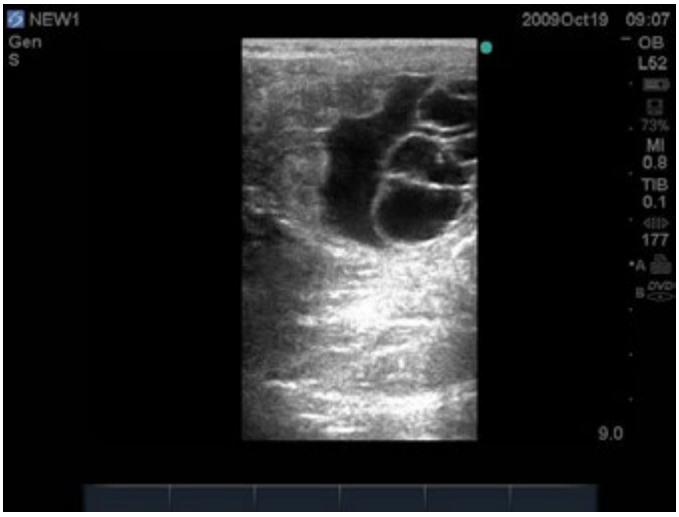

Figure 3e. Multiple cysts associated with a pregnancy at the corpus corneal junction

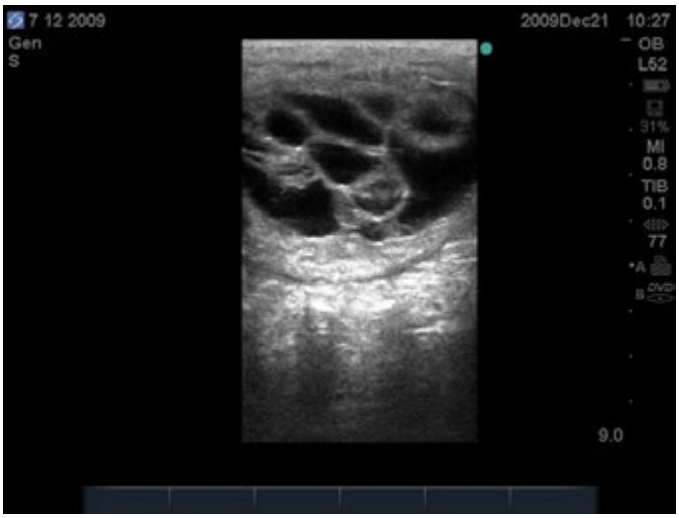

Figure 3f. Larger number of cysts at the corpus corneal junction with a pregnancy. This number of cysts may interfere with absorption of nutrients although there are no definitive studies to prove so.

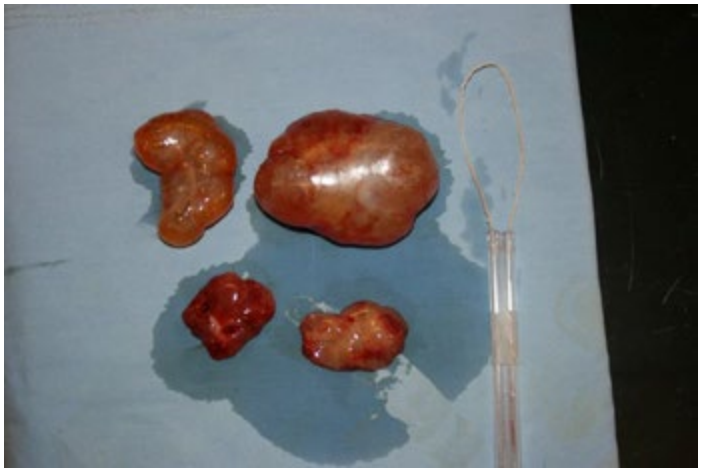

Figure 3g. Pedunculated uterine cysts removed with a snare

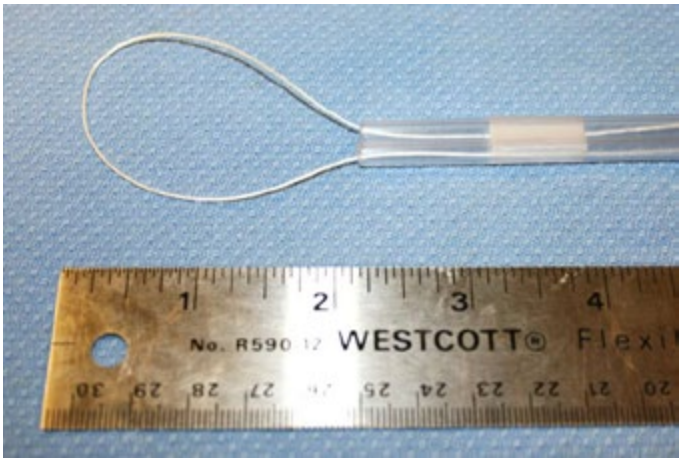

Figure 3h. The suture material in the pipettes is used to saw of the attachment of the cyst while the pipettes protect the uterus from damage.

Intrauterine air

Air is recognised by the presence of multiple, hyperechogenic reflections (Figures 4a and b) (occasionally a ventral reverberation artifact is present, and it appears to be more prevalent slightly cranial to the cervix, although it can be present in the cranial body or uterine horns. When air is present < 24 hr after artificial insemination, we consider it normal. However, we do not expect it to be detected in normal mares > 24 hr after breeding. The observation of air in the uterus of mares that have not been cultured or bred recently is an indication of pneumo-uterus and reflects failure of the competency of the vaginal labia, vestibulo-vaginal sphincter and(or) cervix(McKinnon et al., 1987a). In our experience it has been of use to determine when some mares may need to be caslicked, particularly so when a clinical examination did not suggest the procedure. Occasionally air may be detected in a pregnant mare. In these cases, the prognosis for pregnancy maintenance is poor.

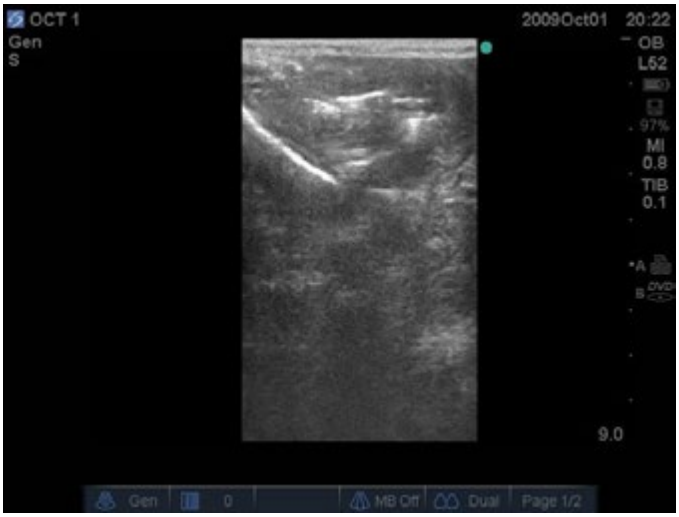

Figure 4a. Air is detected as hyper-echoic reflections in the uterine horn

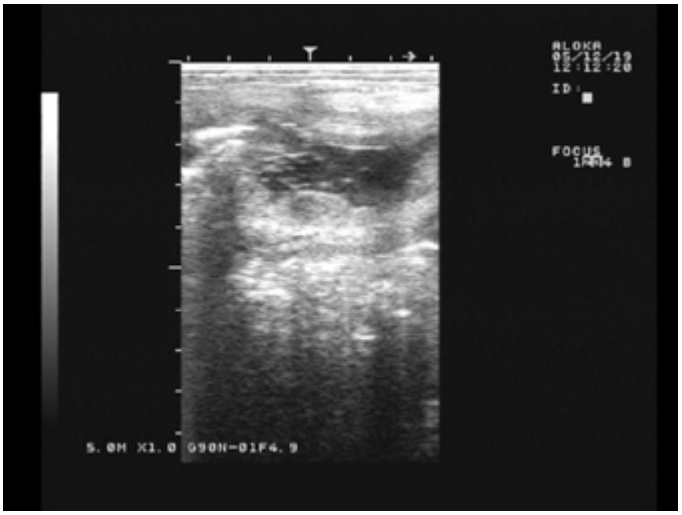

Figure 4b. Air and fluid immediately cranial to the cervix

Foreign bodies

On occasion, strongly echogenic areas in the uterine lumen are observed with a concomitant echo shadow, such as is seen with dense tissue like foetal bone (Figure 5a). This might be expected after mummification. We have also identified a similar ultrasonographic image that was confirmed subsequently as the tip of a uterine culturette (Figure 5b). Another substance that creates a strong echo shadow is the presence of chronically retained foetal membrane. These cases are usually first recognised at the first post-partum examination; however, they may not be apparent until the echogenicity increases. In addition, we have seen a few interesting cases such as calcification on the uterine epithelial surface, suture material after a Caesarean section and post treatment debris after multiple antibiotic suspension treatments into the uterus of a mare that had poor uterine clearance abilities. Marbles placed into the uterus to create a pseudo-pregnant state are easily visualised by ultrasonography (Figures 5 c and d)

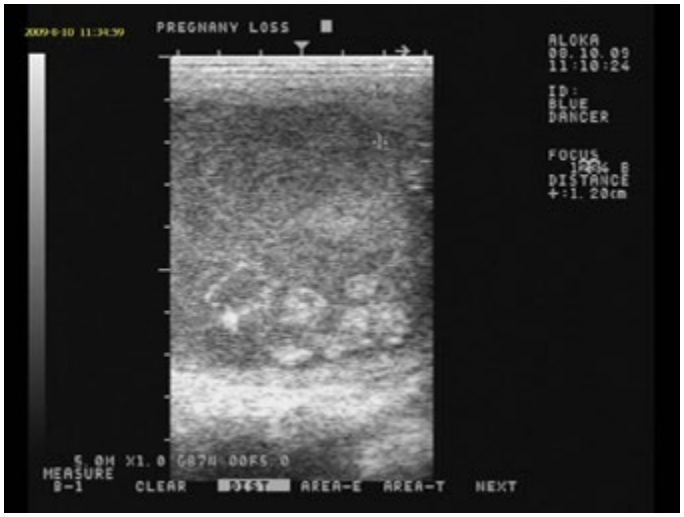

Figure 5a. Foetal remnants in a uterus with large amount of Grade 2 fluid

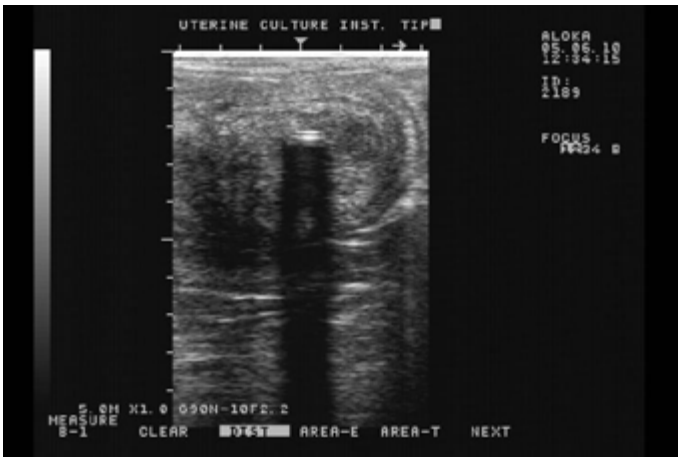

Figure 5b. Echogenic mass that was confirmed to be the tip of a culturette

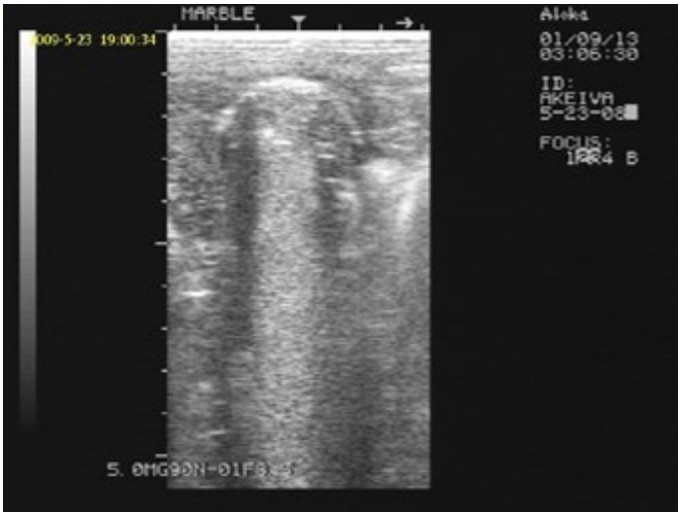

Figure 5c. Glass marble in the uterus

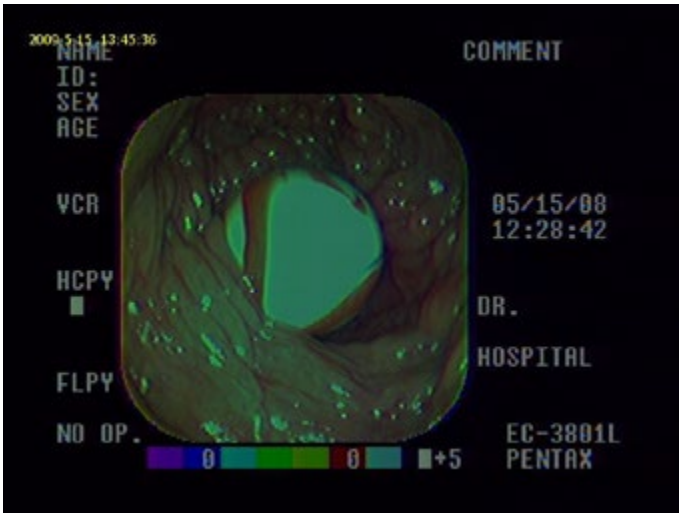

Figure 5d. Glass marble visualised by endoscopy

Retained endometrial cups

In a normal pregnancy the endometrial cups are not detected, however on occasion after EED and expulsion of foetal fluids they may become visible. Mares can in unusual circumstances retain endometrial cups(Silva et al., 1995; Willis and Riddle, 2005) in an active state for a long time (a year or more) despite either foaling, aborting or suffering from early embryonic death(Steiner et al., 2006). These mares present as dry mares in the next breeding season and typically have irregular oestrus cycles with abnormal ovarian function such as follicles that luteinise without ovulating. Presumably the abnormal ovarian function is associated with the high levels of equine chorionic gonadotrophin (eCG) that are secreted from the retained cups. Mares with retained cups may exhibit irregular or prolonged oestrus or anoestrus behaviour. A diagnosis of retained endometrial cups can be suggested by ultrasonography and confirmed by endoscopy of the uterus or by measurement of serum eCG concentrations (Figure 6). Retained endometrial cups will eventually be removed by immunological rejection but it may take months. There is no technique currently available to consistently hasten the removal of aberrant persistent endometrial cups(Steiner et al., 2006).

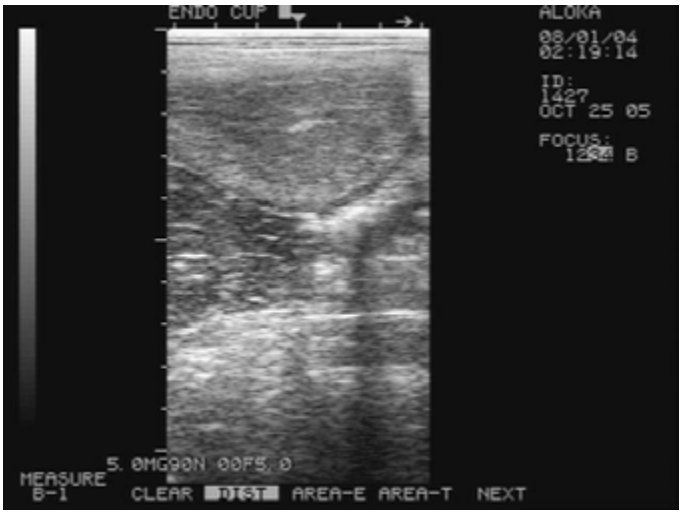

Figure 6. Retained endometrial cups may on occasion be identified as hyper-echoic plaques

Uterine adhesions

Occasionally after severe trauma to the reproductive tract or even after irritation from endometritis, transluminal uterine adhesions will form. Because of continuing secretions from the oviduct or endometritis in the horn proximal to the adhesions, fluid will accumulate in the tip of the affected horn. This fluid is well localised and should make the examiner suspicious of a complete adhesion. Diagnosis can be confirmed manually after first gently dilating the cervix or if that is not possible, infusions of fluid (i.e., 1 L of LRS) into the uterus should demonstrate lack of fluid entering part of the uterus (typically a uterine horn). When adhesions exist, it is not possible to dilate the tip of the affected horn because the fluid has restricted or denied entry. Adhesions may respond to manual breakdown and multiple infusions of an antibiotic extender; however, some appear to get worse with the irritation of treatment and may respond better to either an oily based corticosteroid cream or rest from treatment for at least three weeks.

Less commonly recognised uterine pathology

Uterine neoplasia, abscesses and haematomas can be recognised with ultrasonography. Leiomyomas of the uterus are not uncommon although careful scrutiny may be necessary to detect them as their detectable size varies from 10mm up to as large as 60mm. Leiomyomas are only slightly more echogenic than the uterus and they present as rounded discrete masses (Figures 7a-c).

This season (2021) courtesy Dr Stacy McGregor, Euroa) we recognised a form a neoplastic change in the uterus we hadn't identified before. The mare presented with an intrauterine mass that consistently bled. So much so that fertility (breeding with AI and embryo recovery) was affected. A biopsy confirmed the mass was a hamartoma (Figure 7d). Ultrasonography may be useful in recognition of mucus layers and biofilm in the mares' uterus (Figures 2g-i). Biofilm production is a protective mechanism of certain bacteria and is associated with increased difficulty to eliminate the infection with traditional treatment. Recent therapies showing promise for mucus and biofilm removal or modification are N-acetylcysteine (NAC) (30 ml of a 20% solution in 150 ml of saline)(Gores-Lindholm et al., 2009) or Ethylene-diaminetetraacetic acid-tromethamine (EDTA-tris) (250 ml of 3.5 mM EDTA, 0.05 M tris, pH 8 –(LeBlanc, 2008a). However, in my experience, the use of hydrogen peroxide (1%) as a 60 ml infusion with Lactated Ringer's (LRS) is the most effective and consistently usual treatment for biofilm. We began using H_2O_2 many years ago for yeast infections of mares particularly *Candida albicans*. H_2O_2 has also been useful for intrauterine treatment of bacterial endometritis and is useful to prevent misuse and overuse of routine antibiotic prophylactic treatment post breeding.

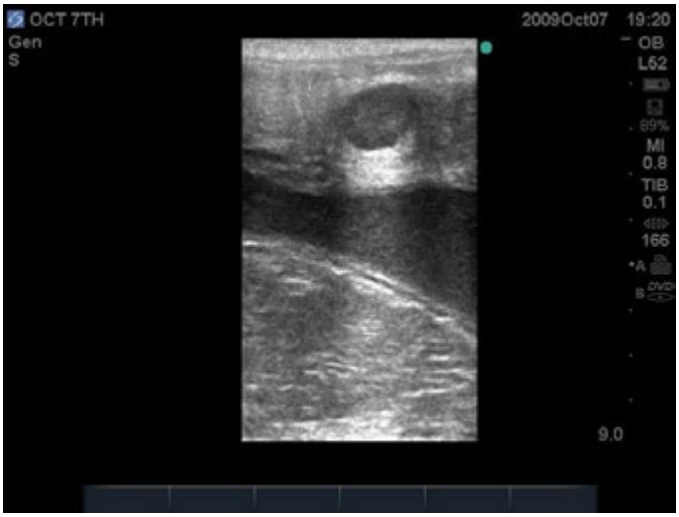

Figure 7a. Small leiomyoma in the uterus. They typically lie in the submucosa or deeper and can on occasion be removed after gently dilating the cervix and tearing them off manually.

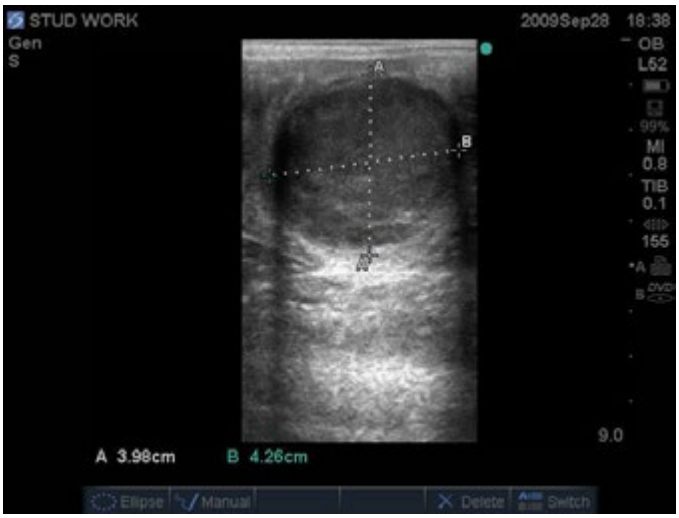

Figure 7b. Large leiomyoma

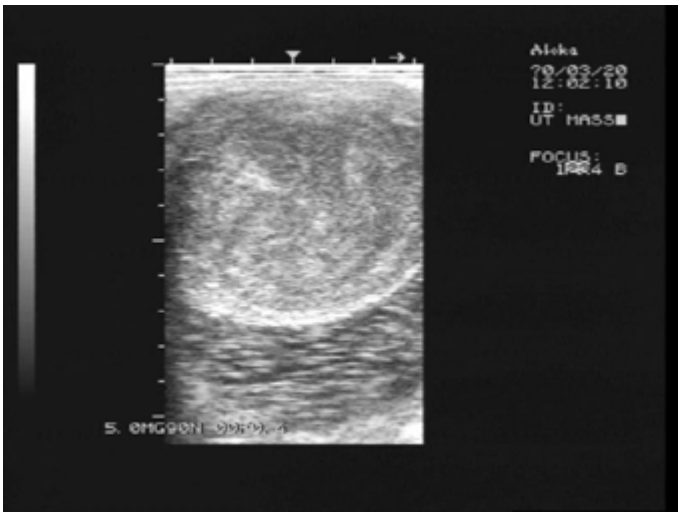

Figure 7c. A large leiomyoma that has been highlighted by infusing LRS into the uterus

Evaluation of the caudal reproductive tract

Ultrasonography has not been particularly useful for diagnosis of cervical conditions, however on a few occasions we have identified pathology such as cysts or scarring of the cervix. In general, manual or speculum examination are more useful. Once the reproductive tract is fully involuted and the mare is in dioestrus (when the cervix should be completely closed), the cervix can be evaluated to determine whether it is capable of closing. Involution is also necessary to allow identification of the muscular and mucosal layers that must be debrided and sutured. We commonly find unidentified cervical tears when referred cases of problem mares. In addition, it is surprising how many mares with cervical defects will become pregnant.

Ultrasonography can be useful in diagnosing cases of vaginal hematomas and abscesses (see above). Careful evaluation may even reveal urine or other fluid in the vagina/vestibule in mares with reproductive problems (Figure 8).

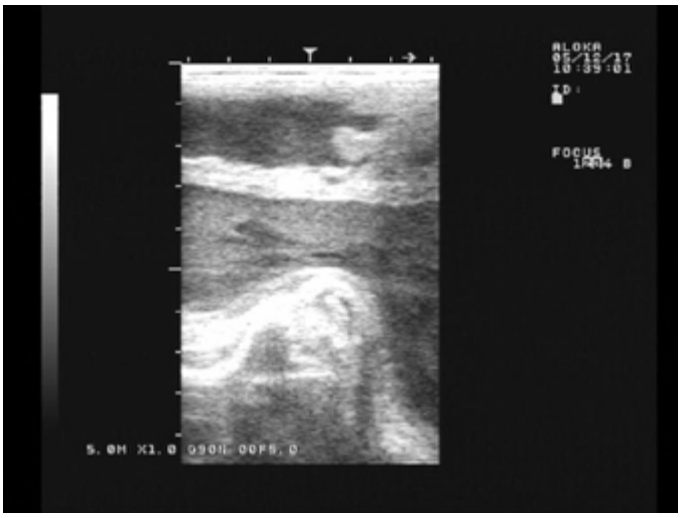

Figure 8. Ultrasonography was useful to visualise urine in the cranial vagina of this mare with vesico-vaginal reflux.

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Management of Cyclic Problems of Mares

Bain Fallon 2022

1

2

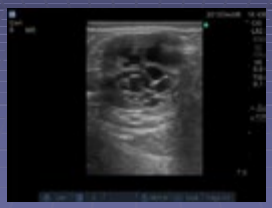
3

Techniques

- Behaviour
- Physical exam
- Palpation per rectum
- Vaginal examination
- Ultrasonography
- Hormonal analysis
- Biopsy techniques
- Karyotyping
- Laparoscopy

4

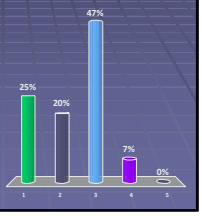
Left ovary



5

What is the likely abnormality..?

1. Normal ovary
2. Epithelial inclusion cysts
3. Granulosa cell tumor
4. Corpus albicans
5. Turners syndrome



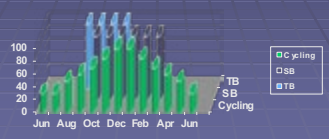
6

The transition

- Vernal (spring) transition
- Autumnal transition

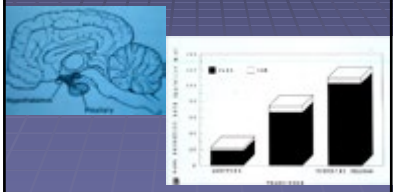
8

Effect of the time of the year on the number of mares cycling



9

Initiation of cyclicity



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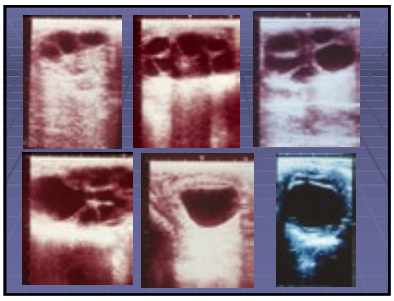


11

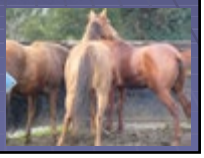
Transitional mares

- Lights
- Nutrition
- Hormones
  - Early – GnRH
    - Pulsatile
  - Mid – GnRH
    - Constant release
  - Late –
    - Progestagens (progesterone like compounds)
    - GnRH
    - Others

12

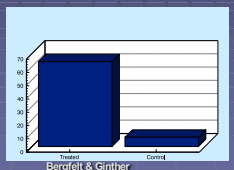
How do we get mares to get through the transition earlier and to cycle…..?

- **Light**
- **Stimulatory drugs**



13

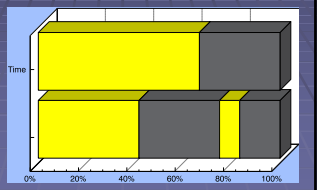
EED & ANOESTROUS OVULATION



Bergfelt & Ginther 1992

14

PHOTO-PERIOD

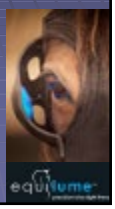


15

Various delivery systems

- Inside/outside/incandescent/strength and beginning and ending of light
- Mobile low level timed lighting to the horse's eye
- Horses can remain outdoors
- Non-pregnant mares:
  - Advances breeding season
- Pregnant mares:
  - Shortens gestation
  - Increases foal birth weight?

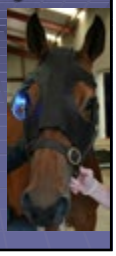
Courtesy Barbara Murphy



16

How much light is enough?

- First study:
  - Determine optimum light intensity
  - One eye versus two eyes?
- Results:
  - 50 lux blue light to one eye is as effective as standard barn Lighting at inhibiting melatonin

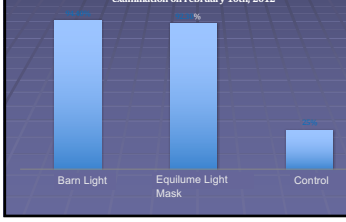


17

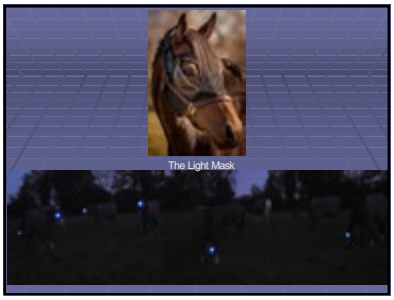
18

Results

% of Mares Reproductively Active as determined by veterinary examination on February 10th, 2012



19



20

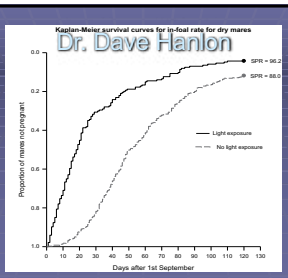
Effect of light exposure – dry mares (Dr. Dave Hanlon)

	Lights[a]	No lights[b]
Mares served in Sept. (%)	98.6	39.5
Mean days to 1st service	8.8	38.5
Median days to conception	19	51
PR to 1st service (%)	89.1	54.2
Twins at 15 days (%)	14.6	9.6
Seasonal pregnancy rate (%)	95.1	88.0

[a,b] All values within the same row are significantly different P<0.05

Light exposure commenced between 1st-15th July

21



22

Hormonal manipulation

- GnRH (constant versus pulsatile)
- hCG
- EPE
- Progestagens
- Dopamine antagonists

23

Drugs to modify transition

- GnRH (Deslorelin)
- Progestagens
- Only late in the transition with larger follicles

24

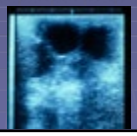
Use of deslorelin (GnRH) for hastening ovulation in the transitional mare

Goulburn Valley Equine Hospital

25

Materials and Methods Experiment 1

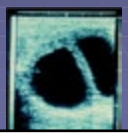
- Early transition (<25mm follicle)
- 21 mares (treated and control)
- EOD Ovuplant till Ov or 6 tx



26

Materials and methods Experiment 2

- Late transition (>30mm follicle)
- 20 mares (treated and control)
- EOD Ovuplant till Ov or 6 tx



27

Results Experiment 1

- Ovulation < 10 days
- Treated 6/11
- Control 0/10
- P<0.01

28

Results Experiment 1

- Time to Ovulation
- Treated 24.6 days +/- 20.1
- Control 29.4 days +/- 12
- (P>0.26)

29

Results Experiment 1

- Interovulatory interval
- Treated 23.2 days +/- 3.15
- Control 22.7 days +/- 2.8
- (P>0.35)

30

Results Experiment 2

- Ovulation < 4 days
- Treated 8/10
- Control 0/10
- P<0.0005

31

Results Experiment 2

- Time to Ovulation
- Treated 3.7 days +/- 3.2
  - range 2-11
- 2.1 implants (average)
- Control 21.9 days +/- 18.4
  - range 4-64
- (P<0.006)

32

Results Experiment 2

- Interovulatory interval
- Treated 25 days +/- 3.8
- Control 19.7 days +/- 1.9
- (P<0.002)

33

Conclusions

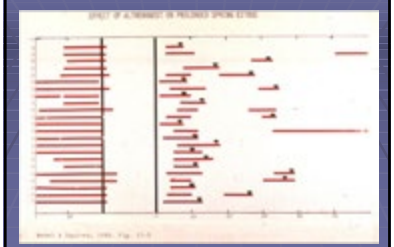
- It is difficult to select which mares will respond to deslorelin unless a follicle > 30mm is present
- Accurate timed ovulation was achieved during the late transition using multiple implants of Ovuplant (deslorelin)
- Same achieved clinically with bio-release Deslorelin

34

Managing mares in transition with progestagens

- Oral Altrenogest
- Injectable bio-release altrenogest
- Progesterone releasing devices (vaginal)

35



36

37

Altrenogest was effective for suppression of oestrous for an extended period of time with no effect on fertility

- Squires et al
- Best results were with > 20 mm follicle at beginning of treatment

38

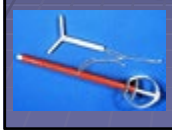
Injectable long acting dose Altrenogest

- 150 mg Altrenogest (Bio-release Altrenogest)
  - Follicle > 20 mm
  - Mares examined at 7 days
  - Burns et al
  - Reasonable results but we prefer PRID's

39

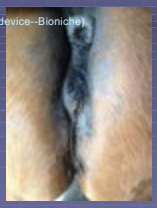
Vaginal Devices

- CIDR
- PRID
- Cue-Mare



40

Cue-Mare (Intravaginal device--Bioniche)
1.72 g progesterone 7-10 days



41

42

Criteria we use

Mid to late transition

Follicle > 20 mm

Insert 10 days

Examine after within 2-4 days of removal (unusual to ovulate less than that)

If there are folds and a large follicle present on first exam schedule for breeding

Don't always show good oestrous behaviour in the first few days after removal (P4 hangover)

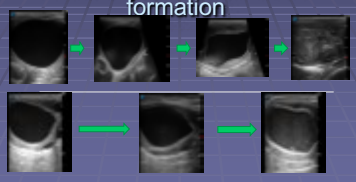
43

Anovulatory Haemorrhagic Follicles (AHF)

Also known as Persistent annovulatory follicles (PAF) or Haemorrhagic Anovulatory Follicles (HAF)

44

Normal ovulation vs. HAF formation



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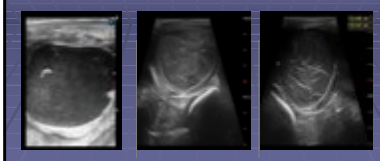
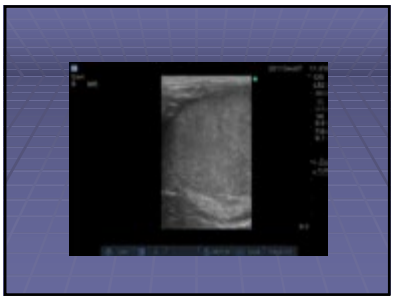


- Most HAF luteinize and secrete progesterone
- Luteinization occurs slower than a normal corpus luteum formed after ovulation

46



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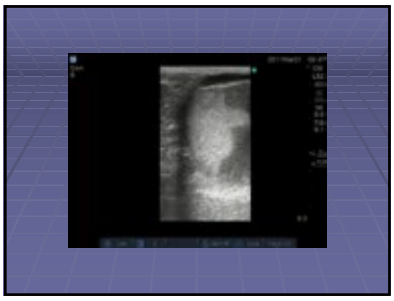

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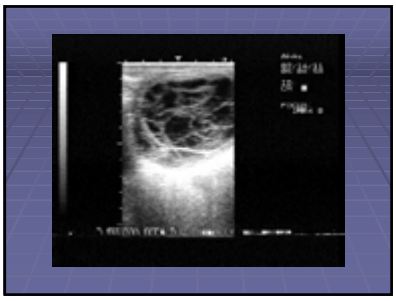
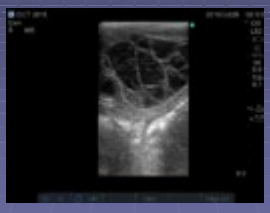


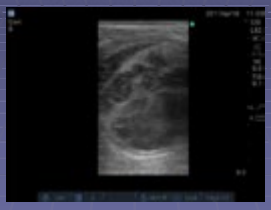
50

AHF, HAF, PAF, GCT or CH?



51

AHF, HAF, PAF or CH?



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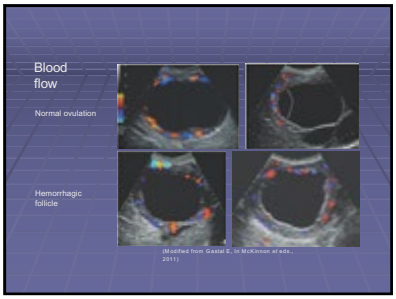


53

AHF, PAF and HAF

- All variations of the same developmental abnormality
- Risk factors:
  - NSAID's
  - Age
  - Previous development
  - PG (?) potentially if have high LH
- Compare with Supplemental CI's
  - High P4 and high eCG

54

Persistent anovulatory follicles in the mare

McCue PM and Squires EL

Theriogenology 58:541-543, 2002

721 mares 1845 cycles

5 years

55

AHF

- Ovulation 91.8%
- AHF formation 8.2%
- AHF Folds (78.3%)
- AHF associated with age
- 6-10 years old (4.4%)
- 16-20 years old (13.1%)

56

AHF

- Pregnancy rates
  - normal ovulation -1251 cycles = 42%
  - AHF – 71 cycles = 0%
- Repeatability during same season = 43%

57

Management

- No prostaglandin ? (low dose or multiple dose) – avoid high LH
- Natural cycle
- Avoid non selective NSAID (Cox-1 and Cox-2 inhibitors- Phenylbutazone and Flunixin) during oestrous
- Can use Cox-2 inhibitor Firocoxib (Previcox)
- Use progesterone/progestin to facilitate return to oestrus (gentle – reduce the effects of endogenous PG)
- Do not PG mares with multiple AHF's if bred

58

59

Management

- Traditional
  - Single PG day 9-10 after AHF formation
  - Poor response
  - Delayed return to oestrus
  - Increased interovulatory interval
  - Majority respond in time

60

Management

- Novel (Dr's Davolli and Pinto LSU)
- Multiple $PGF_{2\alpha}$ for managing HAFs
- Rationale:
  - Prevention of luteal formation after normal ovulation with multiple $PGF_{2\alpha}$ administration soon after ovulation, as opposed to single injection Day 6-10 (Coffman, 2014)

61

LSU

An examination on the effects of serial PGF2alpha administration to prevent and treat hemorrhagic anovulatory follicles

Carlos R. Pinto, E. Oberhaus, C.K. Mak, G.D. Davolli, L.H. Aguiar, V.L. Gomes, D.L. Paccamonti

Serial $PGF_{2\alpha}$ Injections Prevent Luteinization of Experimentally Induced Hemorrhagic Anovulatory Follicles

G.M. Davolli*, V. Medina, C.R.F. Pinto

(JEVS 2018)

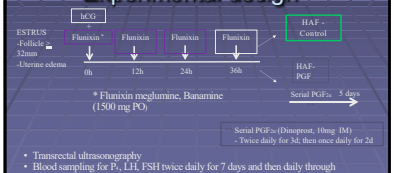
62

Experimental design

- Six cycling mares
- Balanced cross-over design:
  - Subject to two HAF induced estrous cycles.
- After HAF induction:
  - One cycle treated with serial PGF2a protocol **(HAF-PGF)**
  - One cycle left untreated **(HAF-Control)**
- At least one wash-out cycle between experimental cycles

63

Experimental design

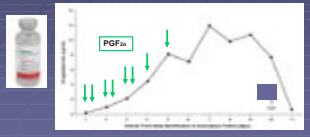


Serial $PGF_{2\alpha}$ (Dinoprost, 10mg IM):
- Twice daily for 3d; then once daily for 2d

- Transrectal ultrasonography
- Blood sampling for P_4, LH, FSH twice daily for 7 days and then daily through beginning of subsequent cycle

64

Multiple $PGF_{2\alpha}$ for managing HAFs



- Clinically, this protocol can also be: daily dose of $PGF_{2\alpha}$ until ultrasonographic demise of HAF or progesterone decrease below 1ng/mL

65

AHF

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- Can use Cox-2 inhibitor Firocoxib (Previcox)
- Use progesterone/progestin to facilitate return to oestrus (gentle – reduce the effects of endogenous PG)

58


Theriogenology

Periovulatory administration of firocoxib did not alter ovulation rates and mitigated post-breeding inflammatory response in mares

59

Management

- Traditional
  - Single PG day 9-10 after AHF formation
  - Poor response
  - Delayed return to oestrus
  - Increased intervulatory interval
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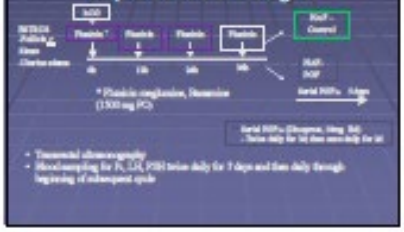

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64

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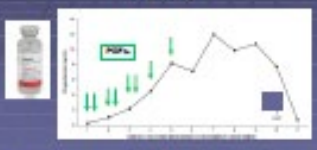


- Clinically, this protocol can also be: daily dose of $PGF_{2\alpha}$ until ultrasonographic decline of HAF or progesterone decrease below 1 ng/mL

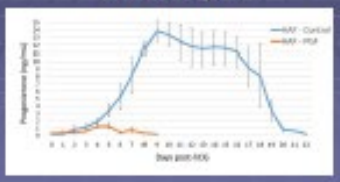
65

Results

- Out of 12 cycles planned (2 per mare), only 75% resulted in HAF induction (5 HAF-controls; 4 HAF-PGF)
- No spontaneous HAFs were formed in the washout cycles

66

Progesterone (P4) in HAF induced cycles



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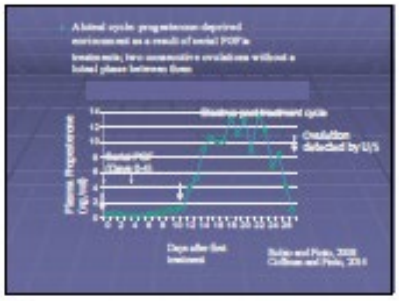


68

Results

All HAFs in control mares underwent luteinization; whereas, PGF2a-treated mares displayed minimal (mean concentrations of plasma P4 between 1-2 ng/mL) and transient luteinization (2 days duration). Maximum mean concentrations of plasma P4 were 1.44 (Day 5) and 11.12 ng/mL (Day 9); P < 0.05), respectively, in the HAF-PGF and HAF-control cycles.

69

Results

- On HAF-control cycles, interval-to-estrus and interval-to-ovulation were 22.5 days and 23.3, in contrast to 11.4 and 14.75 days on HAF-PG cycles (P < 0.05).
- There was no mention of incidence of AHF in subsequent cycles but Carlos said there were none so far

70

Conclusion

- Serial injections of $PGF_{2\alpha}$ can be started as soon as HAF is detected
  - Our model has utilized twice daily injections for 3 days followed by once daily for 2 days
  - Clinically, once-daily treatments can be used for several days until luteal regression is evident (via ultrasonography or blood progesterone)
- Advantages:
  - More rapid return to the subsequent estrus
  - Prevention of formation of excessive large HAF that may take long to resolve
    - Risk of pressure necrosis and atrophy of the ovary

71

Clinical Cases

Courtesy Dr. Davoli Resident LSU with Carlos Pinto)

72

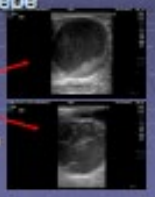
1. Phoebe

11-year-old Appendix

0 hours: 35 mm follicle – hCG treatment
24 hours post-hCG: 50 mm follicle showing signs of antral hemorrhage

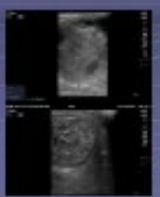
48 hours post hCG (Day 0): 60mm follicle post hCG; signs of hemorrhage

- $PGF_{2\alpha}$ protocol started (10 mg Dinoprost IM) Daily for 3 days and then once daily for 2 days



73

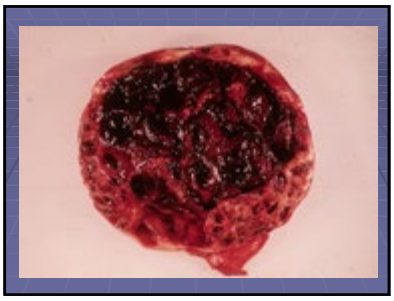
1. Phoebe



Day 1: reorganizing HAF; 61 mm diameter

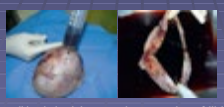
Day 4 (4 days after first PGF injection) HAF, now 39 mm

75



90

- HAFs, exceptionally, failed to luteinize and continue to grow
- These unresolved HAFs may destroy the ovarian parenchyma or be life-threatening.



The condition depicted above started as an anovulatory follicle that eventually became hemorrhagic, failed to luteinize, and continued to grow over the course of two months

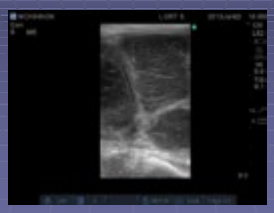
Pinto CRF, Fraser GS. Reproduction. In: Marr TS, Love S, Schumacher J, Smith RKW, Frazer GS (eds.) Equine Medicine, Surgery and Reproduction. London: Saunders Elsevier. 2013:283-308.

91

Supplemental CL

- Definitions !!!!
  - Primary CL
  - Supplementary CL
    - Secondary CL
    - Accessory CL

92



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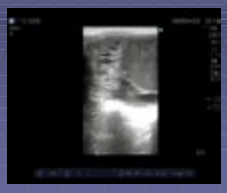

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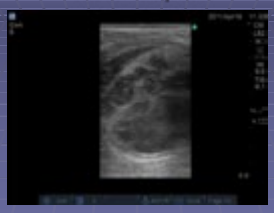
Case Report



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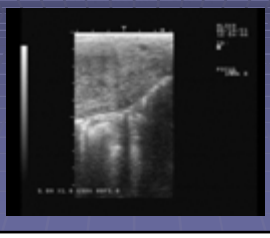
Case Report

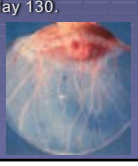


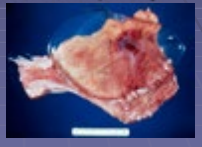
101

PERSISTENT ENDOMETRIAL CUPS

102

- Generally considered to form between 38-40 days of gestation. Reach maturity day 60-70 . Slough beginning 70-100 , complete by day 130.





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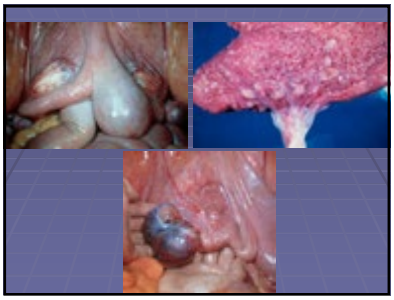


104

Treatment of retained endometrial cups

- Kerosene
- Curettage
- Time

105

Abortion at day 70 (Estrade et al 2016)

- Pre-abortion eCG Mean 32 IU (0.8-86)
- All mares had 0 IU eCG by 70 days
- 50% had ovulated by day 34 ± 10 days
- 90% of ovulating mares became pregnant that season
- Pre-abortion eCG was lower (P< 0.007) in mares that cycled

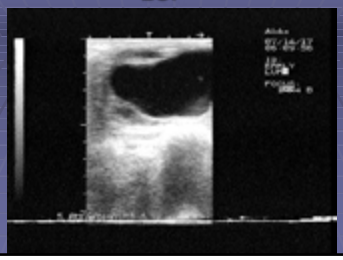
106

Luteinised Unruptured Follicles

(LUF)

107

LUF



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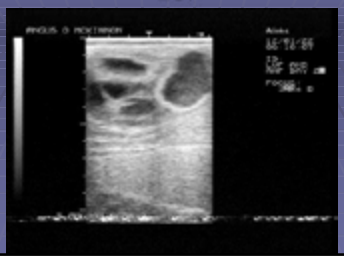
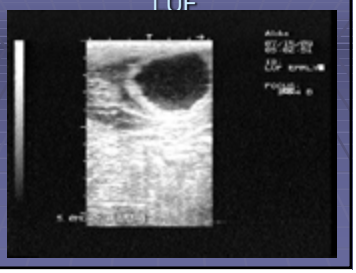
LUF



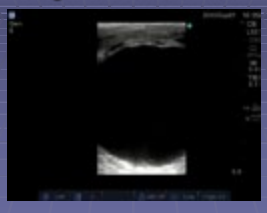
109

LUF



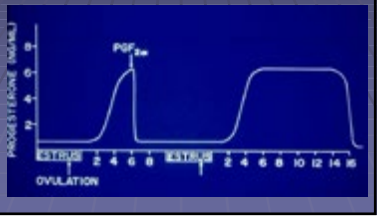
110

Large dioestrus follicle



111

Effect of Prostaglandin

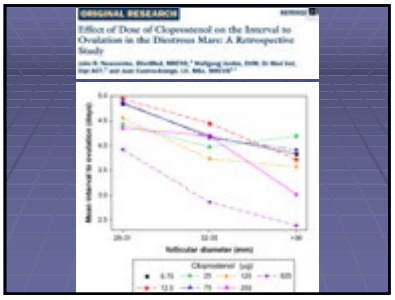


112

Effect of follicle size on response to $PGF_{2\alpha}$

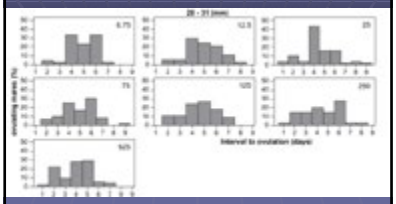
Follicle Size	Days to Oestrous
< 15 mm	6-7 days
30 mm	4-5 days
30-40 mm	3-4 days
> 40 mm	May ovulate in 24-48 hours without oestrous

113



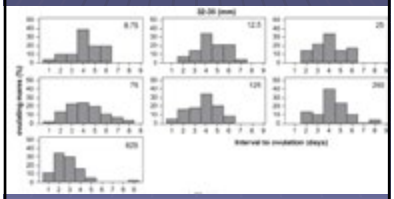
114

Effect of dose 28-31 mm



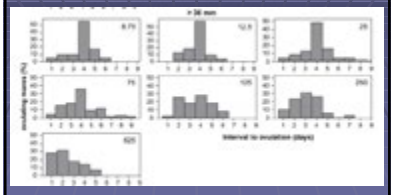
115

Effect of dose 32-35 mm



116

Effect of dose > 36 mm



117

Effect of Cloprostenol Administration on Interval to Subsequent Ovulation and Anovulatory Follicle Formation in Quarter Horse Mares

Chelsie A. Barden, Patrick M. McCue*, Ryan A. Ferris

- 520 oestrous cycles
- PG (Cloprostenol) day 5-12 Ave 8.4
- Mares >35 mm
  - Ovulation within 48 hours (13.4%) variable oedema
  - Ovulation > 48 hours (73.1%) uterine oedema
  - Regression without ovulation (emergence of another follicle later) 13.4%

118

Management strategies

- Normal PG dose and monitor
- Low dose PG
- Monitor
- Serial PG
- Progesterone supplement
- Delaying OV with dexamethasone

119

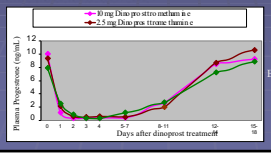
Normal dose PG and monitor

- Acceptable but time consuming as may take quite a while to come into heat if follicle regresses
- Sometimes OK
- Mostly early OV without cervical relaxation or uterine oedema
- Fertility poor

120

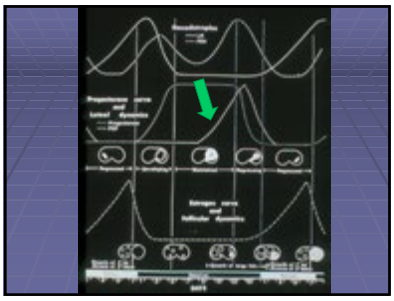
Low dose PG

- Reasonable but not favoured by interns or farm managers
- Must be low dose



Barker and Pinto, 2006

121



122

Monitor

- Difficult as could take quite some time
- Can be used in conjunction with good teasing (TB farms)
- Has a slower PG release and a more normal ovulatory induction profile

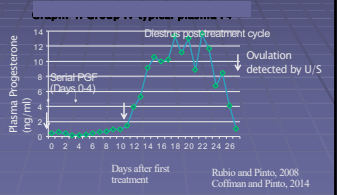
124

Serial PG

- Protocol of Coffman 2014
- If mare ovulates before mare is bred then using the multiple PG approach the mare can be recycled without developing a CL and thus has no progesterone when she is ready to breed and does not need further PG

125

- Aluteal cycle: progesterone-deprived environment as a result of serial PGF2α treatments; two consecutive ovulations without a luteal phase between them



Rubio and Pinto, 2008
Coffman and Pinto, 2014

126

Progesterone supplementation

- Controlled release of altrenogest (eg Biorelease Altrenogest)
- Given without PG
- Can still grow and ovulate but probability is reduced

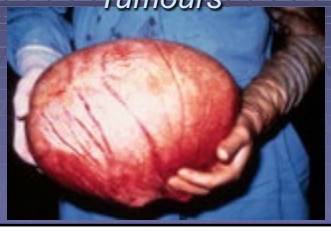
127

Follicular suppression with dexamethasone

- In our hands approximately 50% of the large follicles will regress
- If treatment is delayed until the mare has uterine oedema then follicular suppression does not occur

128

Granulosa Theca Cell Tumours



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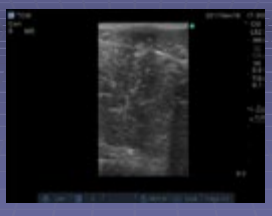

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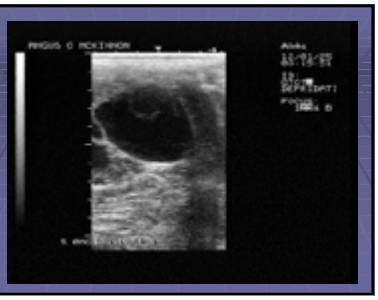

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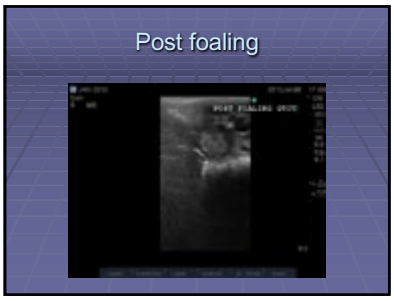

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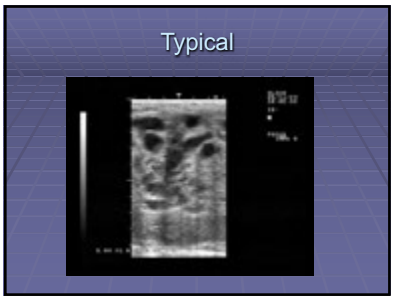

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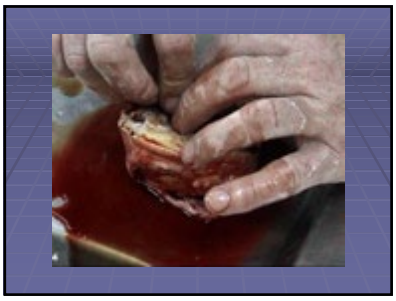

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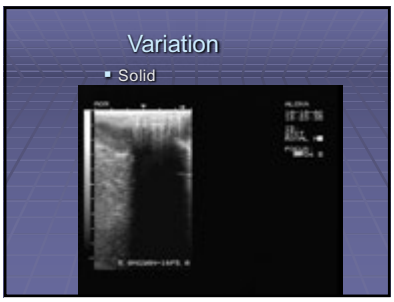

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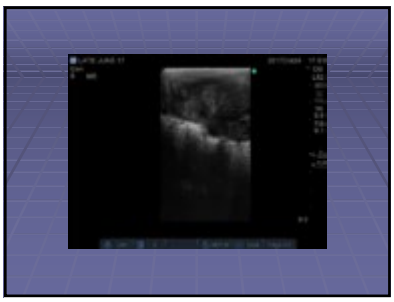

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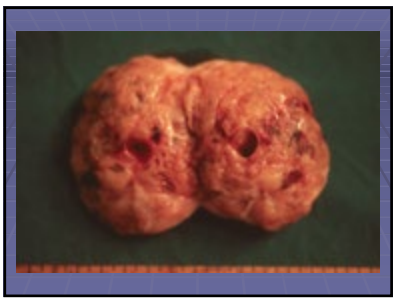

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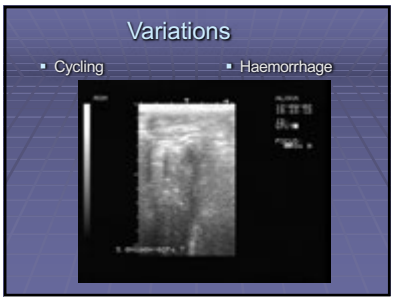

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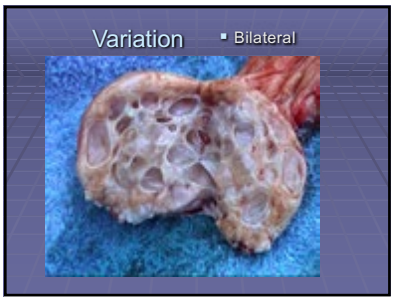

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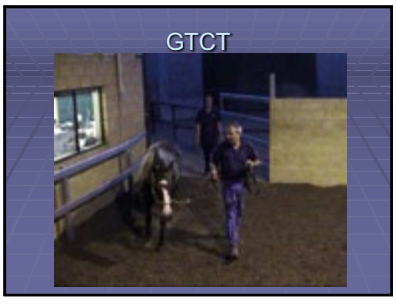

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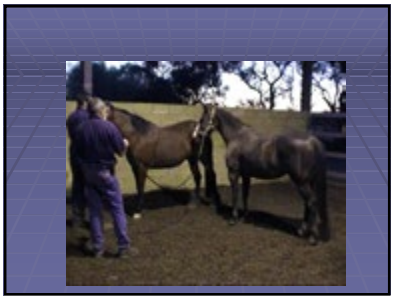

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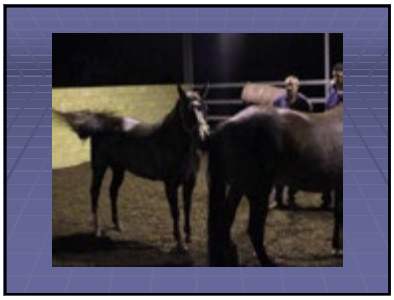

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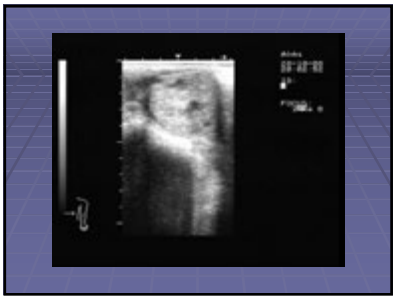

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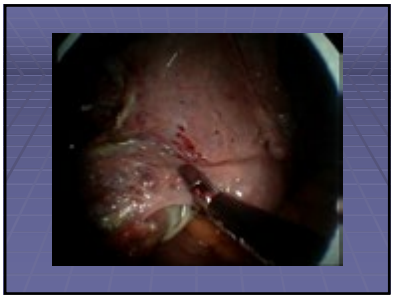

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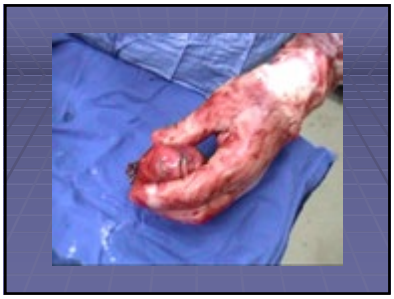

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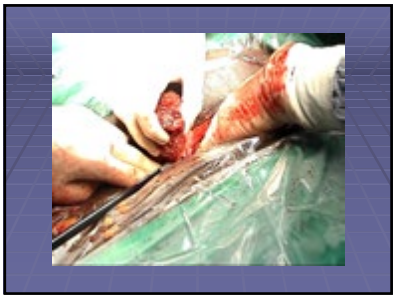

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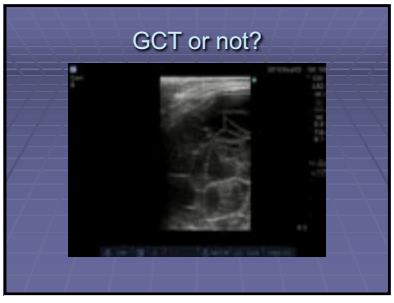

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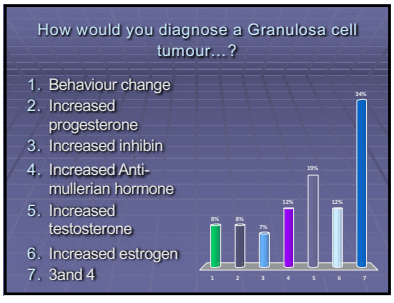

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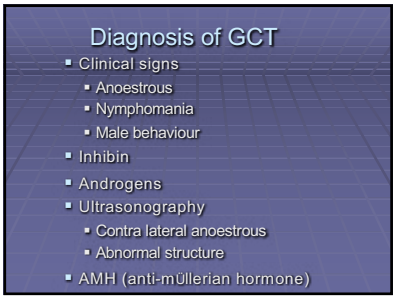
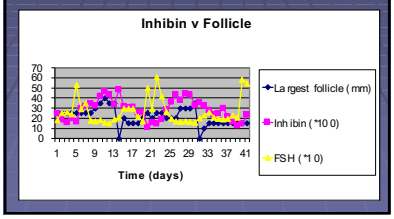


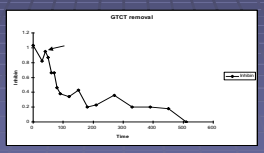
149

Effect of follicle size on Inhibin



150

Granulosa Cell Tumors



151

AMH versus Inhibin

	AMH (ng/mL)		Inhibin (ng/m/L)	
	≥ 4.7	≤ 4.7	≥ 1.65	≤ 1.65
GCT's	25	0	19	5
Abnormal ovaries	2	40	6	36

153

Typical ranges for AMH and Inhibin

AMH
- GCT median - 72.6 ng/mL (4.7-13,768)
- AO's median – 0.70 ng/mL (0.008-17.62)

Inhibin
- GCT median – 2.36 ng/mL (0.39-8.13)
- AO's median – 0.93 ng/mL (0.04-3.9)

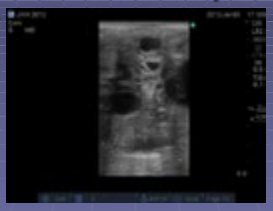
154

Take home message

- AMH > 20 (ng/mL) is more accurate than any other test
- Inhibin has been the industry standard and is reliable but is often elevated late in the development of the tumor and often only when there is inactivity of the contralateral ovary

155

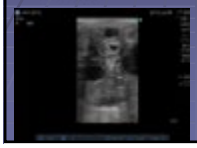
What is this ovary?

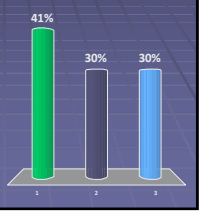


156

What is this ovary…?

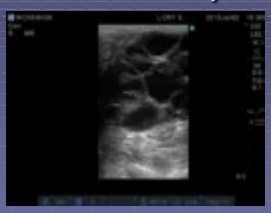
1. GCT
2. Corpus hemorrhagicum
3. Normal enlargement of the ovary





157

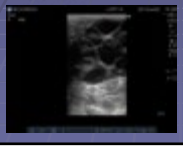
What is this ovary?

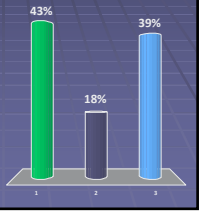


158

What is this ovary…?

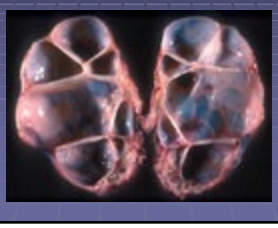
1. GCT
2. Corpus hemorrhagicum
3. Normal enlargement of the ovary





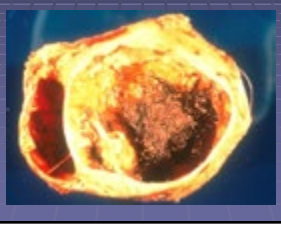
159

Cystadenoma



160

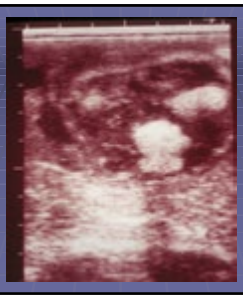
Teratoma and Dysgerminoma



161

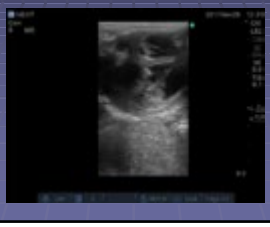
Ovarian Haematoma

163



164

Fibrin in Follicle



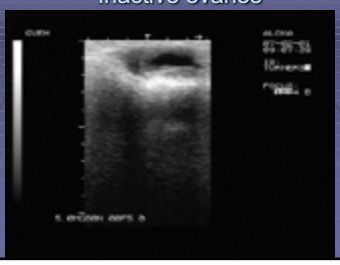
165

Effect of follicle size on response to $PGF_{2\alpha}$

Follicle Size	Days to Oestrous
< 15 mm	6-7 days
30 mm	4-5 days
30-40 mm	3-4 days
> 40 mm	May ovulate in 24-48 hours without oestrous

167

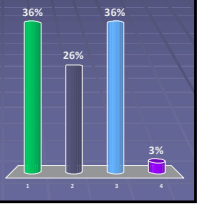
Inactive ovaries



168

What is your approach to diagnosis…?

1. Wait until the longest day
2. Send samples for cytogenetics
3. Get accurate history
4. Destroy the mare



169

Most common reasons

- To early in the breeding season
- Turner's syndrome
- Vaccination against GnRH
- Age

170

Cytogenetic analysis: band karyotyping

- Leukocytes cultured from whole blood sample
- Cells isolated and stained
- 20 cells counted per horse

171

Prolonged ovarian inactivity in broodmares temporally associated with administration of Equity.

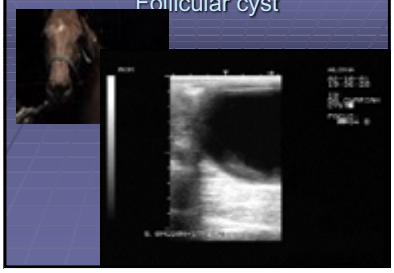
S J Robinson & A O McKinnon
Goulburn Valley Equine Hospital
Shepparton, Victoria

172

Follicular Cysts

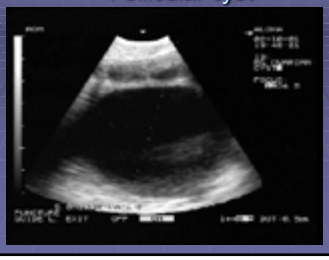
173

Follicular cyst



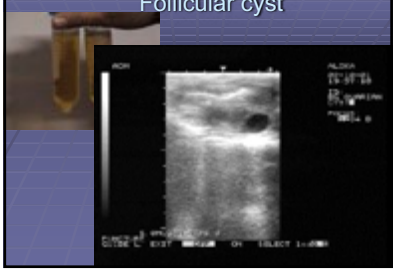
176

Follicular cyst



177

Follicular cyst

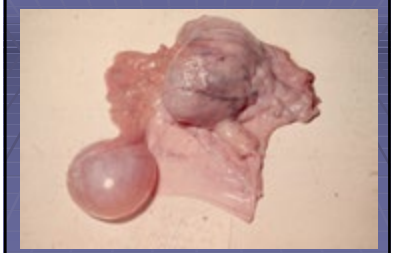


178

Peri ovarian Cysts

(Hydatid of Morgani)

180



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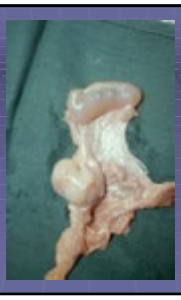

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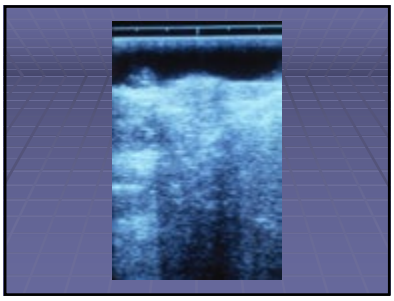

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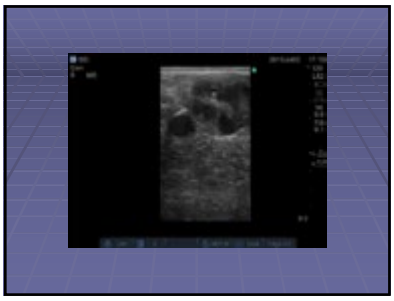

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Hydrosalpinx

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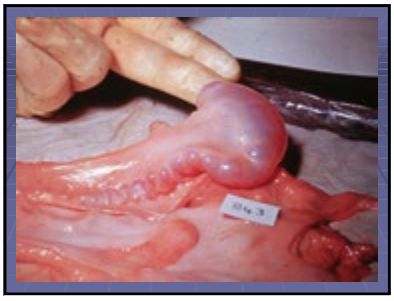

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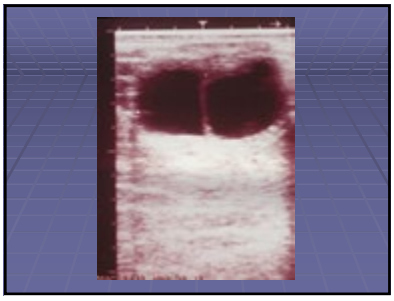

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The End

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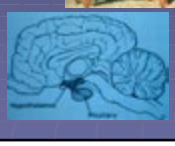
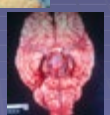
Pituitary Neoplasia





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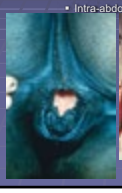
Psuedohermaphrodites

- Male psuedohermaphrodite
  - Looks female like
  - Transition from vagina to penis
  - Intra-abdominal testes



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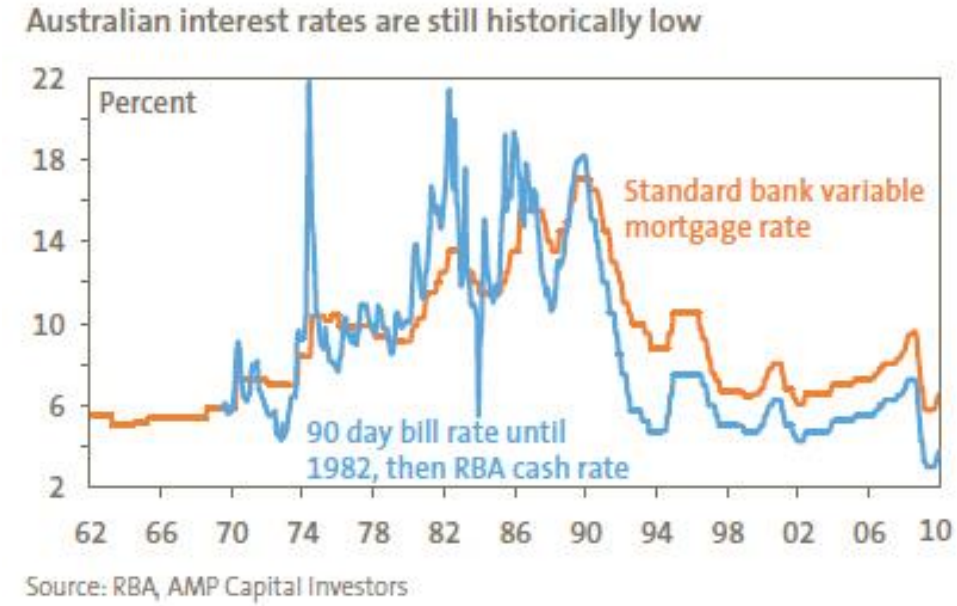
Notes

Some things I have learnt along the way (while working at the Goulburn Valley Equine Hospital)

AO McKinnon

Jim Vasey and I established the hospital in 1988. At the time we first bought the property I was in Colorado. Jim and I had worked together in Guelph in 1979 and 1980, so we had a pretty good idea of our strengths and weaknesses. Our idea was that we would establish an equine referral centre. It was a difficult beginning for a variety of reasons. One eminent equine practitioner said that we had built the biggest "white elephant" ever. Another marvelled that we forced him to scrub in to three colic surgeries in one night☺. Jim and I didn't take a drawing for almost two years. As the interest rates were so high, anything we earnt went towards paying debt. We were lucky enough (sic) to have major loans when interest rates briefly hit 21% PA.



Slowly, the hospital evolved and grew, and we employed more people. At one time, we had 15 veterinarians (including six interns). Over time, we've trained more than 130 interns and many residents. It's been a fun journey. It is still continuing. Many of the interns and residents have gone on to make us proud and become specialists in their area of interest.

The success the hospital was related to a few factors. Firstly, we employed the most talented veterinarians we could acquire; secondly, we all worked hard and had fun doing it; thirdly we were the first specialist non-university practice in Victoria; and finally we developed a wonderful client base that kept us all busy, especially with repeated work such as reproduction and surgery. In time, many of the veterinarians we trained became specialists themselves and became veterinarians to refer to as well. Equine specialist practices flourished. Why would we train people if we didn't want them to develop and succeed? Surely that is the point.

I for one am excited to continue to interact with past interns, residents and veterinary clinicians and enjoy their successes. It's really rewarding. Their successes are intricately

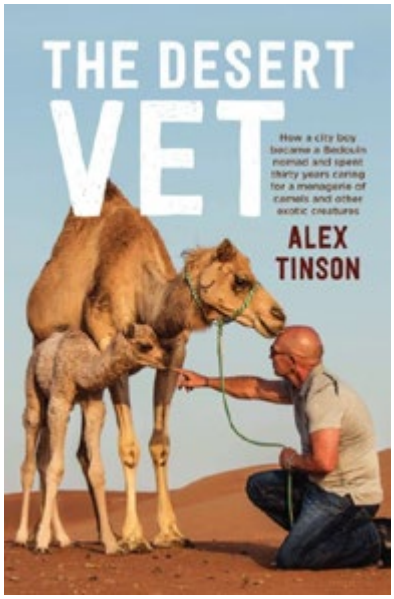
bound to their development at the Goulburn Valley Equine Hospital, and they will be inextricably bound to its future.

The sale of the business to Melbourne University has been another growth in the development of the hospital. Being trained at Melbourne University and for them then to acquire it for continuing education of students was quite a thrill. We were excited by the outcome and happy to be asked to continue to contribute. The sale has resulted in re population of veterinarians in the hospital and expansion and growth in diagnostic facilities to equipment to accommodate the training of students. Working with the students has been a breath of fresh air. They are so enthusiastic and ask such good questions that they keep all of us on our toes. I have always believed that until you can explain something you never are really sure yourself that you understand it properly.

After graduation, my next training after a short and enjoyable 6-month period at Doug Fenwick's practice in Allansford began in Guelph (Ontario Veterinary College, Canada), where I was luckily enough to be appointed as a resident in 1979. Being a young graduate in North America, I was worried that my knowledge and abilities would not measure up to those from North America. Melbourne University did a great job in our early training. They prepared us well (despite the obvious nerves etc). The Ontario Veterinary College provided an awesome training residency program. We were allowed the kind of access and decision making that would never occur today. The senior clinicians were relaxed but always there for guidance. After Guelph I went to Colorado with the aim of continuing to develop my reproduction skills. How fortunate was I to train with Pickett, Squires and Voss? Sadly, Jim Voss and Bill Pickett have passed but Ed Squires and I are working on so many projects together that we have zoom meetings at least once weekly. Ed remains a great friend. The three of them were responsible for giving me opportunities beyond what I ever deserved.

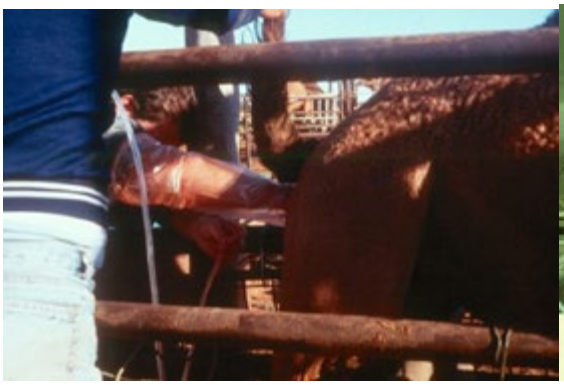
One thing Jim Vasey and had in common was an ability to work hard and long. Nothing else really mattered, and as we watched other practices fracturing, we stayed together as we wanted to succeed in our endeavour. Jim was also able to give me a certain freedom to develop some other avenues of work (in the early days we were quiet in the non-breeding season). When Alex Tinson and Heath Harris called and asked me to join them in the UAE to develop an embryo transfer programme for the Sheikhs' racing camels, Jim was able to recognise the advantages, and so was happy for me to spend months away from the practice in the Middle East during our horse non-breeding season. Later, he was happy for me to take a few days each week over a few months one year to edit the second edition of "Equine Reproduction". I will be forever grateful for his lateral thinking that allowed me those opportunities.

When Heath and Alex called it opened a new chapter for all of us. Alex has gone on to become well recognised for his veterinary prowess in camels. Alex was a year ahead of me in veterinary school at Melbourne University and we were already great mates from Ormond College. His book below has become iconic. Our UAE journey was a fabulous time. In order to study camel reproduction (which was not very well documented), we went to Abu Dhabi. We asked to view camel reproductive tracts but were then informed that due to the Halal method of animal preparation we were not going to be allowed.



The lack of access to the local slaughterhouse resulted in an almost immediate departure to Western Australia. Alex had previously been the veterinarian employed to look after the great Australian camel race, so he had contacts. We were met in Port Headland and flown to Corruna Downs about 45 minutes from Marble Bar. Between Corruna Downs and Warrawagine Station we were able to recruit enough camels for our trials of ultrasonography, electroejaculation and superovulation. By the time we finished camping the desert (May), we had performed the first embryo transfer in Camels in the world and had extensive data on follicular recruitment, electroejaculation and superovulation programmes in camels.

A picture of the rudimentary crush in Western Australia and the multiple crush system in the UAE (designed after the multiple horse crushes at GVEH) are shown below.



The paper below is one of a few (the first one), that recount our achievements that followed on from this. Suffice to say that ET in camels is now well entrenched and has made major genetic gains in racing camels in the UAE (not always appreciated by the Bedouin who used to do all the breeding). After a few years of heading there to transfer the embryos, Alex now does all the assisted reproductive work. Under his guidance they have created the first frozen embryo camel baby and the first camel identical twins and recently he and his team from Korea have created multiple cloned camel calves. Who would have thought how all this would develop from humble beginnings in Marble Bar?

Theriogenology 41:145–150, 1994

EMBRYO TRANSFER IN DROMEDARY CAMELS

A.O. McKinnon[1], A.H. Tinson[2] and G. Nation[2]
[1]Goulburn Valley Equine Hospital, Shepparton, Victoria, Australia and [2]H.H. Sheikh Khalifa Camel Embryo Transfer Research Centre, Al-Ain, United Arab Emirates.

ABSTRACT

Embryo recovery rates per attempt from naturally mated camels that superovulated following treatment with FSH or eCG were 261/68 (384%) and 193/84 (230%) respectively. The rate was affected by interval between ovulation and collection, male fertility and reproductive characteristics of the donor. Best recovery rates were achieved on Day 7.0 or 7.5 after natural mating from multiparous camels that had been superovulated with FSH. An overall pregnancy rate of 32% was achieved from the non-surgical transfer of 296 embryos. Factors affecting pregnancy rate included season, age and parity of recipient, number of recipient CL's, quality of embryo and donor/recipient/embryo synchrony.

Now let's look at some of things that we were able to achieve from a research perspective at GVEH...

Having a large herd of recipients was always going to be an opportunity for us to do funded research. One of our early observations was that there was a product touted for pregnancy maintenance that we noted didn't prevent mares from demonstrating oestrous. Our thoughts were that this probably meant that it didn't bind with equine progesterone receptors and thus was not fit for purpose. Anne Marie Nobelius was a graduate student we then shared with Roger Short (all through the collaborative efforts of Alan Trounson) and progesterone receptor binding became her project. We obtained funding from the Australian Equine Research Foundation (AERF) through Rex Butterfield who was a great support. Later Reuben Rose, who administered RIRDC, was also very supportive or our research. Anne Marie demonstrated that there was no binding of hydroxyprogesterone caproate with progesterone receptors in cells from the mare brain or uterus, despite the advertisements suggesting it was the best current therapy for preventing abortion. The experiment described below also found no benefit in maintaining pregnancy. Still in 2017 it was advertised to manage progesterone deficiency.

Failure of hydroxyprogesterone caproate to maintain pregnancy in ovariectomized mares. McKinnon AO, Tarrida S, Nobelius AM, Hyland JH, Vasey JR: Equine Vet J. 25: 158-160, 1993.

Discussion from the paper:

> "Hydroxyprogesterone caproate has been specifically advocated for use in pregnancy maintenance of mares (manufacturers recommendations). Despite lack of research data supporting the drug for this purpose, its use appears to be widespread. It was not the purpose of this study to examine whether supplementation of progestagens is a rational therapeutic approach (Allen 1984), but if mares are treated with hydroxyprogesterone caproate with the expectation of actually helping them maintain pregnancy, then great caution would appear to be warranted. Our experiment suggests that hydroxyprogesterone caproate is not capable of supporting pregnancy when administered around Day 18. In addition common sense would indicate that it is not likely to be of any more use when administered at a later time in pregnancy. In this study the method of ovariectomy did not appear to influence pregnancy loss, as altrenogest was successful in maintaining all pregnancies until Day 30 and 4 of 5 pregnancies until well past the time when ovarian

steroids are no longer necessary for pregnancy maintenance (Holtan et al. 1979). Mares in Group A received 4 times and those in Group B 15 times the dose of hydroxyprogesterone caproate recommended by the manufacturer. Mares received one (Group A) or two (Group B) treatments before ovariectomy. Because mares in these groups aborted, developed follicles and displayed oestrous behaviour after ovariectomy, it is unlikely that hydroxyprogesterone caproate had much binding to progesterone receptors in these mares or that time from first treatment to ovariectomy (2 days) was responsible for the inability of the drug to maintain pregnancy. Current recommendations for hydroxyprogesterone caproate for pregnancy maintenance in mares are 500 mg every 2 4 weeks. Another point of interest in this study was the relatively quick time to pregnancy loss (mean 3.1 days; range 2-5 days) compared with previous studies using PGF2, (Ginther 1985b) as the method of CL regression. In the latter study the mean number of days to embryonic loss was greater for mares treated with PGF2, on Day 12 (6.8 days) than for mares ovariectomised on Day 12 (3.0 days) (Ginther 1985b). Perhaps these findings demonstrate the effect of immediate total progesterone withdrawal as distinct from a more gradual decline noted after release of PGF2,. In addition, in our study vesicle loss was always associated with a softening cervix and the presence of endometrial folds, although mares did not ovulate for a mean of 7.8 days after unilateral ovariectomy (range 5-1 1 days). The results of this experiment suggest that pregnancy in mares with inadequate luteal function cannot be maintained with hydroxyprogesterone caproate at 4-1 5 times the recommended dose rate. However, pregnancy could be maintained by altrenogest and endogenous progesterone supplementation from secondary CL development is possible despite exogenous progestagen therapy."

The study above led us to examine other progestogens that were also being marketed and sold to maintain pregnancy or keep mares out heat while in training or competing.

The inability of some synthetic progestagens to maintain pregnancy in the mare. McKinnon AO, Lescun TB, Walker JH, Vasey JR and Allen WR. Equine Vet J. 32: 83-85, 2000.

Discussion from the paper:

"This experiment demonstrated convincingly that none of the 4 progestagens, medroxyprogesterone acetate, hydroxyprogesterone hexanoate, norgestomet and megesterol acetate, when administered to pregnant mares at the dose rates recommended by their respective manufacturers, can maintain pregnancy between Days 18 and 30 after ovulation in the absence of endogenous progesterone secreted by a viable corpus luteum. It is therefore reasonable to conclude that the same progestagens will be unlikely to be any more efficacious if administered later in pregnancy, especially after mid-gestation when the placenta becomes the sole source of endogenous progesterone in equine pregnancy (Holtan et al. 1975; Shideler et al. 1982; Knowles et al. 1994). The progestagens used in the experiment are commonly administered to horses by equine veterinary clinicians, either in an attempt to prevent abortion in pregnant mares or to suppress sexual behaviour in performance horses. The dose rates and routes and frequency of administration were those recommended by the manufacturers and it is unfortunately not possible to distinguish whether the abortions occurred as a result of insufficient administration of active compound or, more probably, failure of the progestagen to bind adequately to endometrial progesterone receptors in the treated animals. Altrenogest was used as a positive control in the experiment since we had shown previously that this particular progestogen is able to maintain pregnancy in ovariectomised mares (McKinnon et al. 1993). This earlier trial also showed that

hydroxyprogesterone caproate is unable to maintain pregnancy in ovariectomised mares which may have been the reason why the same synthetic molecule was repackaged under the new name of hydroxyprogesterone hexanoate. This renaming process was not known to us when the present experiment began. Despite the lack of efficacy data to support the administration of any of these 4 synthetic progestagens to mares to prevent abortion, they are used widely in equine stud veterinary practice for this purpose. The unequivocal results of this study demonstrate convincingly that the practice should cease forthwith."

The recognition of the inability of most progestogens to suppress oestrous and maintain pregnancy was an important step in our understanding of not to blindly follow manufactures recommendations especially when not based on scientific evidence. It also led to the formulation of a long-acting form of altrenogest which could be administered every 7 days to maintain pregnancy in the face of lack of luteal support.

A few years before we opened the hospital, whilst I was still working at Colorado State University, I was asked by Thoroughbred Breeders Victoria to speak to their members at Mooney Valley racecourse one evening. It was a very well attended meeting and the ideas we discussed were how the use of ultrasonography should be expanded outside that of just pregnancy diagnosis. What is routine now was not then. During that meeting I came to understand that 60 was a large book of mares and that it was normal to breed mares 2-4 times per cycle. The use of hCG was not routine which was why mares were bred so often and the lack of ovarian ultrasonography meant mares were not being bred at the correct time and ovulations were being poorly detected and managed.

Ultimately, this helped cement my philosophy on breeding management which involved always examining ovarian structures with ultrasonography and to always use ovulating drugs. That philosophy led into researching the use of a GnRH agonist (deslorelin) with Tim Trigg from Peptide technology in Sydney. When deslorelin became available through the commercial production of the implant Ovuplant®, the Thoroughbred industry became aware of the advantages of ovulation induction (and ovulation control). The surprising thing was hCG was already available but underutilised. This information led to a large increase in mare numbers bred to stallions. At Blue Gum Farm we were the first farm ever to breed over 200 mares to a stallion (Encosta De Lago) in a season. This was possible for two reasons. Firstly, we could separate his breedings with hCG and Ovuplant® and secondly, he was extremely fertile which meant we didn't have many return breedings.

Our first paper examining the use of deslorelin for hastening ovulation in mares is below. This research was used to determine the dose rate in the Ovuplant® implant of deslorelin.

Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. McKinnon AO, Nobelius AM, Tarrida Del Marmol Fiqueroa S, Skidmore J, Vasey JR, Trigg TE: Equine Vet J. 25: 321-323, 1993.

Study outline:

The experimental animals were part of a herd of 95 mares in good body condition, kept on pasture and given lucerne hay and grain supplement as necessary. They were studied between October 1990 and March 1991 at the Goulburn Valley Equine Hospital. Mares were teased daily, and ultrasonography was used to monitor follicular size and to determine ovulation and number of ovulations per cycle. Mares were examined every 3 days during dioestrus, daily during early oestrus and twice daily (06:30 and 18:30 h) when a follicle >30 mm in diameter was detected. Ovulation was diagnosed by absence of a previously identified

pre-ovulatory follicle (>30 mm diameter) and visualisation of the characteristic echogenic structure formed by the collapse of the follicular walls. All cycles were induced by luteolysis effected by administration of PGF2, on Days 7-10 after ovulation from mares that had follicles <20 mm in diameter. Mares (N=77) with follicles >30 mm in diameter and showing characteristic signs of behavioural or physiological (endometrial folds, softening cervix) oestrus were randomised according to predetermined draw into 5 approximately equal treatment groups between 1 November 1990 and 15 January 1991. Mares were given subcutaneous (sc) implants of 1.3 mg deslorelin (Peptide Technology Ltd, DeeWhy, NSW 2099, Australia) (25 cycles; Group A), 1.6 mg deslorelin (25 cycles; Group B) or 2.2 mg deslorelin (28 cycles; Group C). Mares in Group D were given 3000 iu hCG iv (26 cycles) and those in Group E were given empty sc implants (26 cycles). Implants were placed sc on the neck by means of a 4-inch 10- gauge needle with obturator.

Results:
The number of mares showing behavioural oestrus before assignment (73/119, 61%) was not different (P<0.05) between treatments (14/25, 17/23, 11/23, 17/23, 14/25) for Groups A-E respectively. Follicle size at assignment was not different, but that at ovulation was significantly larger in control mares (Group E) compared with mares in Groups B, C and D (P<0.0l) (Table I). Time to first ovulation was shorter (P<0.00l) for treated mares in Groups B, C and D compared with controls (Group E) (Table 1). Time to second ovulation was shorter (P<0.02) for treated mares in Group D than in controls (Table 1). Number of mares ovulating 2 follicles per cycle was not different between groups. Number of mares ovulating the first (or only) follicle within 48 h of treatment was greater in Groups A-D (19/25, 15/23, 22/23, 23/23 respectively) than in controls (4/25) (P<0.00l), and greater in Groups C and D than in Groups B (P<0.0l) and A (P<0.05) (Table I).

The best responses were deslorelin was 2.2 mg deslorelin and hCG (3000 IU).

The current recommendation of 1.25 mg Deslorelin in a slow-release oil base supported by another study from our hospital by Sean Finan:

Comparative efficacy of Biorelease Deslorelin injection for induction of ovulation in oestrus mares: a field study: SA Finan, EL Lamkin AO McKinnon Aust Vet J 2016: 94 339-340

Objective:
To investigate the comparative efficacy of BioRelease Deslorelin (BRD) and Ovuplant for induction of ovulation in cyclic mares in Australia.

Methods:
Ovarian follicular activity of 60 mares for a total of 95 cycles was monitored by ultrasonography until they developed a follicle≥30 mm and a uterine oedema pattern of 3. Mares were then randomly allocated to one of three treatment groups:(1) treatment with 1.25 mg BRD, (2) a single Ovuplant pellet or (3) 1 mL compound sodium lactate control. Follicular activity was monitored with ultrasonography every 12 h until ovulation was detected or for at least 5 days post treatment. The injection site on each mare was monitored for reaction for a minimum of 5 days post treatment.

Results
There was no difference in the percentage of mares ovulating within 48 h when treated with BRD (93.75%) compared with Ovuplant (87.09%). Treatment with both ovulating agents

significantly decreased the time to ovulation compared with control mares (P < 0.00005). More mares had injection site reactions with Ovuplant (64.5%) treatment compared with BRD (15.6%) or control mares (0%) (P < 0.00005).

Conclusions
Treatment of mares with 1.25 mg BRD when there is a follicle≥30 mm and uterine oedema pattern of 3 is as effective as treatment with Ovuplant

We also used Deslorelin to research the potential to control ovulation through difficult times such as the transition period:

Repeated use of a GnRH analogue deslorelin (Ovuplant) for hastening ovulation in the transitional mare. McKinnon AO, Vasey JR, Lescun TB Trigg TE: Equine Vet J. 29: 153-155, 1997.

Experiment 1 In 1994, 21 early transitional mares (multiple follicles< 15 mm) were randomly assigned to either a control (n = 10) or treated (n = 11) group on the same day (September 29). Treated mares were implanted every other day with one implant of deslorelin until either ovulation had occurred, or 6 implants had been administered. Experiment 2 In 1995, 20 late transitional mares were randomly assigned to either control or treatment groups after they were identified with a >30 mm follicle, were in oestrus and had endometrial folds demonstrated using ultrasonography. Treated mares were implanted every other day as above.

In experiment 1, utilising mares in the early transitional period, we were able to demonstrate an apparent effect of the implants in hastening ovulation (ovulation in <10 days) in treated mares (6/11) vs. controls (0/10). However, the mean ovulation date was not different between groups and reflected the effect of 5 mares that did not ovulate after treatment. Those 5 mares may have undergone some form of 'down regulation' in GnRH receptors (Irvine and Alexander 1993) as their follicular size decreased quite clearly in comparison to control mares. This experiment highlighted the difficultly in selecting mares randomly for treatment during the early part of the transitional period. The responses were similar to previous reports with GnRH therapies early in the transitional period. In experiment 2, we were able to demonstrate the ability of the implants to hasten ovulation late in the transitional period. The number of mares with a >30 mm follicle ovulating within 3 days of treatment (8/10) suggests that the procedure would be useful in predicting ovulation in transitional mares.

Failure of mares to ovulate during transition into the breeding season is a major source of frustration to clients, breeding farm managers and veterinarians. This is compounded by the number of mares that now are bred after transport from their breeding farm of residence and back again the same day.

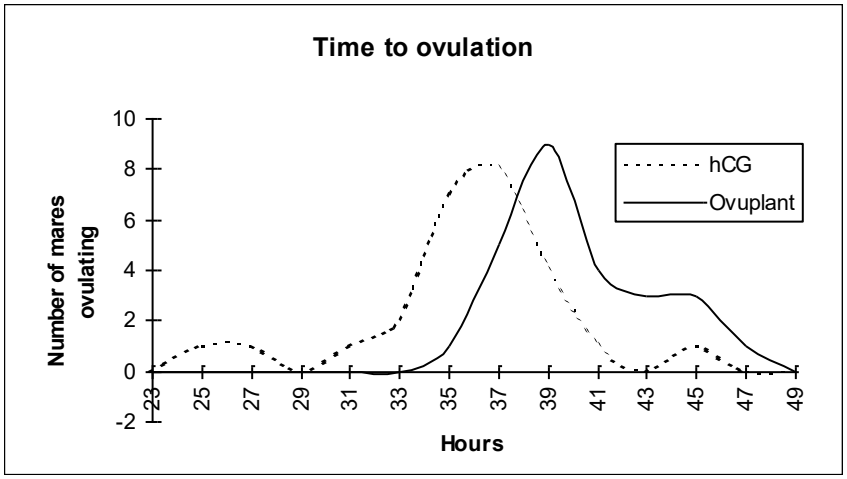
The average number of implants needed was 2.1 and this may result in savings in decreased veterinary costs, decreased boarding charges, decreased teasing and handling and better usage of the stallion. Each year, one mare in the treated group became anoestrus or transitional again and demonstrated, therefore, that ovulation may have been forced upon them prematurely. From this perspective it would appear prudent to wait until a transitional mare has demonstrated maturity of the reproductive axis by maintaining large (>30 mm) follicles for a few days before initiating induction of ovulation. The increased interovulatory

interval detected in treated mares in experiment 2 may have been associated with premature entry into the cyclic season or may suggest a luteotropic action of the implants. No progesterone measurements were taken and would be a logical area for further investigation. In summary, it was demonstrated that accurate, timed ovulation was achieved during the late transition using multiple implants of Ovuplant®. This regime is simple, may be cost effective and is expected to be useful for practising veterinarians.

Despite all mares receiving ovulation inducing agents every season we always have some mares during the transitional period that fail to have their first ovulation withing 48 hours of breeding. When this occurs, an important decision must be made. Do we send the mare for rebreeding, or do we wait and see what will happen? My philosophy is that if the follicle has grown since we last examined it then we administer another Deslorelin injection (or perhaps the first one if hCG was used initially) and watch. If the follicle has not grown it is unlikely that it will ovulate no matter what you administer so we wait and observe. We rarely rebreed due to the excessive costs of transport. If the mare resides on the same farm as the stallion, then she is rebred if he is available.

Other studies with deslorelin clarified the interval from treatment to ovulation for hCG and Deslorelin. The study below showed that Deslorelin results in mares ovulating at 41+/- 3 hours and hCG resulted in mares ovulating at 36 +/- 4 hours. This was the first time the interval from treatment to ovulation had been studied with Deslorelin, however, the interval from treatment to ovulation with hCG was published by Jim Voss back in the 1980's (although they used rectal palpation not ultrasonography). For frozen breeds it means that mares can have the ovulation induction drug deslorelin at 7 pm and then be examined 6-hourly until the day of breeding when she should ovulate around 12 noon (+/- 3 hours).

Effect of a GnRH analogue (Ovuplant), hCG and dexamethasone on time to ovulation in cycling mares. McKinnon AO, Perriam WJ, Lescun TB, Walker J, Vasey JR Trigg TE: World Equine Veterinary Review 2: 16-18, 1997.



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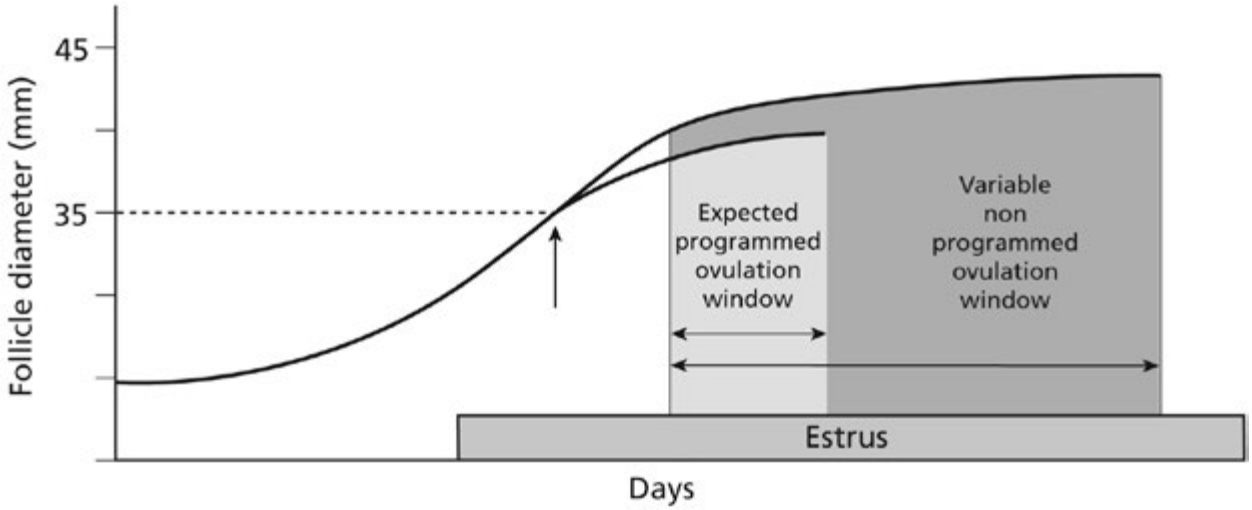
Ovulation induction is a fundamental part of equine reproduction. On Thoroughbred farms it is one of the ways that farm personal can make their judgements on your abilities. When mares continue to fail to respond well to ovulation induction it can be a major reason for clients to look elsewhere (see further discussion below).

The graph below attempts to outline how early in oestrous mares can be encouraged to ovulate at a certain follicle size before the follicle grows to big and we lose control over its ability to respond. Similarly, when the follicle is too small it will not be mature enough to respond.



The size a follicle can be when a mare responds to ovulation induction will vary according to many factors. Induction of ovulation will not occur in a precise manner when agents are administered with a follicle not mature enough to respond. It is expected that ovulation will occur in 24-48 hours after an ovulation-inducing agent is administered. When ovulation occurs earlier than 24 hours it is likely the follicle was already destined to ovulate before the agent was administered. When a follicle ovulates more than 48 hours after administration, it is likely that the agent was not administered when a suitably mature follicle was present. Typically, a follicle will respond to an ovulating agent when the mare has been in oestrus for 2-3 days and the follicle has reached a minimum critical size that is characteristic for the type or breed of mare. The presence and absence of uterine folds is also an important parameter to monitor to help develop an understanding that the mares is actually in oestrous or not.

Recognition of oestrus is paramount and all too often mares are programmed to ovulate without reference to teasing patterns and a softening cervix as more and more reliance is placed on ultrasonography. This happens more frequently on smaller farms that may not have access to a teaser stallion and on farms that rely heavily on artificial insemination. Failure to tease properly may result in mares presented for breeding that are not in oestrus and some mares may even have a corpus luteum present.

The size of a follicle that can respond to an ovulating agent will vary according to the time of the year and the breed. Typically, mares will need two or three follicular waves to develop and subside before the first ovulation from the transitional period to the ovulatory season. The response to ovulating agents in the early transition follicular waves is very poor. In the regular ovulatory season (once the first ovulation has occurred) most Thoroughbred and Standardbred mares could be expected to have a predictable response to programming when follicles reach 35-40 mm and most Warmbloods 40-45 mm. However, many smaller breeds (ponies and some Quarter Horses) will have already ovulated before the follicles have reached that size and will have much smaller follicles that are capable of a predictable

response to programming. Draft horses and Friesians are at the other extreme and will usually not respond to an ovulating agent until the dominant follicle is greater than 50 mm in diameter. When used with care and attention to detail most clinicians should expect a response rate (defined as ovulating between 24-48 hours after administration) of at least 95%. The ability to achieve this will vary with the skill of the person programming, the frequency of examination prior to programming, the use of $PGF_{2\alpha}$ to initiate oestrus, the time of year, and the agents used. On larger breeding farms where clients are very discerning of veterinary abilities and similar to the ability of predicting the response to prostaglandin administration, the response of programming mares to ovulate is an easy target for farm personnel to identify and monitor and to some extent judge the veterinarian.

The most predicable responses are when mares with follicles ≤ 25 mm are given $PGF_{2\alpha}$ and monitored frequently enough to attain follicle sizes listed in Table 1. The optimal follicle size at which to administer an ovulating agent varies according to the breed and even number of potential pre-ovulatory follicles. Mares in oestrus for several days with large mature follicles may spontaneously ovulate sooner than expected based solely on response to an ovulating agent. Table 1 lists mare lists suggested follicle size by breed for either 1) ideal size to program, 2) unlikely to respond or 3) follicles that may already be destined to ovulate imminently.

Breed	Follicle size below which mares are unlikely to respond	Ideal follicle size to respond	Mature follicle size and may ovulate spontaneously or not respond predictably
Thoroughbred and Standardbred	< 35 mm	35 - 45 mm	> 45 mm
Quarterhorse, Riding ponies and Arabians	< 30 mm	30 – 40 mm	> 40 mm
Miniatures	< 30 mm	30-35 mm	> 35 mm
Draught horse	< 40 mm	40-55 mm	> 55 mm
Friesian	< 45 mm	45-55 mm	> 60 mm

Table 1: Follicle sizes recommended for programming mares to ovulate within a 24-48 time interval according to breed and during the cycling season. The follicle sizes are guidelines only as mares do not always respond exactly as expected.

A specific managerial problem occurs in mares that are given $PGF_{2\alpha}$ is when large follicles (> 35 mm) are present in mid diestrus. These follicles may ovulate quite quickly (< 48 hours), often before attaining characteristics that allow accurate programming. In 1999 we identified a technique of using multiple low doses (1/8-1/6 typical dose given 4 times per day until uterine oedema is visible) of $PGF_{2\alpha}$. The idea was to mimic the more natural endogenous prostaglandin release seen in mares in late diestrus and avoid the large and rapid drop in progesterone associated with luteal regression and exogenous $PGF_{2\alpha}$.This technique allows a more natural return to oestrus and commonly allows programming of follicles once oestrus is demonstrated and uterine oedema identified, but before the follicle development has progressed so rapidly that ovulation is imminent or occurs unexpectedly. More recently work from Louisiana State University (Amy Coffman and Carlos Pinto 2014) has identified that multiple $PGF_{2\alpha}$ shots (twice daily from the day of ovulation for 4 days) will prevent the formation of the CL and mares will recycle and develop follicles in a more controlled manner. In this situation seeing that there is no rapid drop-in progesterone from PG effects on the CL there are no rapid follicular responses. It is a very good way to handle the problem of large

follicles ovulating quickly after $PGF_{2\alpha}$. Another specific problem is the presence of more than one large follicle which can make it difficult to predict if one, two or three follicles will ovulate, or which is the dominant follicle and which is destined to ovulate first. It is usually more accurate to program using the largest follicle unless the rapid growth of another smaller follicle has been detected. Use of ovulating agents increases the number of twins detected, possibly by reducing the time interval between ovulations rather than 'forcing' more follicles to ovulate.

Occasionally, a mare does not ovulate in response to an agent and subsequently develops and ovulates another follicle 4-8 days later after the original dominant follicle regresses. It is likely that these mares were programmed too early to respond initially. It is difficult to detect these mares clinically before the problem occurs. Teasing patterns are often useful in this situation and mares that normally tease well should be programmed with caution if the teasing pattern does not match the follicular size.

Factors that may affect results:

Time of the year
The most consistent response is when mares are programmed to ovulate during the natural cycling season. Restrictions from breed registries that encourage foals to be born from breeding of mares not in the normal physiologic cycling season means that many programming events occur during transitional oestrus. Once mares have had their first ovulation of the year to enter the cycling season their response to programming agents will be more predictable.

Ovulatory agent
There are considerable differences in ability of ovulatory agents to achieve the desired effect that are related to dose and specific ovulatory agent. In general, deslorelin is more reliable than hCG and smaller follicles will respond to deslorelin better than hCG. In addition, mares with a late transitional follicle are more likely to respond when deslorelin is administered rather than hCG.

Age of the mare
Older mares are less likely to respond accurately to any ovulatory agent and both hCG and deslorelin have been demonstrated not as efficacious in older mares.

Size and maturity of the follicle
Size of the follicle does not always equate to maturity of which a specific example is the large mid diestrus follicle that will not respond well to ovulating agents while progesterone levels are elevated. Often mares are presented with large follicles and minimal uterine oedema visible with ultrasonography. It is useful with these mares to examine the vagina for mucosal colour and degree of softness of the cervix. However, teasing behaviour is critical to making accurate decisions with these mares.

Occasionally, mares do not ovulate despite ultrasonographic recognition of uterine oedema, a soft cervix and a typical sized follicle that could be expected to respond. Often in this situation, the follicle will continue to grow and will respond to administration of the same or a different ovulatory agent 48 hours or more after the initial dose.

Mares presenting with small follicles that have good uterine oedema and have been demonstrating good teasing behaviour are also difficult to program. Prior history of the

mares' cycle may be useful in determining if the mare is likely to ovulate a small follicle and thus respond to an ovulatory agent.

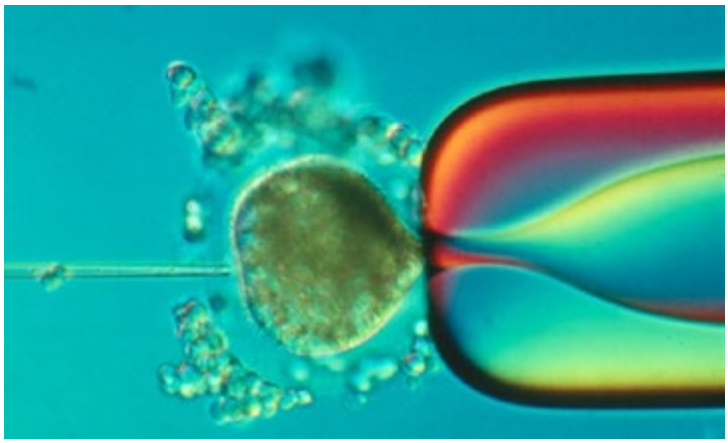
On occasion, mares in oestrus are examined that already have large dominant follicles. It may be difficult to predict the ovulation response of these mares to either hCG or deslorelin. These mares may ovulate spontaneously within a few hours or may ovulate in the normal interval in response to an ovulating agent. In these instances, we would normally program the mare as it is surprising how often they will ovulate within the predicted 'ovulatory window'. In any case, more examinations after programming are necessary if frozen semen is being used to ensure mares are bred within 6 hours before and 6 hours after ovulation.

Programming time.
We visit most breeding farms at the same or similar time every day. As deslorelin is most likely to cause mares to ovulate within a 41 ± 3 hour window, if mares are programmed when bred in the evening (7pm) and then examined two days later in the morning (7am) only 36 hours has elapsed since programming and many mares will have not ovulated at that time. Enthusiastic farm managers often need to be restrained from wanting to re-breed the mare that has not ovulated at 36 hours. The wishes of stud managers to re-breed every 48 hours are annoying when the stallion's fertility is normal, especially if compounded by a lack of understanding due to communication breakdown. Fertility of normal stallions can be expected to be maintained even if the mare does not ovulate until 3 days after last breeding. It is not uncommon for stallions to achieve pregnancies when the interval from breeding to ovulation is as long as 5 or more days.

Multiple follicles
It is not always possible to predict which one or all of 2 or 3 large follicles will ovulate when mares are programmed. On occasion, a mare in oestrus with good uterine folds will have a 30-mm follicle on one ovary and two 35- to 40-mm follicles on the other ovary, and yet the smaller follicle will ovulate within 48 hours of programming and the others will not ovulate and gradually recede. More often than not, this occurs in cases where we do not have the ability to examine the mare frequently enough to identify the accurate growth pattern of follicles. Another similarly frustrating scenario is when mares are programmed and bred and then do not ovulate the intended follicle, which then gradually decreases in size, and despite the mare staying in oestrus, the next follicle may take a week to grow to a size that the mare can be re-bred. Fortunately, those problems do not happen very often as most mares conform to the expected patterns of ovulation.

Assisted reproductive techniques (ART)

We were fortunate enough to collaborate with Alan Trounson from Monash University back in our early days. His knowledge, enthusiasm, and the team around him were all able to help achieve the first (and second) ICSI (intracytoplasmic Sperm Injection) pregnancy from live mares (Ed Squires produced one from an abattoir egg a year earlier). The foal naming competition that Monash University organised was quite fun. We named the first foal ART (for the above acronym). We wanted the second name to be SMART for the same method as ART, but the only person who thought of that was a work experience student working with us that week. However, our staff chose (from hundreds of entries from all around the world) the name MUSIC (method using sperm injected cell). Which we all thought was quite clever.



Pregnancies produced from fertile and infertile stallions by intracytoplasmic sperm injection (ICSI) of single frozen-thawed spermatozoa into in-vivo matured oocytes. A. O. McKinnon, O. Lacham-Kaplan, and A. O. Trounson: J.Reprod.Fert.Suppl. 56:513-517, 2000.

Our ICSI programme revolved initially on the use of in vivo matured oocytes which meant that we could aspirate only once per cycle and often we missed the opportunity as we waited right up to when the mare was ready to ovulate. It meant we didn't culture either the oocyte or the resultant embryo which we put back in surgically.

The first ICSI foal was born in 1997, our two which were the second and third ever were born in 1998.

Nowadays ovum pickup (OPU) is used vaginally to typically provide at least 5 immature oocytes for in vitro maturation (IVM) from each aspiration attempt. Cesare Galli in Italy, I think, was first to develop a laboratory devoted to ICSI and throughout Europe people perform OPU

and send the oocytes to his laboratory, and he cultures the oocytes, performs ICSI and freezes those embryos that develop into blastocysts. It is a mature (no pun intended:-) programme with good, consistent results. It should also be mentioned here that my good friend Lee Morris has also developed an ICSI lab in NZ and has enjoyed good success. Labs devoted to ICSI are also active in the USA and South America (Brazil and Argentina). For a variety of reasons, we have not been actively pursuing OPU and IVM, ICSI pregnancies. However, client pressure is such that we will re-visit our OPU/IVM/ICSI programme this coming season.

Throughout those early times with ICSI we continued to work with oocyte transfer (the transfer of oocytes that were matured in vivo and placed into the oviduct of a recipient mare that was bred). We first pioneered the technique in Colorado and continued after we established the hospital here:

Heterogenous and xenogenous fertilization of matured equine oocytes. McKinnon AO, Carnevale EM, Squires EL, Voss JL, Seidel GE Jr: J Eq Vet Sci 8:143-147, 1988.

The issue with that technique was that we had to remove any oocytes that could potentially be mature in the recipient and result in the establishment of a pregnancy from the correct semen but the incorrect mare. It only happened to us one time and thankfully the client was warned before and during the transfer process and thankfully she adored the foal (WB X STB) regardless.

During that time at the hospital, we also had some fun performing techniques that others had already developed. One was the transfer of an endangered Donkey embryo into a mare. Twink Allen was very helpful here as he had produced multiple donkey-in-horse foals. Our efforts resulted in the production of Poitou donkey foal. Apparently, there were only 300 Poitou donkeys on the world, mostly in France where they were developed as larger more powerful versions of other donkeys exclusively for farm work- also referred to as Baudet du Poitou. Once again, the foal naming competition was inspiring. This one resulted in the foal being named R2D2 in deference to Poitou.

Throughout the next decade, we continued to publish on reproductive techniques, but it wasn't until one day we accidentally discovered something that changed our approach to breeding management. It wasn't a novel idea it was just an extrapolation. I was asked by Henry Plumtree at Darley Victoria to provide him with a method of ensuring mares coming to the farm were free from reproductive pathogens such as *Klebsiella* and *Pseudomonas*. An internet search revealed the use of Chromagar for UTI (urinary tract infections) of humans. Importantly both bacteria are common pathogens of human UTI's. The discovery of the potential use of Chromagar for our hospital was quite enlightening.

It resulted in the immediate ability to diagnose reproductive pathogens based on their ability to change bacterial colonies into different colours (based on biochemical reactions). It saved an important time-consuming step of sending individual suspicious colonies for ID. It proved to be a real advantage in time so much so that when an empty pregnant mare was cultured before breeding, we could give a definitive answer that she was negative to *Klebsiella* and *Pseudomonas* well before the lab could let us know. Not to mention that in house diagnostics could be done accurately over the weekend. It was big profit driver of the practice and also saved time. One of the publications that we used to publicise the techniques is appended below.

It has been fun to relive some of our adventures. I was surprised how much I had forgotten. "May you live in interesting times" has been a truism for all of us. I was trained in palpation and percussion and physical examination had the excitement of being introduced to ultrasonography back when none of really knew what we were going to find. A textbook I co-edited a long time ago (Rantanen NW, McKinnon AO (eds) Equine Diagnostic Ultrasonography. Williams and Wilkins, 1998) was where I first recorded that I would not want to practice again if ultrasonography was not available to us. It is just so useful. Also revolutionary to me was digital x-rays and instruments such as the Ligasure®. We always used to tell the interns and now tell both them and the students that 'if you don't look you won't find'.

Use of Chromogenic Agar to rapidly identify specific reproductive tract bacteria
AO McKinnon and DP Beehan

The traditional approach to the detection of bacteria associated with the reproductive tract has been to culture either the mare or stallion and then inoculate one or more general purpose media such as Horse Blood Agar (HBA) and MacConkey agar, followed by aerobic incubation at 37°C for 24-48h. Additional culturing techniques are used for anaerobic and microaerophilic pathogens.[1,2,3] Identification of the suspect pathogens has been based on colony size, morphology, pigmentation, haemolytic patterns, and Gram-stain which rarely permit more than a presumptive identification. Biochemical and/or serological tests are then used for definitive diagnosis. This approach frequently necessitates the testing of commensal bacteria that resemble pathogens which is often more expensive and labour intensive.[4] This is especially true in a veterinary practice wishing accurate and expedient bacterial diagnoses.

Since 1990, a wide range of chromogenic culture media has been made commercially available providing useful tools for diagnostic clinical microbiology. These chromogenic substrates release specific coloured dyes when hydrolysed by enzymes of pathogenic microorganisms, thus resulting in readily identifiable coloured colonies.[5] The principle of chromogenic agar is the use of substrates incorporated into the agar that reveal genus- or species-specific enzyme activities of microorganisms.[6] By the inclusion of chromogenic enzyme substrates targeting microbial enzymes, these media are able to target certain pathogens with high specificity. The inclusion of multiple chromogenic substrates into culture media facilitates the differentiation of polymicrobial cultures, thus allowing for diagnosis of clitoral sinus cultures that if taken correctly should be highly contaminated with multiple different colonies. Selective chromogenic media was first used in 1976 for the direct identification of *Escherichia coli* in the primary culture of urine.[7] Since then, a wide range of chromogenic culture media has been developed for the use in diagnostic clinical microbiology. For example, in human medicine a chrome agar has been developed that allows the rapid and highly specific identification of methicillin-resistant *Staphylococcus aureus* (MRSA). A positive result can now be available as early as 18 to 24h. This early diagnosis allows improvement of infection control measures and reduces the cost associated with MRSA in human and veterinary hospitals.[8,9] Other examples of interest are isolation of Salmonella sp which are typically hard to isolate and yet thought to be commonly associated with equine diarrhea and for enhanced discrimination of yeasts such as Candida sp. which are slow growing.[10,11,4]

The most common microbiological pathogens of the reproductive tract disease in the horse are *Streptococcus* sp*, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Specific bacterial venereal pathogens of horses are *K. pneumoniae, P. aeruginosa* and *Taylorella equigenitalis* (See chapter Uterine and Clitoral cultures Ricketts). Some of the previously mentioned bacteria (*Streptococcus* sp*, E. coli, K. pneumoniae, and P. aeruginosa*) are also common pathogens of the human urinary tract. There is now a number of commercially prepared chromogenic agars have been developed for the accurate and rapid identification of pathogens of the human urinary tract. Urine cultures in human hospitals contribute greatly to the daily workload of a microbiological laboratory.[12] Hence, there has been much research devoted to the development of highly specific chromogenic agars that have the same ability to detect urine pathogens as the combination of traditional blood agar and MacConkey agars and reduce laboratory workloads. Brilliance Urinary Tract Infection (UTI) Agar[a] is one such commercially prepared media and is the chromogenic media used at the Goulburn Valley Equine Hospital (GVEH) to detect potential reproductive tract pathogens.

The potential of chromogenic agar for use in our veterinary practice was first realised after a 'Google' search was performed by one of us (AOM) in 2006 to find a practical 'in house' rapid and accurate laboratory technique to diagnose *P. aeruginosa* and *K. pneumoniae*.

The use of chromogenic agar had also been reported to allow identification of other less common pathogens of the human urinary tract including *Streptococcus* sp, *Staphylococcus* sp, *Enterobacter aerogenes, Enterococcus faecalis, Citrobacter* sp, *Proteus* sp and *Candida* sp. Similarly these pathogens are also isolated from the reproductive tract of horses and recently we reported on the use of chromogenic agar in the rapid identification of these pathogens.[13,14]

Detection of potential equine reproductive tract pathogens

A total of 1374 endometrial swabs and 1158 clitoral swabs were collected from Thoroughbred mares on farms serviced by practice veterinarians at the GVEH and from other regional veterinary practices.[13] Endometrial samples were taken using double-guarded swabs to minimise vaginal or perineal contamination. The swabs were transported to the laboratory in transport media. On arrival the name of the mare, the breeding farm, the stallion farm, the date and time were recorded, and the swabs were then streaked on chromogenic agar, HBA and MacConkey split agar plates[a]. Plates were incubated aerobically at 37ºC, and any bacterial growth was registered at 24h and 48h. Examination at 24h was required to identify heavy mixed cultures that may have required sub-culturing so individual colonies could then be identified at 48h. Also, at 24h presumptive identification of pure uterine cultures permitted antimicrobial sensitivity testing to begin, with results often available at 48h. All plates were then re-examined at 48h for a final evaluation. A negative result was only confirmed at 48h. Any further sub-culturing or antimicrobial sensitivity testing was performed at this time. When all microorganism identification was complete and antimicrobial sensitivities were analysed, a complete report was made available to both the stallion farm and brood mare farm. In general, for reasons of discretion, a positive result was only made available to the broodmare farm.

Using chromogenic agar microorganisms are generally identified by their colour and Gram stain appearance, however, to confirm the presence of *P. aeruginosa* and *K. pneumonia* biochemical testing is always performed. The 3 biochemical test kits used at the GVEH practice laboratory are the RapID™ NF Plus, the RapID™ ONE, and the RapID™ SS/U identification tests[b]. These tests are designed to be easy to set-up, easy to interpret, accurate and allow same day results , as these tests only require 2-4 hour incubation periods.[15,16] The RapID™ NF Plus test was used for the diagnosis of the medically important glucose non-fermenting (catalase positive and oxidase variable) gram-negative rods e.g. *P. aeruginosa*, the RapID™ ONE test for the identification medically important *Enterobacteriaceae* and other selected, oxidase negative, Gram negative bacilli e.g. *K. pneumoniae*, and the RapID™ SS/U test was for general identification of microorganisms commonly isolated from the reproductive tract when it was not clear that either of the before mentioned tests were more appropriate (Table 2).

Pseudomonas sp
Klebsiella sp
Escherichia coli
Staphylococcus sp
Enterobacter sp
Citrobacter sp
Enterococcus sp
Proteus sp
Candida albicans

Table 2. Common reproductive tract bacteria identified by the RapID™ SS/U test

The colour and colony appearance guide provided in Table 3 was used to identify the bacteria isolated. The identification of microorganisms is made at both 24h and 48 h. The similar appearance of *Staphylococcus* sp colonies and those of *P. aeruginosa* required an oxidase test to be performed. Oxidase Test Strips[a] were used to perform this test (Figure 1, oxidase strip).

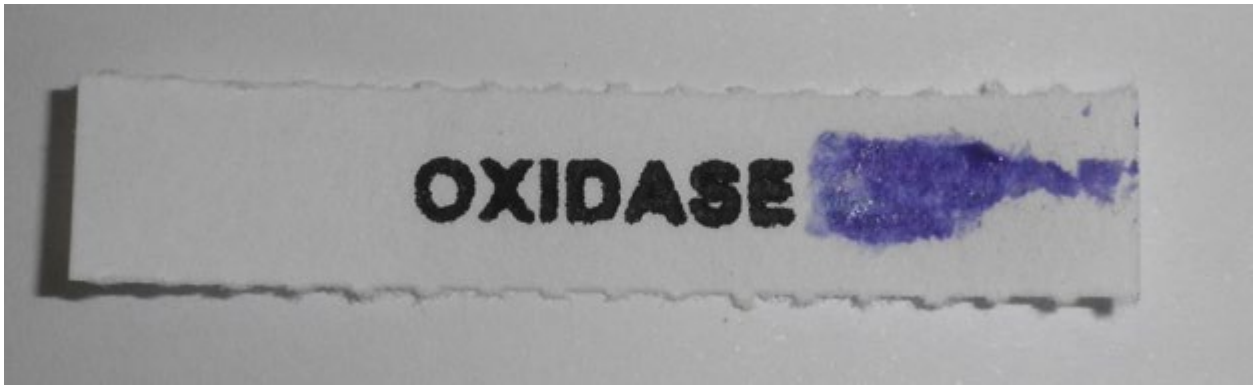


Figure 1. A positive Oxidase test result. A purple colour within 30 seconds is a positive result. Oxidase tests are performed by lightly rubbing the suspect colony on the test strip. Typically the colour change occurs in < 10 seconds.

Species	Isolation % at GVEH	Appearance of colonies on Chrome UTI Agar
Pseudomonas aeruginosa	1.17	Variable. Usually cream/pale green or tan/brownish. Oxidase positive. Good but slow growth. 2.5-4mm.
Klebsiella pneumoniae	1.21	Good growth, large purple/deep blue/metallic navy colonies. 2-4mm.
Escherichia coli	33.58	Good growth, darkish pink colonies. 2-3mm.
Streptococcus sp	27.76	Good growth, light blue colonies. 0.5-1mm.
Staphylococcus sp	18.02	Good growth, white colonies. Oxidase negative, always catalase positive. 2-3mm.
Enterobacter aerogenes	3.72	Dark blue/ navy colonies. Similar but subjectively smaller than Klebsiella sp. 2-4mm.
Citrobacter sp	1.41	Dark blue/ navy colonies. Usually smaller than *Klebsiella* sp and *Enterobacter* sp.
Enterococcus sp	5.41	Good Growth, light blue colonies. 0.5-1mm.
Proteus sp	7.60	Good growth, brown colonies. Swarming
Candida sp	0.12	Good but slow growth. Typically blue/white colonies.

Table 3. Colony Identification Guide and percentage of isolates from reproductive cultures at the GVEH

The diagnosis of *P. aeruginosa* is suggested based on colony appearance (Figure 2a-b), a positive oxidase test, identification by gram-stain of medium sized gram-negative rods and then after sub-culturing to ensure pure colonies, a RapID™ NF Plus identification test is performed as the final confirmation. It was noted that the emergence of *P. aeruginosa* colonies was somewhat slower than other tested microorganisms, and therefore a 48h examination of incubated plates, despite a previous presumptive diagnosis, was required in all cases. Also noted was the unique effect that pyocyanin and pyoverdin (Figure 2c), a blue-green pigment typically produced by *P. aeruginosa*, had on sensitivity plates. Another interesting aspect of *P. aeruginosa* is fluorescence in dark conditions when exposed to an ultraviolet light (e.g., Wood's UV light) (Figure 2d). This affect can also be seen on the vulvar labia of affected mares (Figure 2e).

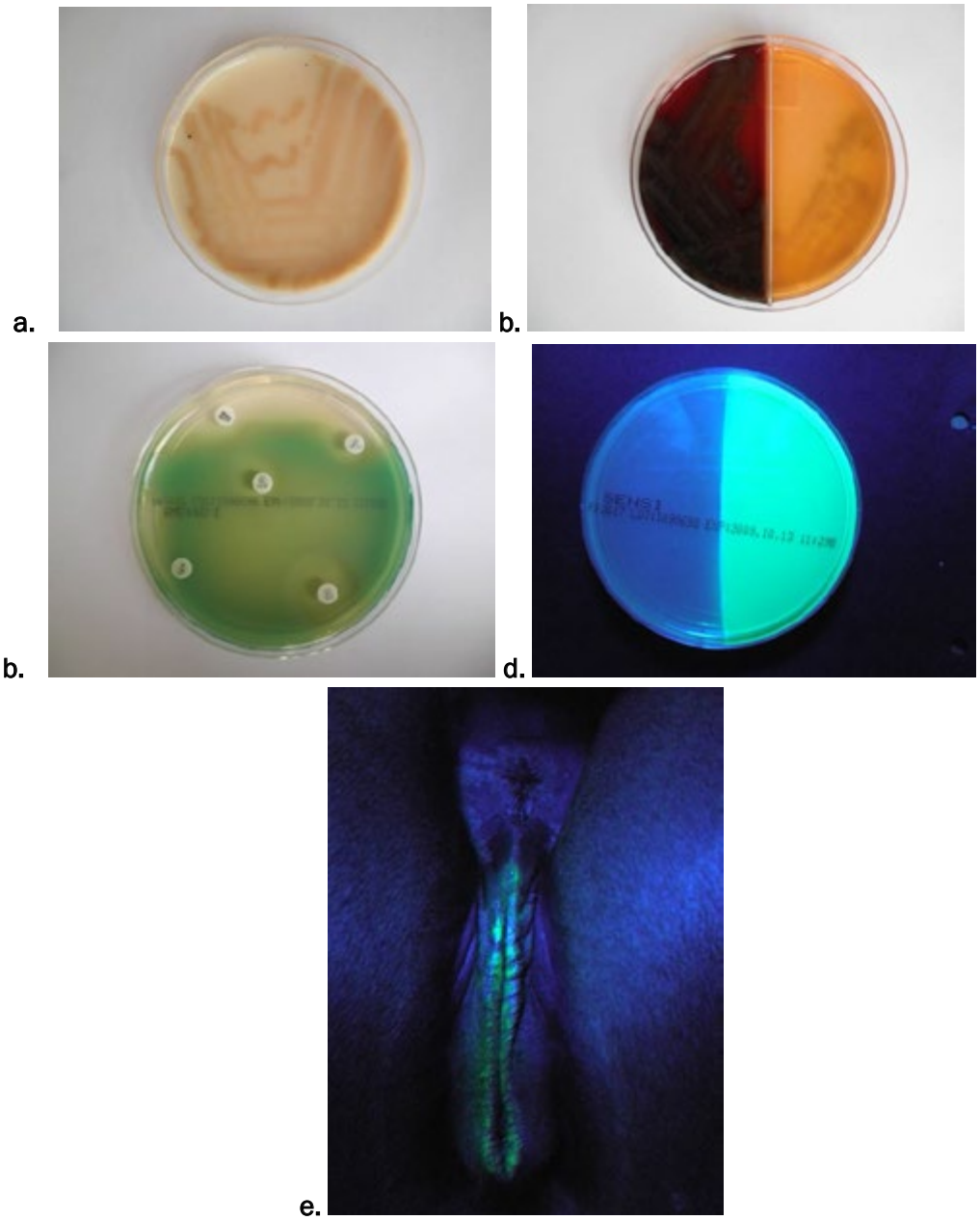


Figure 2. a. *Pseudomonas aeruginosa*. The cream to light brown coloured colonies are the most common colony types; b. *Pseudomonas aeruginosa* on a split plate (Horse Blood Agar -HBA on LHS and MacConkey's agar on RHS); c. *Pseudomonas aeruginosa*, demonstrating effects of pyocyanin and pyoverdin on a sensitivity plate; d. *Pseudomonas aeruginosa* demonstrating fluorescence (RHS) and *Klebsiella* (LHS) demonstrating no fluorescence with UV stimulation; e. *Pseudomonas aeruginosa* on the vulval lips of a mare demonstrating fluorescence with UV stimulation

The diagnosis of *K. pneumoniae* (Figure 3a) required the differentiation of these colonies from those of *Enterobacter* sp (Figure 3b) and *Citrobacter* sp as their similar colour appearance on chromogenic agar (Figure 3c) prevented differentiation among them(Samra *et al* 1998). They were differentiated by sub-culturing when necessary to isolate single colonies, a gram stain to confirm a gram-negative rod and then the RapID™ One test was performed for final identification (Figure 3d).

a. b. c.

d.

Figure 3a. *Klebsiella pneumoniae;* b. *Enterobacter aerogenes;* c. *Enterobacter aerogenes* (left) and *Klebsiella pneumoniae* (right)*;* d. RapID™ One test with various colour reactions (*Klebsiella pneumoniae*)

The presumptive diagnosis of the other main offending bacteria i.e. *E. coli*, *Streptococcus* sp and *Staphylococcus* sp was made by using the colony identification guide shown in Table 2. *E. coli* grew rapidly and were the only bacteria that produced pink colonies (Fig 4a and b) on the chromogenic agar.

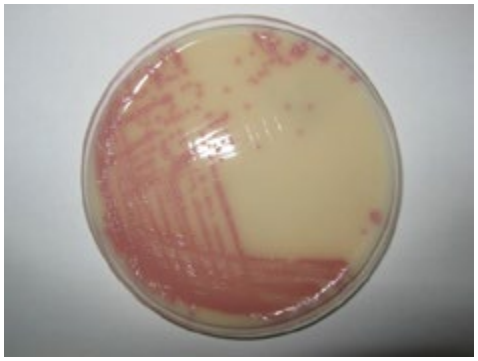
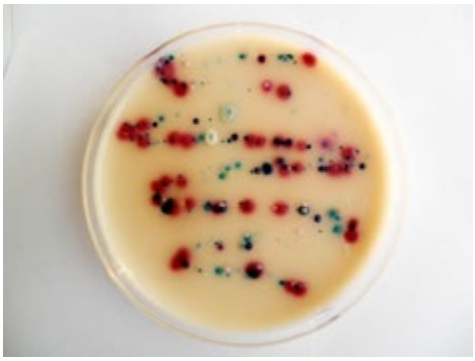
a.  b. 

Figure 4a. The pink colonies of *Escherichia coli; 4*b. Large pink colonies are indicative of *E. coli* in this example taken from the clitoris which also demonstrates multiple other colonies that can be presumptively identified from the colour guide

The most significant *Streptococcus* sp associated with endometrial disease are the β-haemolytic *Streptococcus* sp e.g. *Streptococcus equi var. zooepidemicus*. To diagnose the presence of a β-haemolytic *Streptococcus* sp, the haemolytic pattern must be taken from the bacterial growth on HBA as chromogenic agar will not show any haemolytic patterns. This also allows the differentiation from the non-haemolytic and α-haemolytic *Streptococcus* sp. The presence of β-haemolytic pin-point colonies that are only present on HBA (Figure 5a), in conjunction with the presence of light blue colonies on chromogenic agar (Figure 5b) allows the presumptive diagnosis of β-haemolytic *Streptococcus* sp.

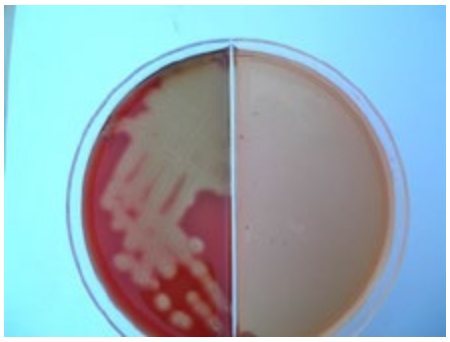
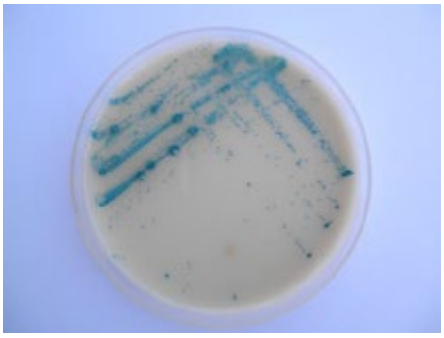
a.  b. 

Figure 5a. β-haemolytic *Streptococcus* sp on HBA/MacConkey split plate; 5b. *Streptococcus* sp on chromogenic agar

Enterococcus sp (Gram positive cocci formerly classified in the Streptococcus group) is the other main differential for the growth of light blue colonies on chromogenic agar. However, *Enterococcus* sp are unique in that they have the ability to grow on both HBA and MacConkey agar, thus aiding in the differentiation from *Streptococcus* sp (which do not grow on MacConkey agar).

Staphylococcus sp can be differentiated from *P. aeruginosa,* as it is oxidase negative and catalase positive (Figure 6). The catalase test is another simple test that can be used to

identify a species of bacteria. It is especially useful for distinguishing between *Staphylococcus* sp and *Streptococcus* sp when both may show haemolysis on blood agar, and both are coccoid on Gram stain (typically Streptococcus sp are ovoid as they divide only in one plane and are present in chains while Staphylococcus sp are perfectly round and are present in clusters as they divide in two planes). The catalase test is performed by placing a drop of 3% hydrogen peroxide on a microscope slide and smearing a bacterial colony from chrome agar through the drop. A positive result is bubbling, or frothing associated with generation of oxygen (Fig 6). *Staphylococcus* sp are strongly catalase positive and Streptococcus sp and *Enterococcus* sp, are catalase negative. The catalase test should not be performed on blood agar as it contains catalase.

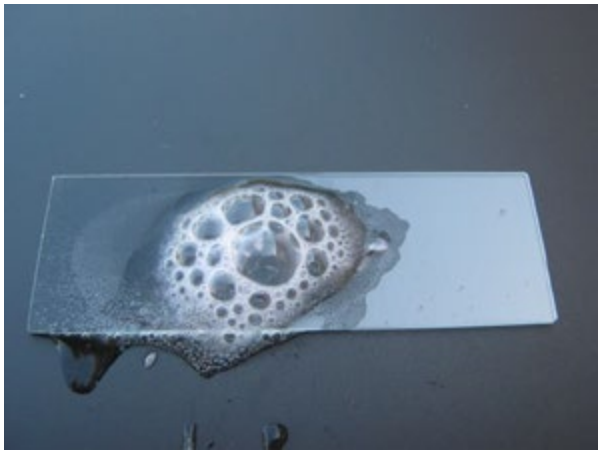


Figure 6. A catalase test is performed by placing a drop of 3% hydrogen peroxide on a microscope slide, smearing a bacterial colony from chrome agar into the hydrogen peroxide and a positive result is to observe frothing.

To confirm the presence of *Staphylococcus aureus*, a coagulase test must be performed. Coagulase is an enzyme produced by particular *Staphylococcus* sp and correlates with pathogenicity. The test is performed by mixing a suspension of staphylococci with rabbit plasma on a slide. A positive reaction is indicated by clumping of bacteria within 1-2 minutes. *S. aureus* is coagulase positive, whereas many commensal *Staphylococci* sp are negative. This test is not routinely performed at the GVEH. *Proteus* sp are a reasonably common isolate and can be recognised by 'swarming' of the colonies and brown pigment on chromogenic agar (Figure 7).

Figure 7. *Proteus* sp. can be recognised by 'swarming' of the colonies and brown pigment on chromogenic agar.

Candida sp and *Staphylococci sp* can have similar colour colony formation on both HBA/MacConkey Agar and chromogenic agar (Figure 8a and b) and can occasionally confuse the diagnosis as both may produce white or pale coloured colonies that are oxidase negative. A Gram stain easily differentiates these microorganisms and *Staphylococcus* sp invariably are catalase positive. A specific chromogenic agar is also available for yeast identification[a] (Figures 8c and d) and a biochemical test (RapID yeast plus)[a] can separate the identities of up to 304 clinically significant yeasts.[17]

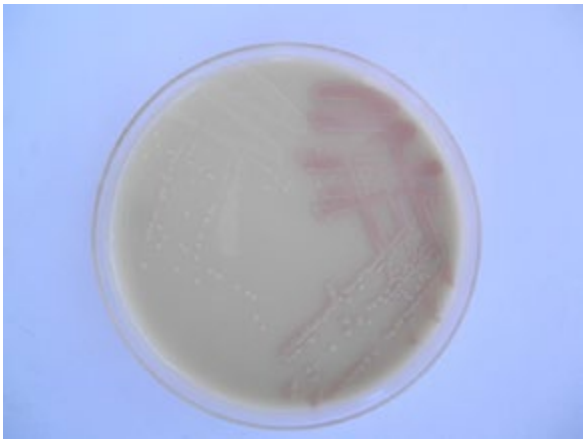
a.  b. 

Figure 8 a and b *Staphylococci sp* (left side of plate) and *Candida sp* (right side of plate) can have similar colour colony formation on both HBA/MacConkey Agar (8a) and chromogenic agar (8b);

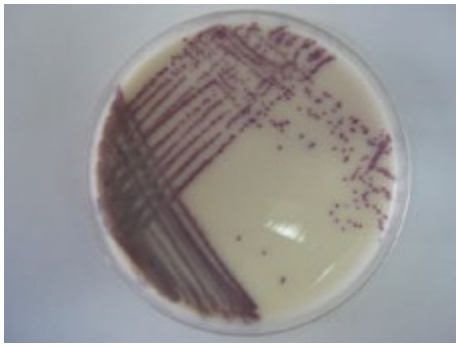
c.  d. 

Figure 8 c. *Candida albicans* on specialised yeast media; d. *Candida tropicalis* on specialised yeast media

The value of chromogenic agar compared to HBA/MacConkey traditional plates for rapid identification of potential pathogens can be appreciated when examining multiple colonies in heavy concentrations from clitoral sinus cultures (Figure 4a and Figures 9a and b).

Figure 9. Multiple colonies in heavy concentrations from clitoral sinus cultures on HBA/MacConkey (a) and chromogenic agar (b)

In our laboratory an endometrial culture is considered positive if 5 colonies forming units per plate are identified and had no more than three different bacterial pathogens present. Any positive endometrial swabs that have four or more different bacterial colonies are presumed to be contamination and re-swabbing of the affected mare is advised. If an endometrial swab result has a positive growth, antimicrobial sensitivity testing is performed, using colonies taken from the chromogenic agar. Some antibiotics tested at our facility for microbial sensitivity are Penicillin, Ticarcillin, Gentamicin, Neomycin, Ceftiofur and Enrofloxacin (Figure 10 a and b).

a. b.

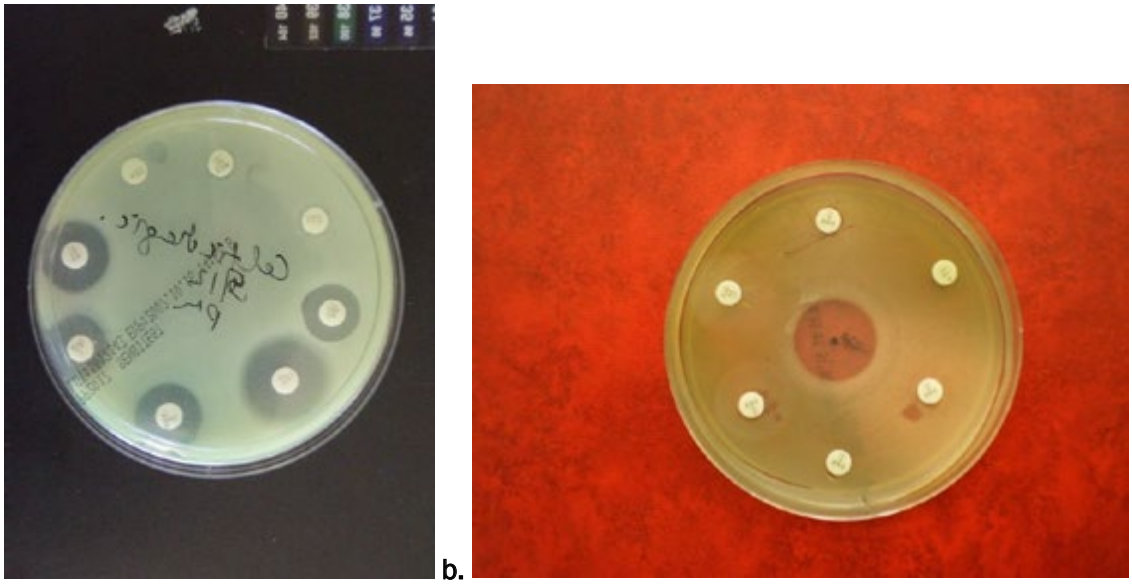


Figure 10. Antimicrobial sensitivity testing of a bacteria (a) and a yeast (Candida albicans. b) The clear zone in the centre of Figure 10b is 3% hydrogen peroxide

Quality control measures are used to ensure that results are accurate and reliable. These consist of standard pure cultures of bacterial specimens that have been previously validated by an accredited microbiology laboratory and can be used to compare colour and biochemical tests results. In-house biochemical testing is routinely used on all bacteria that are from positive endometrial cultures. As shown in Table 2, the RapID™ SS/U test can be used to identify many of the bacteria that are expected to be reproductive pathogens. During our first year using the new procedure independent diagnosis of bacteria was also confirmed on selected samples by an accredited microbiological laboratory.

Potential use of chromogenic agar in routine reproductive practice

The use of chromogenic agar can help to identify the pathogens of the equine reproductive tract. It has revolutionised our practices' microbiological management due to rapid and accurate identification of common reproductive tract pathogens.

It has several advantages over traditional methods of microbial identification. These include the reduction in the time required for the laboratory processing of samples; the ease of colony identification; the low-level of skill required to interpret results; the reliability of colonies taken from chromogenic agar for pathogen identification and antimicrobial sensitivity testing and the proven high level of sensitivity of chromogenic agar in identifying pathogens (Kitch *et al* 1992,Kitch *et al* 1994). The slightly higher cost of chromogenic agar per plate ($ 1.15 AUS) compared to conventional HBA/MacConkey split plate media ($0.58 AUS) was offset by a reduced need for complementary reagents and less labour associated with the processing of culture plates and suspect pathogens (Perry and Freydiere 2007). In addition, the technique removed the often-identified problems associated with samples submitted immediately prior to the weekend.

The disadvantages of this technique include the slightly higher cost of chromogenic agar plates, especially in facilities processing small numbers of samples and chromogenic agar is of *no benefit* for the screening and diagnosis of *Taylorella equigenitalis*, the causative agent of Contagious Equine Metritis (CEM), another potential venereal pathogen that recently has emerged again in the USA.

The use of chromogenic agars does not differentiate reproductive pathogenic from non-pathogenic bacteria and any interpretation of the significance of uterine bacteria isolated must be related to endometrial cytology, endometrial biopsy, or other forms of clinical examination.

In summary due to a limited availability of regional laboratory facilities that offer a rapid, accurate and continuous weekly screening service in many areas, the introduction of chromogenic agar has been very useful. It has resulted in a fast and accurate service which was especially important when samples are collected from mares close to the weekend when traditional services are not able to provide a report at a time suitable to facilitate breeding when mares are required to be presented for breeding with a negative culture to potential reproductive pathogens.

Footnotes

[a]Oxoid Limited, Hampshire RC248PW, England
[b]Remel, Lenexa, KS 66215-3594, USA

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Notes

Clinical applications of assisted reproductive techniques in the mare

Chelsie A Burden
Goulburn Valley Equine Hospital
905b Goulburn Valley HWY, Congupna, VIC 3633

With increasing research and understanding of assisted reproductive technologies (ARTs), various assisted reproductive techniques are becoming commonplace in breeding management of the mare. From artificial insemination (AI) to embryo cryopreservation and cloning, assisted reproductive techniques have been used to optimise equine reproduction and genetic preservation of the horse. There are numerous publications and reviews written on equine assisted reproduction as techniques continue to develop and be incorporated into the equine industry worldwide.[1-6] These proceedings are intended to describe the clinical applications and limitations of ART with a focus on embryo technologies.

Embryo recovery and transfer

While commonly summarised by the term "embryo transfer" (ET), the process of embryo recovery and transfer requires multiple procedures. Following breeding, recovery of an embryo from the uterus of a donor mare is performed via embryo flushing, followed by transfer of the embryo to the uterus of a suitable recipient mare. Embryo transfer in the mare is considered a well-established equine assisted reproductive technique.[3,7]

In clinical practice, it is common to monitor the cyclicity of the donor mare via transrectal palpation and ultrasonography to establish the ultimate timeframe for breeding and confirmation of ovulation (Day 0). The donor mare may require further post-breeding management to ensure the best uterine environment prior to embryo recovery. While the embryo enters the uterus on Day 5.5-6 post-ovulation, the embryo flush is typically performed on Day 7 or 8 post-ovulation.[8,9] There are multiple variations of the technique utilised in embryo recovery that all follow the same basic procedures: flushing is performed by filling the uterus with flush solution followed by draining the fluid through a filter to catch the embryo in the fluid retained within the filter. The embryo is then identified, washed, and prepared for transfer. Prior to transfer, the embryo will be given a grade or score that identifies the health or quality of the embryo. Embryo grading is an attempt to evaluate abnormalities of individual embryos as they relate to the potential of establishing a pregnancy after transfer. Grade 1 being assigned to normal embryos and Grade 4 being severely damaged or degenerated. Poor quality embryos have lower associated pregnancy rates.[9]

Historically, embryos were transferred using surgical techniques.[10] Non-surgical, transcervical techniques became more commonplace as the clinical demand for more embryo flush and transfer procedures increased. The initial transcervical transfer rates were lower than surgical transfers.[11] It is likely that excessive manipulation of the cervix and/or contamination of the uterus leads to inflammation and potential prostaglandin release resulting in poor pregnancy rates. With experience, practitioners performed non-surgical transfers with success suggesting that the skills of the clinician performing the transcervical transfer is the significant factor when using properly synchronised recipient mares. A transfer method that helps reduce the influence of the clinician, is the Wilsher technique. A vaginal speculum and cervical grasping forceps (Wilsher's forceps) facilitate the passage of the transfer pipette through the cervix, minimising manipulation and reducing the risk of introducing contamination or misplacing the embryo.[12] Despite the ease and high pregnancy rates achieved when using the technique, it has not been widely adopted by clinicians overall. Still, for operators with less experience, results demonstrate that high pregnancy rates

(>90%) can be achieved when the Wilsher technique is used. Further, the experience of the clinician performing the transfer compared to pregnancy rates is greatly reduced with the Wilsher technique compared to conventional transcervical transfer methods.[13]

Embryo recovery and transfer is routinely used to produce multiple foals per one donor mare each year, to produce foals from mares in competition or that are unable to carry a foal due to risk or physical limitations and to obtain pregnancy from mares deemed problem breeders due to pathologies of the uterus or cervix and inability to carry a foal to term.[14] With a fertile donor mare, the expected embryo recovery rate is approximately 75% and the expected pregnancy rate after transfer about 75%. This results in approximately 50% recipient pregnancy rate per cycle.[3,7] Although many practitioners may report overall higher rates, factors such as donor mare suitability, semen selection, recipient mare availability and embryo grade all affect the overall pregnancy rate following ET.[15]

One of the main limitations to embryo transfer is suitability of the donor mare. Recovery of embryos requires that the donor mare have a functional reproductive tract enabling breeding, spermatozoa to reach the oviduct, normal ovulation, and fertilisation along with an oviductal and uterine environment suitable to support the developing embryo until the embryo flush at Day 7 or 8 post-ovulation.[9] Season, temperature and humidity may play a role in successful embryo recovery.[16] Donor mares in competition or heavy work can be complicated by the effects of stress.[17-19] Donor mares with multiple breeding and ET attempts undergo repeat manipulation of the reproductive tract that can be associated with induced endometritis that is associated with reduced embryo recover rates and more intensive management.[17] Another limitation to ET in the horse compared to other species such as cattle is the lack of successful superovulation and ability to recover numerous embryos per embryo flush procedure.[20]

A significant component of successful embryo transfer is the availability of a suitable recipient mare. To have a recipient mare in the right stage of her oestrous cycle can be the biggest challenge a clinician faces. This is especially true when managing recipient mares for cooled, shipped embryos flushed at another facility. The cost and labour of managing recipient mares for expected embryo transfer can pose a further challenge. Several equine embryo transfer units have large herds of recipient mares to ensure a few mares are in oestrus at the same time as each donor mare. Ovulation of recipient mares is induced once an ovulation of the donor mare is confirmed. The selection of the recipient used on the day of transfer is influenced by transrectal palpation and ultrasonography findings, ensuring good uterine tone, a tightly closed cervix, and the echo structure of the uterus should be uniform with no oedema or fluid.[15]

Oocyte Recovery

While embryo transfer is widely accepted across multiple equine disciplines in Australia and worldwide, the clinical use of other ART procedures such as oocyte transfer (OT) and intracytoplasmic sperm injection (ICSI) is a quickly evolving sector of equine reproduction. Initially, the clinical use of these assisted reproductive technologies has focused on producing foals from mares or stallions with compromised fertility.[2] In the mare with ovulation failure, oviductal pathology, or pathology of the uterus or cervix that make embryo recovery unsuccessful, oocyte recovery and either transfer to the oviduct of an inseminated recipient mare (oocyte transfer), or intracytoplasmic sperm injection (ICSI) and embryo culture can be used to obtain pregnancies. Further, ICSI can be used when sperm numbers or quality is low. As the procedures become more efficient, oocyte recovery and ICSI are now being used more commonly for management of normally fertile mares and stallions along with problem breeders.[21] In fact, a key influence for the use of ICSI comes from the stallion owner. The efficiency of frozen semen is greatly increased by ICSI, thus allowing breeding of mares by

top stallions that have aged or died, and so have only limited supplies of frozen semen available. Frozen semen can also be thawed, diluted and refrozen in a large number of straws of "ICSI doses" or a "cut" portion of the straw can be used at one time for fertilization of numerous oocytes.[22]

It should be noted that the procedures required for oocyte collections are more invasive than for embryo transfer.[23] During transvaginal, ultrasound-guided oocyte collections (OPU), a needle is punctured through the vaginal wall and into the follicular antrum. Transrectal manipulation is necessary to ensure the ovary is in an appropriate position and in direct apposition with the vaginal wall. It is also important to palpate the ovarian surface to avoid puncture of the oviduct or ovulation fossa. Damage to either structure could limit future attempts at breeding or embryo transfer.[6,23]

In Australia the primary disadvantages of OT or ICSI are increased cost and less convenience because few facilities commercially provide equine ICSI procedures. With increasing international success and client demand, it is likely the procedures will soon become commonplace.

Ovum Pick Up (OPU)

There are two methods utilised to obtain oocytes from the live donor mare. One method of ovum pick-up or follicular aspiration is performed on the dominant, hormonally stimulated follicle (gonadotropin such as hCG or GnRH) just prior to ovulation. In this case, an in vivo-matured oocyte is recovered. There are several advantages to recovery of the mature oocyte from the dominant, stimulated follicle, especially for the practitioner just starting to work with follicle aspiration. The large follicular size makes ultrasonographic visualisation and puncture of the follicle much easier. There is also a high recovery rate reported. This is due to the weakened cumulus-oocyte complex and detachment from the follicular wall that occurs with maturation just before ovulation.[24] The disadvantages of recovery from dominant, stimulated follicles are the increased requirements of ultrasonographic monitoring of follicular growth, the precise timing of hormone stimulation and coordination with the OPU team and ICSI laboratory. Aspiration must be done when the follicle has responded to the gonadotropin to ensure expansion of the cumulus, but before ovulation; 24–35 hr after gonadotropin stimulation. As superovulation in the horse is not rewarding, it is typical to only aspirate a single follicle per procedure. Since oocytes from matured follicles are in the process of meiosis, they need to be kept at body temperature until the time of ICSI. Portable incubators should be utilised to ensure temperature control, and the oocyte(s) should be shipped in culture media rather than embryo holding medium.

The second approach is the aspiration of all immature follicles on the ovaries of the donor mare without hormone stimulation. The immature oocytes must then be matured in vitro. Several detailed descriptions of the OPU procedures are available.[4,25] The collection of oocytes from the multiple small follicles will require multiple ovarian punctures per session; however, few adverse problems have been reported.[26-28] Despite the number of punctures made, the clinician and owner should be aware of the risks and monitor for complications such as peritonitis, ileus, colic, haemorrhage, rectal tear and ovarian abscessation.[6,28] A major advantage of immature oocyte collection is that aspiration can be done on a predictable set schedule, without following ovarian activity between aspirations providing ease of scheduling for the clinician and the breeder.[29,30] This is especially beneficial for mares in competition to plan around busy show schedules. Alternatively, donor mares can be monitored to select a time for follicle aspiration when an adequate number of small follicles are present.[5] Typically, all follicles on the ovary over 5-10mm diameter are aspirated. The number of oocytes recovered depends upon the number of follicles present and the skill

of the clinician. Different breeds and individual mares tend to have different follicle numbers, oocyte recovery rates and may have different rates of embryo production after ICSI.[5] Aspiration of immature follicles can be performed year-round, providing further advantage to already busy clinician and owner.[31] The procedure can be performed during less demanding times or out of breeding season, with normal maturation and blastocyst rates obtained from oocytes recovered in the non-breeding season.[31] The oocytes recovered from the immature follicles are in the "resting phase" of meiosis. This allows the clinician to handle the oocytes at room temperature and transport them in embryo holding media to the ICSI laboratory with no detrimental effect.[22,32,33] At the lab, the oocytes can be placed in direct maturation or held overnight before being put into maturation. This allows ICSI to be performed at a convenient time.[32] The main disadvantage to the aspiration of immature follicles is the difficulty of the technique. Small follicles are more difficult to puncture and require a "scraping" technique to separate the tightly adhered cumulus-oocyte mass from the follicular wall. It is impossible to differentiate between juvenile growing follicles and atretic follicles during aspiration. Only about 60% of the recovered oocytes will mature in culture and be available for ICSI.[3] Despite this, the overall number of blastocyst development post-ICSI is higher than for single mature follicle aspiration.

Clinically, aspiration of immature follicles has become the more efficient method of oocyte recovery. The cost of OPU and ICSI is typically higher than embryo transfer. However, for difficult embryo donors, the additional cost per cycle may be balanced by a higher success rate per cycle with OPU and ICSI.[30] This may be especially true for the aged mare. Oocyte viability declines with increasing mare age.[24] ICSI allows observation of the early cleavage stage of embryo development. After in vitro maturation and ICSI, the success of oocyte maturation and development of blastocysts after are comparable between age groups.[34] It should be noted that ICSI is not a helpful option if the cause of subfertility in certain mares is due to oocyte quality. A mare that fails to produce in vitro produced embryos may not be a viable candidate for future OPU-ICSI procedures.

By utilising these advanced reproductive techniques in clinical practice, oocytes can be recovered from donor mares by the attending veterinarian and shipped to a laboratory for ICSI, without any decrease in oocyte or embryo viability.[32,35]

Postmortem Oocyte Recovery

In cases of unexpected death of a mare, ovaries or oocytes can be transported to the ICSI laboratory for production of embryos. An overall maturation rate of 41%, cleavage rate of 40%, and blastocyst rate of 19% has been described.[36,37] If the ovaries are to be shipped, they should be allowed to cool slowly to room temperature during shipment but not chilled.[38] The ovaries should be recovered and shipped quickly to the laboratory, with the best results obtained if the ovaries are received within 6 hours of the mare's death. Alternatively, the ovaries can be processed to recover immature oocytes, and the oocytes shipped to the ICSI laboratory with less damage.[39] Recovery of oocytes from follicles requires scraping of the granulosa cell layer to dislodge the oocyte from the follicle wall, as is done during OPU of immature follicles. A common technique involves opening the follicle with a scalpel and scraping the surface with the needle or curette, followed by rinsing with medium.[6] Embryo holding medium may be used to wash the collected cells into a Petri dish. The oocytes are identified and then handled as for immature oocytes.

Although often uncontrollable, referring veterinarians can improve successful outcomes by understanding the importance of variables such as time of death to oocyte recovery and making every effort to ship ovaries while maintaining temperatures between 15–25°C.[36]

Intracytoplasmic Sperm Injection (ICSI)

As traditional in-vitro fertilisation is ineffective in the horse, ICSI is utilised for in vitro embryo production and has become a commonly used clinical procedure in equine reproduction world-wide. Extensive clinical research has established and continually works to improve the steps of oocyte maturation, ICSI and embryo maturation prior to transfer into the recipient mare. The laboratory-based materials and methods are variable but well described between laboratories.[6,40,41] In general, when oocytes arrive at the lab, the first step is maturation in cell culture or maturation media. The cumulus cells surrounding the oocyte must be denuded, and mature oocytes showing a polar body are selected for ICSI. Spermatozoa can be prepared and selected by swim-up, direct wash or density-gradient centrifugation. A novel and potentially superior method of sperm selection is via a microfluidic (MF) chip, which utilizes natural rheotactic behaviours of sperm to select for a highly motile and viable population of cells that would be optimal for IVP.[42,43] Using micromanipulation techniques on an inverted microscope, the selected sperm cell plasma membrane is damaged; the sperm is then injected into the cytoplasm of the mature oocyte. This step may be performed with a conventional micropipette or Piezo pipette system. Some reports suggest a higher blastocyst rate with Piezo-driven ICSI.[44] Following ICSI, the fertilised oocytes, presumptive embryos, are cultured in embryo culture medium and evaluated for blastocyst development between days and 12 post ICSI. Blastocysts can be transferred after culture or vitrified for later transfer.[45,46]

Depending on location, the ICSI laboratory may have a herd of recipient mares, or the embryos maybe shipped to an embryo transfer facility. In vitro-produced blastocysts are generally considered comparable to Day 6 embryos despite the number of days in culture. These embryos should be transferred to recipient mares that are 4–5 days post-ovulation.[47] There are no significant differences in foal general health, size or weight, or placental morphology among foals produced by natural conception, embryo transfer or ICSI.[48]

One limitation of the laboratory-based procedures is variability between laboratoriesand in Australia, the lack of established ICSI laboratories.[49] To become a commercially viable program, the clinician must work closely with the ICSI laboratory to establish effective methods for successful embryo, pregnancy, and foal production. One experienced clinician reports an expected in vitro maturation rate of ≥50%, the blastocyst rate per injected oocyte of ≥20%, the pregnancy rate after transfer of blastocysts to recipient mares ≥65% and the foaling rate per established pregnancy of ≥80%.[3]

Oocyte Transfer

While OPU and ICSI are prevalent ART procedures, some locations are limited by the availability and proximity to ICSI laboratories. An alternative is to perform oocyte transfer (OT). The procedure is performed by aspiration of a mature follicle and transfer of the mature oocyte(s) into the oviduct of the recipient mare. The recipient mare is bred, fertilisation occurs in the oviduct of the recipient mare, and the recipient mare will carry the resulting pregnancy.[1,49]

The main disadvantage of OT is the required surgical transfer into the oviduct of the recipient mare.[49] The recipient mare must also be inseminated with an adequate dose of semen to achieve pregnancy. The recipient mare should also be in oestrus, allowing her reproductive tract to manage the sperm after insemination and supporting fertilisation of the transferred oocyte. After the transfer, the recipient mare must form a functional corpus luteum or be supplemented with progesterone so that her tract allows normal embryo development and pregnancy. This can be assured by choosing a recipient mare in oestrus with a dominant, preovulatory follicle or using an anoestrous or early oestrus recipient treated with oestrogen

before the transfer and progesterone afterward.[50,51] The recipient follicle must be aspirated to remove the oocyte to avoid ovulation and fertilisation of the recipient mare oocyte.[52]

Embryo Biopsy

Prior to transfer, embryos of both in vivo and in vitro origin can be biopsied for genetic evaluation. A biopsy is performed via microblade (<300 µm diameter, small embryo) or micromanipulation with a small number of trophoblast cells removed using the micropipette.[53,54] The latter technique is preferred for larger embryos to achieve transfer with normal viability.[54,55] The recovered cells are utilised for genetic diagnosis and selected for sex, or determination of embryos carrying disease-related mutations.[56,57] In locations where laboratories are available to process the embryos, embryos can be shipped to the laboratory overnight, biopsied, and shipped back for immediate transfer with a resulting normal pregnancy rate.[53]

Embryo Cryopreservation

Unlike the limited success in cryopreservation of unfertilized oocytes, equine embryos can be cryopreserved by slow freeze or vitrification methods. Each technique is well described and can be used for both in vivo and in vitro produced embryos.[46,58] In vivo produced embryos can be collected, cooled and transported for cryopreservation.[59] Embryos less than 300 µm in diameter can be frozen or vitrified successfully as described.[46,60] When flushing a mare to obtain a small embryo, recovery must be performed on Day 6 after ovulation which may result in lower embryo recovery compared to that for the normal Day 7 or 8 embryo flush.[61] Cryopreservation of expanded equine blastocysts (>300 µm) has become less problematic with the availability of embryo collapse.[62] Biopsy methods are utilised for embryo collapse prior to cryopreservation. The major limitation is the availability of a laboratory with a micromanipulator to perform the procedure. A recent study suggests that vitrification of larger, collapsed blastocysts is beneficial over the slow-freeze procedure.[63]

Cloning (Nuclear Transfer)

The procedures used to perform nuclear transfer reflect the steps involved in fertilization and are beyond the scope of this publication. The efficiency of cloning and the health of the cloned foal depend greatly on the techniques used in the cloning laboratory.[3] There are numerous techniques described for each step of the process, and nuclear transfer continues to be significant in clinical research.

There are limitations to nuclear transfer in the horse. Like other species, cloned pregnancies can be lost throughout gestation, and cloned offspring may suffer complications such as neonatal maladjustment syndrome, enlarged umbilical cord, and front leg contracture.[64,65 66] Abnormalities of the placenta have also been reported, including placental oedema, detachment from the uterus, presence of cystic structures, and placentitis.[67,68] Another disadvantage of cloning is that few breed registries currently allow registration of cloned horses.

Assisted reproductive technologies (ARTs) in research and clinical practice have vastly increased the efficiency and availability of management strategies for the clinician and breeder. With enhancing clinical skills and availability, assisted reproductive techniques will continue to shape the future of equine reproduction.

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Notes

Conditions of the Arytenoid Cartilage

Brian Anderson
ba@bvp.com.au
Ballarat Veterinary Practice
Ballarat, Victoria
Australia

Conditions of the Arytenoid Cartilage

The most common and important condition involving the arytenoid cartilage other than RLN is arytenoid chondropathy, and this is described in detail below. Other non-RLN conditions that result in respiratory obstruction involving the arytenoid carilage are ventromedial luxation of the corniculate process of the arytenoid cartilage (VLAC), equine laryngeal dysplasia and dynamic laryngeal collapse of the arytenoid cartilages associated with poll flexion in standardbreds and sport horses. These will be mentioned briefly.

Arytenoid Chondropathy

Disease of one or both arytenoid cartilages resulting in abnormal enlargement is referred to as arytenoid chondropathy. Pathologic changes identified in affected cartilages include chondritis, chondrosis, chondroma formation, dysplasia, necrosis, perichondritis, granulating laryngitis, and abscessation.[1] The precise aetiopathogenesis has not been determined, but arytenoid chondropathy is likely to be the end result of a process that starts as mucosal injury followed by ascending infection and subsequent inflammation of the underlying cartilage.[2-9] This can result in superficial chondritis with granulation tissue formation and a fistulous tract, or if deeper infection occurs, the development of an abscess. Arytenoid cartilage mucosal injury could have multiple and coexisting causes (Figure 1). The inflammatory processes not only involve the laryngeal mucosal surface and the arytenoid cartilage(s) but also peri-arytenoid tissues and dorsal muscular structures.[1,9,10] The end result is usually fibrous tissue lamination, necrosis, deposition of poor-quality cartilage matrix and mineralisation of the laminar portion of primarily the body of the arytenoid cartilage.[1,11] Frequently, the affected cartilage has reduced mobility. Partial or complete failure of abduction is caused by the laterally positioned thyroid cartilage physically restricting movement and/or impaired function of the dorsocricoartyenoid joint because of extension of the inflammatory process to affect the joint or the dorsal cricoarytenoid muscle. The net outcome is a reduced *rima glottidis* aperture and airflow compromise.[2] Classification of arytenoid lesions and suggested management has been proposed (Table 1).

Figure 1: Suggested pathogenesis and outcome of arytenoid mucosal injury

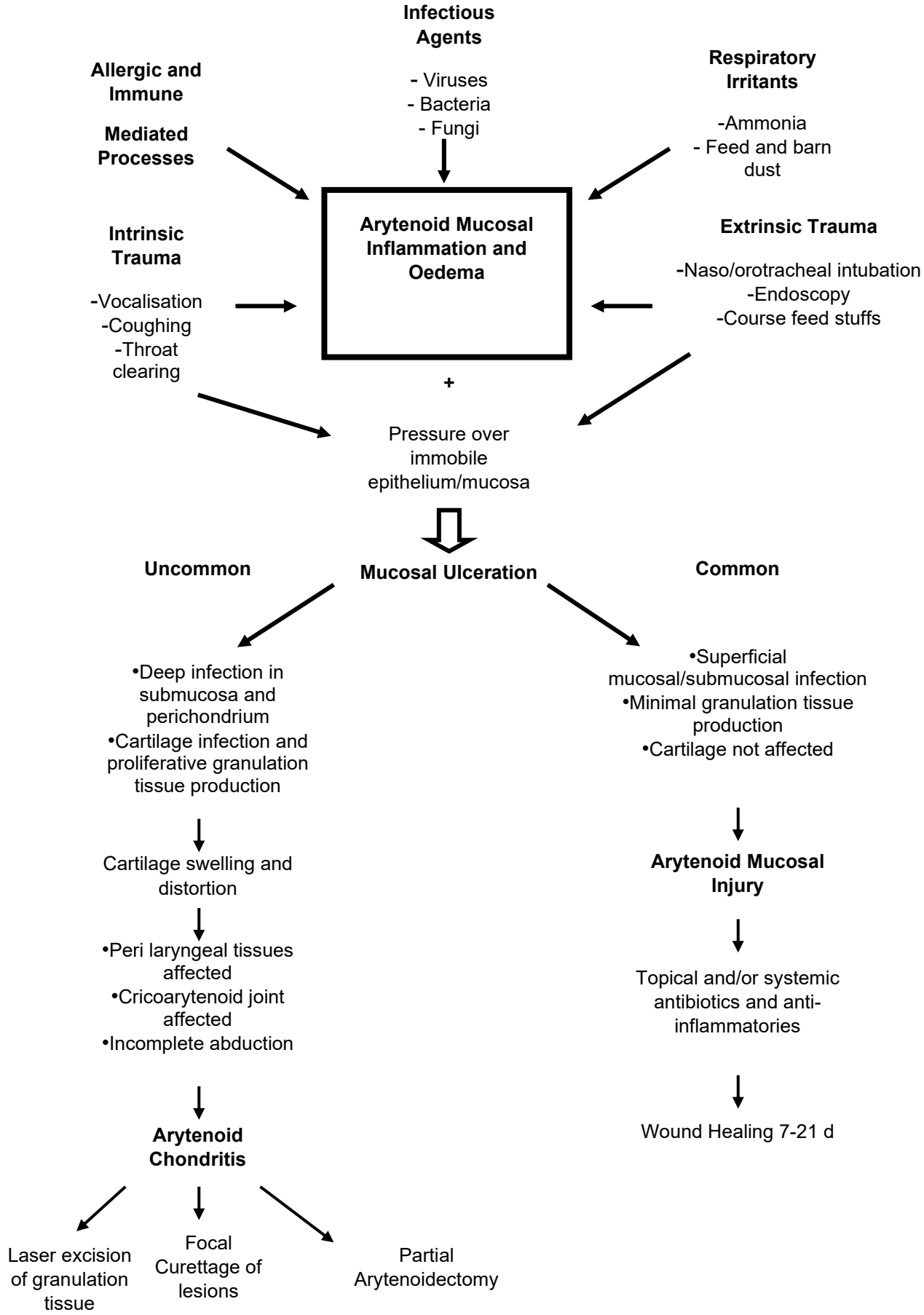

Table 1: Classification and management of arytenoid lesions

	Mucosal Lesion	Intra-laryngeal granulation tissue/mild Arytenoid Chondritis	Moderate Arytenoid Chondritis	Severe Arytenoid Chondritis	Arytenoid Chondroma
Unilateral or bilateral	Frequently bilateral	Mostly unilateral 'Kissing" lesions on opposite cartilage	Mostly unilateral "Kissing" lesions on opposite cartilage	Usually unilateral in younger horses, frequently bilateral in TB broodmares	Usually unilateral
Epithelial erosions/ulcers or raised areas on vocal process of arytenoid cartilage	+	+/-	+/-	+/-	Mucosa usually unaffected
Intra-laryngeal granulation tissue	-	+	+/-	+/-	-
Enlargement of arytenoid cartilage(s)	None	Mild, often focal	Obvious	Extensive	Usually markedly enlarged
Arytenoid movements	Normal	Often Normal	Inability to achieve full abduction	Inability to achieve full abduction. All movements markedly reduced	Inability to achieve full abduction. All movements markedly reduced
Diameter of rima glottidis	Unchanged	Reduced	Reduced	Severely reduced	Severely reduced
Treatment	None or medical	Medical, laser resection or sharp resection and focal curettage	Laser or sharp resection of granulation tissue. Focal curettage. Partial arytenoidectomy	Partial arytenoidectomy Permanent tracheostomy	Partial arytenoidectomy Permanent tracheostomy
Prognosis	Excellent 10% form granulomas 5% progress to chondropathy[7]	Very good to excellent. Recurrence responds to repeat surgery	Laser treatment 50% for racing[22] Arytenoidectomy approx. 66% for racing[19-21]	Approx. 66% for racing successfully following arytenoidectomy [19-21]	Good for non-athletic endeavours, recurrence not reported

History and Clinical Signs

Arytenoid chondropathy can be acute but is more commonly chronic. It affects all breeds and ages of horses, causing respiratory obstruction and exercise intolerance. In performance horses, typically young racing horses, signs are *not detected at rest* but occur during strenuous exercise, where even small changes in the cross-sectional area of the rima glottidis caused by the chondropathy manifest as respiratory stridor (inspiratory and expiratory noise) and exercise intolerance.[9,12] The disease can be asymptomatic, but generally there is a slow progression of clinical signs. It is important to note that the history for racehorses with the disease, particularly where cartilage enlargment is not severe, is very similar to RLN. Therefore a careful clinical and endoscopic evaluation is required to differentiate the two conditions.[1,13] In some horses it is surprising how advanced the disease can be on URT endoscopy when little or no clinical signs have been reported. Acute disease has also been observed in yearling Thoroughbreds associated with severe upper respiratory tract infections, including strangles, but respiratory noise at rest is uncommon in our experience.

In contrast, because of the reduced respiratory demand in non-performance horses, the effects of arytenoid chondropathy are not recognised until there is marked airway narrowing and chronic disease is present. Therefore, these horses commonly present with *obvious respiratory noise or dyspnoea during mild exercise or at rest*.[9] Typically affected horses are older and have an insidious onset of clinical signs with gradual worsening over months to years. However, in some horses (commonly broodmares) with advanced unilateral or bilateral chronic chondropathy, life-threatening respiratory obstruction occurs, necessitating emergency tracheotomy.

Acute inflammatory chondropathy results from generalised laryngitis (perichondral submucosal oedema), causing a markedly reduced diameter of the *rima glottidis* and therefore, stridor or dyspnoea at rest. Acute chondropathy is more common in older non-performance horses, where clinical signs can be rapid in onset and progress quickly, and depression, fever, and leucocytosis may be present.[9] In these horses, the acute form of the disease may be an intermittent or a severe manifestation of chronic inflammatory chondropathy.

Diagnosis

A diagnosis of arytenoid chondropathy can usually be made during endoscopic examination of the larynx. On initial observation, RLN might be suspected because the arytenoid cartilage is in an adducted position and shows little movement. However, the affected cartilage is usually mishapen or distorted (Figure 2). Mucosal disease of the arytenoid cartilages in young Thoroughbred horses in Australia and New Zealand has been described.[4,7] Bilateral kissing lesions are found on or near the vocal processes just proximal to the attachment of the vocal cords (Figure 3). The aetiology is uncertain; therefore, the condition has been termed idiopathic mucosal lesions.[4] These lesions manifest as small erosions/ulcers, or alternatively, raised areas of epithelial injury. They are often hyperaemic and can have small, slightly purulent centres. The incidence of mucosal lesions in two surveys of Thoroughbred yearlings presented to public auction sales (5629 horses) was 0.6%, and the incidence of arytenoid chondritis was 0.21%.[4,7] In a population of 774 racing Thoroughbreds, 2.4% had mucosal lesions.[14] These lesions do not affect subsequent racing performance and many heal uneventfully with or without medical therapy.[4,14] However, treatment is recommended, because in one report 10% of affected horses had lesions progress to become granulomas and 5% to arytenoid chondritis.[7] Broad spectrum antibiotics, NSAIDs, and pharyngeal throat washes are recommended for 7 to 10 days.

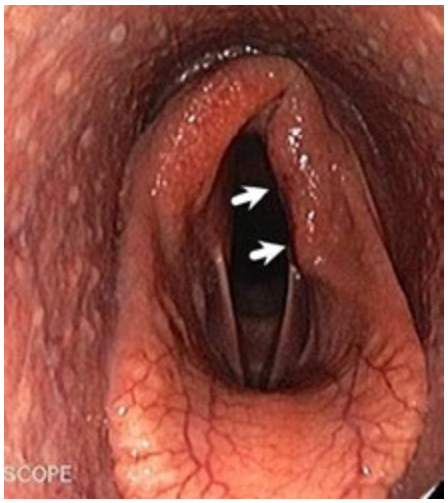

Figure 2. The left corniculate process of the arytenoid cartilage is misshapen and distorted and in an adducted position (white arrows) typical of arytenoid chondropathy

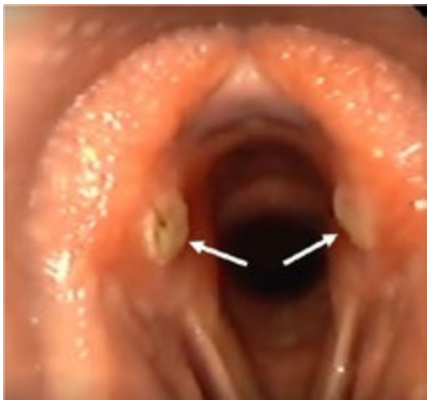

Figure 3. Arytenoid mucosal lesions (white arrows)

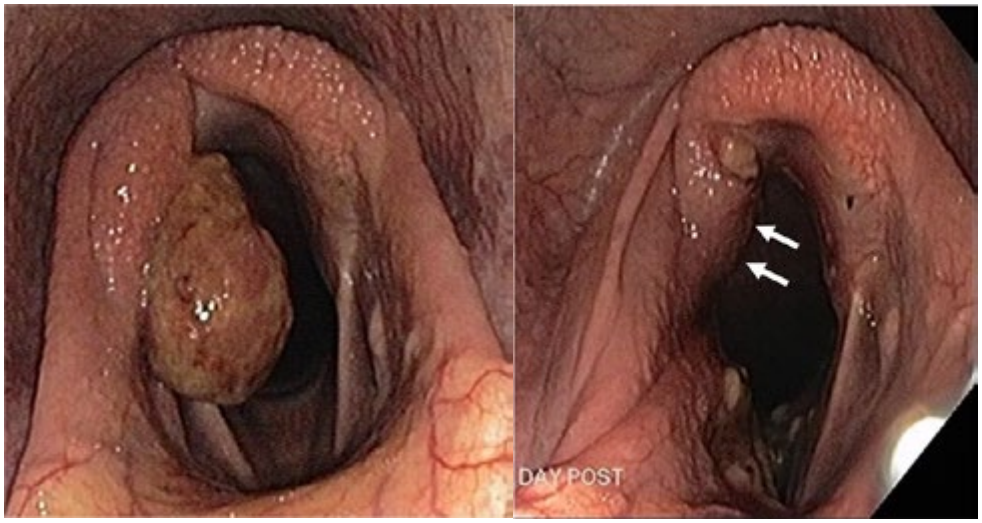

Figure 4. Large granuloma on the axial surface of the right arytenoid cartilage in the picture to the left and after a partial arytenoidectomy in the picture on the right

One of the endoscopic hallmarks of arytenoid chondritis is the presence of intraluminal projections of granulation tissue (Figure 4). Frequently, a fistula or sinus tract in the centre of the granulation tissue communicating with the body of the arytenoid cartilage is present. A contact ulcer or projection of granulation tissue is often present on the opposing cartilage ("kissing lesion"). The palatopharyngeal arch on the affected side is often more prominent. Because significant RLN of the right side of the larynx is rare, if it present on endoscopic examination, arytenoid chondropathy should always be a primary differential and ruled out by careful endoscopic and clinical examination. In acute chondropathy, the predominant endoscopic feature is mucosal and submucosal perichondrial oedema and peri-larygeal swelling with intense hyperaemia. The *rima glottidis* diameter can be markedly reduced often to a slit-like opening. The degree of underlying cartilage pathology may be highly variable and difficult to determine until medical therapy resolves the oedema.[9]

Careful palpation of the larynx, with attention to comparing the percutaneous prominence of the muscular processes on either side of the larynx, is important. In cases of significant RLN, the left muscular process is more obvious. In contrast, thickening around the muscular process can be palpated in cases of arytenoid chondropathy and might be the only diagnostic finding. In general, the larynx may feel less resilient to digital palpation and firm manual pressure may cause stridor or dyspnoea.[9,15] Ultrasonography may be useful in further defining the disease. It can help detect abaxial arytenoid cartilage lesions (including abscessation), and determine if the disease is confined to the arytenoid cartilages or extends into the peri-arytenoid tissues.[16] Radiographic features of arytenoid chondropathy include abnormal patterns of cartilage mineralisation; increased density of the arytenoid cartilages; abnormal contour or size of the corniculate process; obliteration of the larygneal ventricle; and laryngeal masses.[9] Some mineralisation in the larynx occurs with aging in horses, particularly of the thyroid and subsequently the dorsal arch of the cricoid cartilage. Extensive mineralisation of the larynx is a poor prognostic sign for successful surgical treatment.[1, 9]

Treatment

Management of arytenoid chondropathy depends on the stage of the disease (acute or chronic), the degree of airway dysfunction and whether athletic activity is required. In the acute form, horses may present in respiratory distress (stertor) with laryngeal oedema. Medical therapy with broad-spectrum antibiotics, NSAIDs and/or steroids and pharyngeal throat sprays often resolves the condition, but occasionally an emergency tracheotomy is required. A 7 to 10-day course of treatment is prescribed with endoscopic review. In some animals, the chondropathy regresses, and arytenoid cartilage function returns to normal or near normal. In other animals, chronic chondropathy ensues, but if airway obstruction is not severe, it is possible for some athletic animals to continue to compete. Because arytenoid chondropathy is frequently progressive, repeated endoscopic monitoring is recommended, especially if respiratory noise increases. Table 1 outlines the management or treatment approach to the various forms of arytenoid chondropathy. Some considerations on treatment include:

- To treat mild to moderate chondropathy and limit disease progression, ceftiofur alone, or in combination with gentamicin, appears the drug(s) of choice based on 83% of bacterial isolates being sensitive.[17] A wide variety of bacteria and multidrug resistance were identified. Because of this, surgery is indicated if medical therapy is not effective in a short period.
- If arytenoid function is acceptable, arytenoidectomy should be avoided to optimise athletic function.
- Partial arytenoidectomy is the preferred method for arytenoid cartilage removal. Standing partial arytenoidectomy has recently been reported and is less invasive, more cost effective and less arduous for the horse.[18] Not enough long-term data is available to determine if it is a superior technique to arytenoidectomy under general anaesthesia. The corniculate mucosa is not sutured, and the formation of excessive

granulation using this technique may prove a disadvantage. Current techiques have reduced the morbidity associated with dysphagia and coughing, but chronic low grade aspiration is likely leading to inflammatory airway disease (IAD). Collapse of unsupported abaxial mucosa, IDDSP and inspiratory noise remains in many horses.

- If the condition is bilateral, unilateral arytenoidectomy of the most affected cartilage may be sufficient. Other surgeons believe bilateral arytenoidectomy is inevitable and prefer a single stage procedure.[9] Bilateral arytenoidectomy is considered a salvage procedure. Permanent tracheostomy is a valid procedure for bilateral disease in nonathletic animals and has been used with good results in Thoroughbred broodmares with the disease.

Prognosis

The prognosis following treatment for arytenoid chondropathy depends on the nature and extent of the disease and the respiratory demands placed on the patient.[1,9, 19,20,21] If treatment is instituted early, the condition is unilateral and arytenoid function is minimally affected, medical management can halt progression, and in one study, palliative treatment allowed some horses to race for 1 to 2 years.[1] The likelihood of Thoroughbred horses with idiopathic arytenoid mucosal lesions racing more than three times is not different to unaffected control animals.[4] Excision of intralaryngeal granulation tissue in non-racehorses has an excellent prognosis. In race horses prognosis is determined by degree of cartilage thickening, degree of cartilage abduction and if co-existing RLN is present. A group of 5 racehorses with only slight cartilage thickening and no other larygneal pathology, all returned to racing three or more times with only one having reduced performance after treatment. However, if moderate cartilage thickening is present or concurrent laryngeal pathology exists, only 50% of treated horses returned to racing and usually at a lower level of performance.[21]

Partial arytenoidectomy is very effective in restoring *rima glottidis* diameter but prognosis depends on the respiratory demands for exercise. Six of 8 horses (75%) with occupations other than racing were useful after partial arytenoidectomy was performed for unilateral chondropathy (5), bilateral chondropathy (2), or failed laryngoplasty (2).[1] In general, the prognosis in non-working horses treated with partial arytenoidectomy is favourable, but is reduced in cases of advanced unilateral chondropathy accompanied by complicating lesions, such as contralateral laryngeal paralysis, peri-arytenoid inflammation, and pharyngeal cicatrices. Even horses suffering from bilateral arytenoidectomy may have a better prognosis for survival compared to those with advanced unilateral chondropathy and accompanying lesions. In these horses, a permanent tracheotomy might be a better treatment option.[9]

In Thoroughbreds, partial arytenoidectomy will allow approximately two-thirds or more of treated horses to train, race, and earn money. A shortened athletic career is likely, and although there is some evidence that shows a reduced performance after surgery, other studies show similar postoperative earnings per start and similar earnings compared to age and sex-matched controls. Bilateral partial arytenoidectomy for bilateral arytenoid chondropathy is a salvage procedure and carries a poor prognosis for continued racing (22%).[1] In addition, it is clear that horses treated with partial arytenoidectomy for arytenoid chondropathy, still have some limitation to airflow when exercising at racing speed.[23,24] Dynamic collapse of unsupported soft tissue on the left side of the larynx following partial arytenoidecotomy is the likely cause. Similar outcomes are observed regardless of whether the arytenoidectomy is performed with or without mucosal closure.[1,19-21,23-26]

Ventromedial luxation of the apex of the corniculate process of the arytenoid cartilage (VLAC)

In this condition, collapse of the apex of one or both corniculate processes of the arytenoid cartilage occurs, but the ventral part of the corniculate process remains abducted. In mild forms, the size of the *rima glottidis* is mildly affected, but in more severe forms, there is a significant reduction in the cross-sectional area, causing an abnormal (predominantly inspiratory) respiratory noise and reduced performance.

The appearance of the *rima glottidis* can vary:

- Ventromedial displacement or "subluxation" of the apex and body of the corniculate process of the arytenoid cartilage (freqently the left) under the contralateral cartilage. This can be associated with axial deviation of the aryepiglottic fold or vocal cord collapse. It can be observed at rest (Figure 5) but is frequently a progressive condition during high-speed exercise
- Bilateral ventromedial collapse of the corniculate processes. This typically occurs during high-speed exercise or with excessive poll flexion (Figure 6)

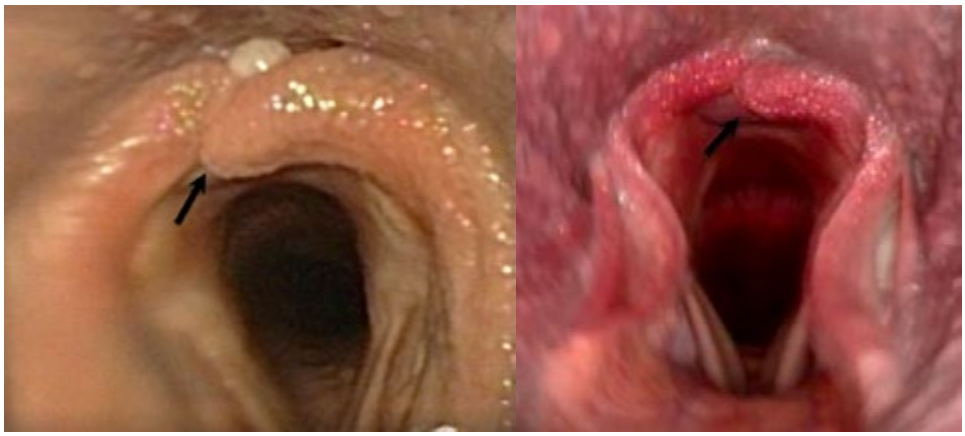

Figure 5. Ventromedial luxation of the apex of the left corniculate process (black arrow) of the arytenoid cartilage under the right corniculate process in a yearling Thoroughbred at rest – left picture (courtesy of Dr Andrea Ritmeester); and in a 3-year-old Thoroughbred racehorse during exercising videoendoscopy– right picture.

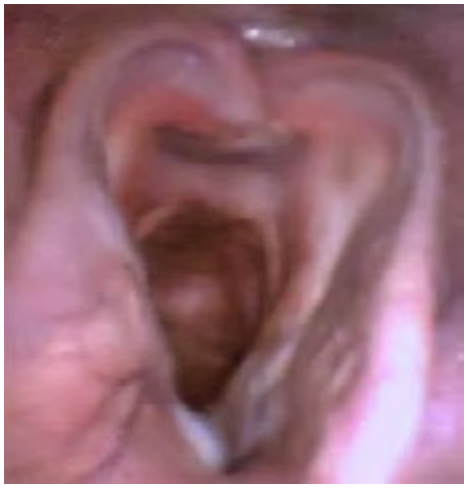

Figure 6. Bilateral ventromedial collapse of the corniculate cartilages in a Colombian Paso
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The precise pathogenesis is unknown or if the different forms of arytenoid subluxation are related. In early reports, it was suggested the condition may be an unusual manifestation of RLN or an advanced stage of adductor muscle dysfunction (transverse arytenoid muscle) during the progression of RLN before full abductor dysfunction is appreciable.[27] Abnormalities of the inter-arytenoid ligament or structure of the corniculate process cartilage may also occur.[28] The incidence, based on endoscopic surveys or dynamic endoscopy, is reported to be 5.2% of Clydesdale horses examined at rest, 4.9-5.5% of Thoroughbreds evaluated for poor performance, 11.6 % of Standardbreds evaluated under racing conditions, and 10% of Colombian Criollo Paso horses examined using overground endoscopy.[27-31] The observation of ventromedial luxation of the apex of the corniculate cartilage in yearling Thoroughbreds on presale endoscopy is considered a risk by some veterinarians for future athletic performance. This is due to some of these horses being later diagnosed with a reduction in *rima glottidi*s airway diameter during fast exercise, inspiratory respiratory noise, and poor performance.

No effective treatment for this condition is available. Some horses can be competitive despite the problem. In horses concurrently affected with RLN, a tie-back would be appropriate, but luxation of the corniculate process can continue to obstruct airflow. Therefore, in horses with significant airway obstruction at exercise and in those with co-existing RLN, arytenoidectomy is currently the preferred option. Long term follow-up on a large number of cases is unavailable, but in a small number of cases racing performance was unaffected by what treatment was used – no treatment, tie-back or arytenoidectomy (Dr D Shaw personal communication – Singapore Turf Club).

Equine Laryngeal Dysplasia (previously known as Fourth Branchial Arch Defect (4-BAD))

In this condition, abnormal development of structures derived from the 4th, and to a lesser extent the 6th branchial arch, result in a range of laryngeal cartilage and muscle anomalies.[32] The thyroid, cricoid and arytenoid cartilages can be involved, together with the *thyropharyngeus*, *cricopharyngeus*, cricothyroideus, *cricoarytenoideus dorsalis* and *cricoarytenoideus lateralis* muscles. Deformity or absence of one or more of these structures unilaterally or bilaterally results in the range of symptoms observed. Early studies relied on detailed post-mortem examination to determine pathology, but in recent years, advanced three-dimensional imaging (CT and MRI) have identified more complex abnormalities. Affected horses frequently make poor athletes due to exercise-induced respiratory noise secondary to dynamic collapse of soft tissues or cartilaginous laryngeal tissues. This condition is a cause of failure on post-sale endoscopy of yearlings at all major public auction sales.

The condition is congenital with an incidence of 0.02%, but age of clinical onset is 3 months to 7 years. In 22% of cases, horses are asymptomatic, only identified during endoscopy, frequently at the time of sale. The most common presenting sign is abnormal respiratory noise during exercise. Diagnosis is by laryngeal palpation, endoscopy at rest, dynamic URT exercising videoendosopy, ultrasonography and advanced imaging. Endoscopic evaluation at rest usually shows inability to abduct the affected arytenoid cartilage (65% of cases are right sided, 24% left sided and 14% bilateral), partial or complete rostral displacement of the palatopharyngeal arch (RDPA) (51% of cases). Results of exercising endoscopy in 26 horses showed 22% had an arytenoid abduction grade A, 65% grade B and 12% grade C. Ipsilateral vocal fold collapse was identified in 50% and 19% had bilateral vocal fold collapse. Aryepiglottic fold collapse was right sided in 35%, bilateral in 12%. Other abnormalities included DDSP, palatal instability, RDPA and nasopharyngeal collapse.[32]

Conformational defects of the larynx can be palpated as an abnormal gap between the dorsal two-thirds of the cricoid and thyroid cartilages due to a loss of the caudal cornu of the thyroid cartilage. An inability to palpate the muscular process of the arytenoid cartilage is due to a dorsal extension of the thyroid lamina. The muscular process of the arytenoid cartilage remains covered with a normal CAD muscle despite reduced arytenoid abduction, helping differentiate between RLN and laryngeal dysplasia. Aerophagia due to aplasia or hypoplasia of the upper oesophageal sphincter muscles can result in eructation and tympanic colic and is observed as gas in the upper oesophagus on lateral radiography. Dysphagia is reported but rare.

Treatment and prognosis for horses with laryngeal dysplasia depend on the underlying abnormalities and degree of respiratory compromise at rest or during exercise. In mild cases, no treatment may be necessary. Horses intended for racing that have laryngeal dysplasia are frequently retired. Because horses with laryngeal dysplasia can have multiple causes of airway obstruction, an OGE or treadmill endoscopic examination is recommended. A treatment plan can then be formulated, and unilateral or bilateral ventriculocordectomy, aryepiglottic fold resection, prosthetic laryngoplasty or arytenoidectomy could be considered. While surgical treatment focusing on the underlying pathology is rational, the complexity of the disorder and loss of cartilaginous and muscular support means success is likely to be modest at best and treated horses only able to participate in lower-level disciplines.

Dynamic Laryngeal Collapse (DLC) associated with Poll Flexion

This condition is characterized by bilateral collapse of the arytenoid cartilages and vocal folds occurring only during periods of exercise in “high” poll flexion. After it is induced, progressive collapse results in severe inspiratory obstruction and poor performance. First described in 2004 in Norwegian cold-blooded trotters (NCT), it has also been documented in other breeds and disciplines.[33] For Scandinavian harness racehorses, the condition occurs when the horse is “on the bit” during training/racing or restrained to prevent premature exhaustion during racing. However, it can be reversed immediately when tension on the bit and reins is released, and the horse allowed to exercise with free head carriage. A decrease in the angle between the mandible and ventral neck of only 12^{o} has been shown to induce DLC in predisposed harness racehorses. The cause is thought to be due to compression of a rostrally positioned larynx by the hyoid apparatus within the intermandibular space in anatomically-predisposed horses. It is well known that peak inspiratory tracheal pressures become more negative in normal horses when the poll is flexed and likely become greater in horses affected with URT disorders such as DLC. Horses experiencing DLC have a more rostral position of the larynx relative to the hyoid apparatus. The position of the larynx appears to be important in this disorder, as demonstrated by a report in three harness horses who did not have the condition prior to a tie-forward procedure but developed it afterwards during periods of poll flexion. It was hypothesised that the rostral advancement of the larynx caused by the tie-forward procedure in a predisposed breed induced the condition.

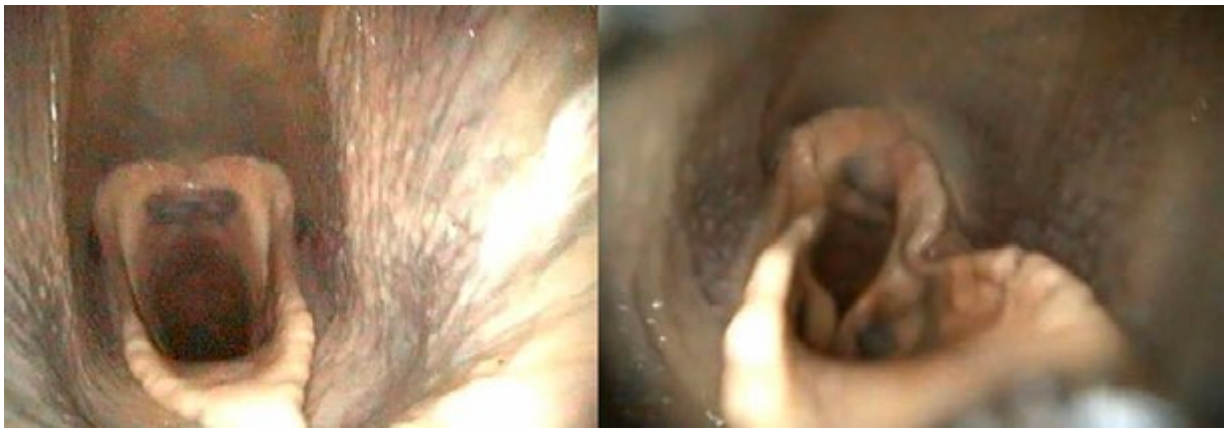

Figure 7. High-speed treadmill videoendoscopy showing larynx before tie-forward procedure during poll flexion and after surgery during poll flexion showing DLC

From: Vermedal H, Strand E. Dynamic laryngeal collapse associated with poll flexion as a complication of laryngeal tie-forward surgery in three harness racehorses. Veterinary Surgery. 2019;1–7. https://doi.org/10.1111/vsu. 13351

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Notes

Additional Proceedings

Update on FEI and EA Medication Regulations

Kirsten Neil
Sporthorse Veterinary Specialists
PO Box 409, Berwick, VIC 3806

Fédération Equestre Internationale (FEI) is the international governing body for most non-racing equestrian disciplines. Equestrian Australia (EA) follows and uses the FEI Equine Anti-Doping and Controlled Medication Regulations (EADCMRs).

Prohibited Substances

The FEI prohibited substance list and database is divided into two sections: controlled medications and banned substances.[1] Controlled medications are considered to have therapeutic value and/or to be commonly used in equine medicine. Controlled medications have the potential to affect performance and/or are a potential welfare risk to the horse. Controlled medications are prohibited in competition and cannot be detectable during competition. Banned substances are those that have been deemed to have no legitimate use in the competition horse and/or have a high potential for abuse. Banned substances are not permitted for use in the competition horse at any time. Sanctions for adverse analytical findings (AAF, i.e. "positive swab") are more severe for banned substances than controlled medications.

The FEI prohibited substance list is extensive; however, several medications do not appear on the list. If an unlisted medication has the same chemical structure or biological effect as a substance on the list, they are also prohibited. Conversely, some medications do not appear on the list as they are allowed during competition. Medications which are allowed during competition include gastroprotectants, oral and injectable vitamins/amino acids, electrolytes, homeopathics, joint support, altrenogest (mares only) and oral antimicrobials. There are some exceptions – for instance, administration of IV calcium, magnesium or excessive doses of IV B vitamins which are being given with the aim of having a calming effect on the horse would not be allowed.

During competition, two veterinary forms may be used. Form A is an authorisation for emergency treatment with controlled medications. This form can also be used retrospectively for treatment prior to competition. The FEI veterinary delegate and the ground jury for the event must approve this, and approval is not guaranteed. Form B is an authorisation for the use of medication not listed as prohibited, such as antimicrobials (except procaine penicillin) and intravenous fluid therapy. Injections can only be administered by an FEI Permitted Treating Veterinarian (PTV). Morning of competition injections are no longer allowed. Veterinarians are considered to be support personnel and can be subject to sanctions under the FEI veterinary regulations and if involved in EADCMR violations.

Detection Times versus Withdrawal Times

The FEI produces a list of detection times for commonly used medications (Appendix 1).[2] A detection time is the approximate period of time for which a drug, or its metabolite, remains in a horse's system, such that it can be detected by the laboratory and is provided as a guide only. The withdrawal time for a drug must be decided by the treating veterinarian based on the detection time with an added safety margin which is determined to allow for differences between horses such as bodyweight, metabolism, fitness level, hydration status, recent illness or disease etc. It is important to be aware that the FEI tribunal expects an appropriate withdrawal time, and the safety margin is at least double the detection time.

Calculating a withdrawal time requires knowledge and consideration of the substance's metabolism and clearance, route of administration, and known potential drug interactions. Detection times are usually established in healthy resting horses administered one medication under controlled conditions. Hepatic and renal compromise may reduce drug clearance of medications. Re-uptake of drugs (e.g. clenbuterol, flunixin, dipyrone) through ingestion of the horse's manure or contaminated bedding can result in prolonged detection times.[2] The FEI publishes threshold limits for some substances but does not publish screening limits, tests used, nor notify when the testing laboratory can detect lower levels of medications or new tests become available.[3]

Atypical Findings and Specified Substances

The FEI introduced the concept of specified substances in 2016. Specified substances are substances which are more likely to have been ingested by horses for a purpose other than the enhancement of performance, such as through a contaminated food source. Specified substances may be controlled medications (e.g. caffeine) or a banned substance (e.g. synephrine, sparteine). Synephrine contamination was particularly problematic at FEI events overseas in 2018-2019. Synephrine has been detected in Teff hay in Australia.[4] In 2021 the FEI introduced the concept of atypical findings (ATF) for results when contamination is suspected. ATF are defined as results that require more investigation before they can be treated as an adverse analytical finding (AAF). Substances considered ATF are specified substances, endogenous substances, ractopamine and zilpaterol.

The FEI Medication Logbook[5]

Competitors/persons responsible (PR) are required to maintain an FEI medication logbook that lists all medications and supplements given during and outside of competition. In the prosecution of any EADCMR violation, the FEI tribunal may request to see the mediation logbook. Failure to produce it may result in an adverse inference being drawn against the PR. The PR should keep samples of feed, bedding and supplements in case they need to be tested in the future to establish the source of a prohibited substance.

FEI and EA Adverse Analytical Findings

Prohibited substances which have resulted in adverse analytical findings from FEI and EA competitions (EA-affiliated events only) are depicted in Tables 1 and 2. Some horses had more than one prohibited substance detected at the same time. Select common medications will be briefly discussed.

Non-steroidal anti-inflammatories (NSAIDs) and corticosteroids remain the most common sources of AAF. Most NSAIDs have a short elimination half-life and a high degree of plasma protein binding, with a prolonged duration of action attributed to sequestration at sites of inflammation.[8] Pharmacokinetics of phenylbutazone are dose-dependent, accumulative with repeated dosing and absorption from the gastrointestinal tract depends on both the formulation administered as well as timing in relation to feeding.[8] Detection times of NSAIDs following topical administration are variable and cannot be easily extrapolated from oral dosing studies. Topical administration of ketoprofen is not recommended.[2] Topical use of NSAIDs can result in prolonged detection times. Diclofenac sodium could be detected for longer in urine than in plasma following topical administration.[9] Urine concentrations are variable, and there is also possibly a depot effect due to sequestration in liposomes. Consideration should be given to using hyoscine (N-butyl scopolamine) only preparations rather than hyoscine/dipyrone combinations close to competition.

Table 1: EA Adverse Analytical Findings[6]

Controlled Medications	Banned Substances
Phenylbutazone*	Reserpine*
Flunixin*	Stanozolol
Acepromazine metabolite*	Testosterone
Metformin*	Dextrorphan
4-methylaminoantipyrine (dipyrone metabolite)	
Hyoscine butylbromide	
Triamcinolone acetonide	
Dexamethasone	
Lignocaine	
Meloxicam	
Butorphanol	
Cyproheptadine	
Pergolide	
Ibuprofen	
Clenbuterol	
Altrenogest	
Caffeine	

Pharmacokinetics of corticosteroids depends on the formulation used as well as the route of administration. Intra-articular administration of triamcinolone acetonide results in higher plasma concentrations compared to intramuscular administration.[10-11] Triamcinolone was still detectable for 52 days in plasma and 60 days in urine following intramuscular administration.[11] Of note, triamcinolone was detectable in urine for longer than in plasma following intra-articular administration.[11] The dose administered, which joints and the number of joints medicated, as well as injection technique can impact detection times following intra-articular administration of corticosteroids. Longer detection times are to be expected when injecting into joints where the risk of subcutaneous and extra-articular deposition is high, or the medication could be administered into a fat pad or ligament.

Registered equine antihistamines containing chlorpheniramine are controlled medications. Care should be taken if using human antihistamines, e.g. bromodiphenhydramine, as a number will be reclassified as banned substances in 2022. Cyproheptadine administration as a treatment for head shaking has resulted in an AAF at an EA event. Although the FEI detection list reports a 4-day detection time for oral cetirizine, care should be taken when considering a withdrawal time as serum hydroxyzine and cetirizine concentrations were still above the limits of quantification 96 hours following a single oral dose of hydroxyzine in one study.[12] Concurrent administration of cetirizine and ivermectin is known to increase the half-life of cetirizine.[13]

Tiludronic acid and clodronic acid are classified as controlled medications. All other bisphosphonates, including zoledronic acid, are now banned. The FEI detection time for tiludronic acid is based on a published pharmacokinetic study.[14] The detection time was shorter in healthy horses compared to lame horses. Clodronate is rapidly cleared from the blood and has a variable and biphasic urinary excretion.[15] Plasma concentrations were below the limit of detection by 40 days in one study.[16] Bisphosphonates are taken up by bone and then slowly excreted over a sustained period of time. There have recent concerns in the racing industry following the detection of tiludronic acid in horses nearly three years post-administration.[17]

Table 2: FEI adverse analytical findings (2016-2021)[7]

Controlled Medications	Controlled Medications (Specified Substances)	Banned Substances	Banned Substances (Specified Substances)
Phenylbutazone	Caffeine	Diisopropylamine	Synephrine
Dexamethasone	Theophylline	Medroxyprogesterone Acetate	
Flunixin	Theobromine	Stanazol	Oripavine
Triamcinolone	Morphine	Boldenone	Ergonavine
Meloxicam	Scopolamine	Ractopamine	Demecolcine
Diclofenac	Codeine[B]	Testosterone	Sparteine
Diclofenac	Paraxanthine[A]	Tramadol	
Acepromazine		o-desmethylvenlafaxine	
Lidocaine		Clomethiazole	
Altrenogest		Nandrolone	
Harpagoside		Strychnine	
Salicylic acid		Trometamol	
Flumetasone		Erthyropoietin (EPO)	
Clenbuterol		Arsenic	
Cobalt		Tolfenamic acid	
Dipyrone		Desoximethasone	
Isoflupredone		Oxycodone	
Mepivicaine		Venflaxazine	
Xylazine		Ergonavine	
Atropine		Pramoxine	
Romifidine		Mephentermine	
Betamethasone		Oxetacaine	
Hydroxydetomidine		Arpiprazole	
Trometamol[A]		Minoxidil	
Dembrexine		Guanabenz	
Ketoprofen		Piroxicam	
Prednisolone		Paracetamol	
Salbutamol		GW1516 sulphone	
Tiludronic acid		Reserpine	
Hydrocortisone hemisuccinate		Colchicine	
Procaine		Oxethazaine	
Aminorex			
Propanolol			
Furosemide			
Methocarbamol			
Gabapentin			
Cetrizine			
Paracetamol (acetaminophen)			
Bupivicaine			
Ambroxol			
Ipratropium			
Isoxsuprine			

[A] Originally classified as banned substances; [B] Codeine is classified as a specified substance if detected as a metabolite of morphine.

No reproductive treatments are allowed during competition and only altrenogest is permitted for use in mares. Progesterone is a controlled medication. Cobalt is also considered to be a controlled medication, and the urinary and plasma thresholds are the same as the current Australian racing thresholds. Care should be taken with the administration of herbal and natural supplements. Although some substances such as Traumeel and arnica are allowed during competition, other substances such as valerian, harpagoside (Devil's claw) and capsaicin are known to result in AAF. Many supplements do not list all ingredients or undergo testing for contaminants, and detection times are unknown. Supplements such as hemp oil which is supposed to be cannabinoid free, could still result in an AAF. Pitcher plant extract is considered a controlled medication. There are also several vitamin/vasodilator products available in Australia which contain the banned substance diisopropylamine.

Pergolide mesylate and metformin have created the most controversy in recent years in Australia. Both are considered controlled medications and, as such, cannot be used nor detectable during competition. There are no published detection times. The detection time for pergolide is likely to be around 7 days; however, detection in serum has been suggested to be up to 10-14 days in some horses.[18,19] This is long after clinical signs could recur, or ACTH could be expected to return to pre-treatment levels. The possible detrimental effects of continually taking horses off pergolide to avoid an AAF is a concern. There have been a number of EA AAF's to metformin, particularly in show horses. The bioavailability of metformin following oral administration is extremely poor and is further reduced if given with feed (7.1 and 3.9% respectively).[20] The reported half-life is variable from 2-12 hours, with low peak concentrations after oral administration.[20-21] Based on these studies, metformin could be expected to be cleared within a few days, but pharmacokinetics appear to be different in healthy horses compared to those with insulin dysregulation. Unfortunately, more research is required before a recommended detection time can be ascertained.

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Appendix 1: FEI List of Detection Times

Taken from the FEI List of Detection Times[2] – refer to website for important notes and considerations. This list is updated by the FEI periodically without warning.

Substance	Preparation	Dose	Route of administration	Number of horses	Detection time (hours)
BETAMETHASONE	Celeston/ Soluspan	30mg total body dose in up to 2 joints	i.a.	8	168 (7d)
BUTORPHANOL[6]	Torbugesic® Fort Dodge Animal Health	100µg/kg	i.v.	6	72 (3d)
CETIRIZINE[2]	Allacan	0.38 mg/kg b.i.d for 9 doses	oral	2	96(4d)
CLENBUTEROL*	Ventipulmin	0.8µg/kg b.i.d. q 8 days	oral	6	168 (7d)
DEMBREXINE[2]	Sputolysin	0.3 mg/kg/ 9 doses at 12 hr intervals	oral	6	120 (5d)
DETOMIDINE[2]	Domosedan	0.02 mg/kg	i.v.	10	48 (2d)
DEXAMETHASONE	Aqueous solution	10 mg Na-phosphate	i.v.	6	48 (2d)
DIPYRONE*[2] (METAMIZOLE)	Vetalgin	30 mg/kg	i.v.	10	72 (3d)
FIROCOXIB[4]	Equioxx	0.1mg/kg s.i.d for 5-14 doses	oral	5-20 in 4 different studies	336 (14d)
FLUNIXIN*[2]	Finadyne	1 mg/kg	i.v.	4	144 (6d)
KETOPROFEN**[2]	Ketofen	2.2 mg/kg/5 days/1x/day	i.v.	6	96 (4d)
LIDOCAINE[2]		60-300 mg	s.c.	6	48 (2d)
MELOXICAM[2,5]	Metacam,	0.6mg/kg/14 days	daily oral	8	72 (3d)
		0.6mg/kg/14 days	i.v.	8	72 (3d)
MEPIVACAINE[2]	Intra-Epicaine	0.07-0.09 mg/kg (2ml/40mg)	s.c. lateral lower limb	6	48 (2d)
		0.28-0.35 mg/kg (8ml/160mg)	s.c. neck	6	48 (2d)
METHYLPREDNISOLONE ACETATE	Depomedrol	200mg in 3 joints 100mg in 2 joints	i.a. i.a.	5 5	672 (28d) 336 (14d)
N-BUTYL SCOPOLAMINE[2]	Buscopan mono***	0.3 mg/kg	i.v.	6	24 (1d)
PHENYLBUTAZONE***[2,]	Equipalazone	4.4 mg/kg/5 days/2x/day	Oral	2	168 (7d)
	Phenylarthrite	8.8 mg/kg	i.v.	6	168 (7d)
	Equipalazone	8.8 mg/kg/2x/day 1 + 4.4 mg/kg/2x/day for 10 days	oral	6	168 (7d)
ROMIFIDENE[7]	Sedivet® Boehringer	80µg/kg	i.v.	8	60 (2.5d)
SALBUTAMOL[3] (also known as albuterol)	Ventolin	0.5mg (5 actuations) q.i.d,	inhaled	6	96 (4d)
TILDRUONATE[1]	Tildren	0.1mg/kg/day for 10 days	i.v.	6	672 (28d)
TRIAMCINOLONE ACETONIDE	Kenacord retard 40 (40 mg/ml)	12 mg in one joint	i.a.	6	168 (7d)

Notes

Chlamydia psittaci as a cause of respiratory disease in neonatal foals

Kristopher Hughes
School of Agricultural, Environmental and Veterinary Sciences
Charles Sturt University
Wagga Wagga, New South Wales, 2678 Australia

Introduction

Chlamydia psittaci, a gram-negative obligate intracellular bacterium, is a pathogen of birds globally. The organism may undergo successful cross-host transmission, resulting in sporadic infection in other species, including horses. In humans, infection with *C. psittaci* typically results from direct contact with birds (most commonly parrots) or bird excreta. It causes a systemic infectious disease, psittacosis, characterised by fever, atypical pneumonia, malaise and myalgia. Recently, *C. psittaci* has emerged as a cause of placentitis, abortion and neonatal disease in horses. Further, outbreaks of psittacosis in humans have developed after exposure to products of equine abortion or critically ill equine neonates, reflecting the emergence of a novel source of zoonotic infection.[1]

Chlamydia psittaci infection in foals

In mares, *C. psittaci* can cause placentitis, abortion (last trimester) or stillbirths.[1-3] Histologically, non-suppurative placentitis, foetal pneumonia and non-suppurative hepatitis are typically present.[1,2] On affected stud farms, *C. psittaci*-associated abortions can be single or multiple events and can occur over multiple years. While serological testing may detect increased serum concentrations of antibodies against *C. psittaci* in affected mares, increased titres are unpredictable and often short-lived.

In foals, *C. psittaci* infection has been associated with neonatal acute respiratory distress syndrome (nARDS) and high rates of mortality.[3,4] Foals with *C. psittaci*-associated nARDS are usually less than to 7 days old and some affected foals demonstrate respiratory dysfunction shortly after birth. Affected foals are usually recumbent, inappetent, markedly obtunded and hypothermic. Heart rates and respiratory rates can vary considerably: in one study foals with *C. psittaci* infection, ranges were 40-160 beats/minute and 4-106 breaths/minute, respectively.[4] Common haematological and blood biochemical findings include leucopenia, hypoglycaemia, hyperlactataemia, azotaemia and increased concentrations of acute phase proteins. Arterial blood gas analysis typically reveals hypoxaemia, hypercapnoea and mixed acidosis. Radiographically, marked, diffuse interstitial changes to the lungs are usually present.

Prognosis for survival of affected foals is poor: despite intensive management, including administration of antimicrobial drugs, intravenous fluid therapy, positive inotropes, parenteral nutrition and respiratory support, most foals demonstrate progressive clinical deterioration. In one study, only 2 of 13 foals survived to discharge.[4]

Diagnosis is achieved by consideration of clinical, clinicopathological and radiographic changes, post-mortem examination findings of diffuse bronchopneumonia, pulmonary congestion and atelectasis, and detection of *C. psittaci* nucleic acid by PCR. Ante-mortem, appropriate samples for PCR are swabs of foal nasal secretions and rectal mucosa and the foetal membranes/vagina of mares.[4] At post-mortem examination, swabs/fresh tissue should be obtained from the lungs and foetal membranes for *C. psittaci* PCR.[3,4]

Recent experiences indicate that *C. psittaci* should be considered as a differential diagnosis for late-term abortion in mares and neonatal foals with signs of systemic disease, including

nARDS. Further, the emergence of associations between exposure to products of equine abortion and sick equine neonates have important implications for zoonotic disease and prevention and control of psittacosis. Personnel involved in the reproductive management of foaling/aborting mares and management of critically ill neonatal foals may be at increased risk of exposure to *C. psittaci* and development of psittacosis. As such, appropriate use of personal protective equipment and the risk of manoeuvres that increase the likelihood of *C. psittaci* aerosolisation and human exposure (e.g., cardiorespiratory resuscitation or assisted ventilation techniques) should be considered during these high-risk situations.

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Notes

How I Manage Glandular Gastric Ulceration

Leanne Begg
REC Specialist Equine Centre
373-379 Wallgrove Road,
Horsley Park, NSW 2175

Horses have a single-chambered compound stomach with ulceration occurring in the upper squamous portion (Equine Squamous Gastric Disease; ESGD) around the margo plicatus and the lower glandular portion (Equine Glandular Gastric Disease; EGGD). Horses have evolved as grazing animals with continuous gastric acid secretion. ESGD has been attributed to low gastric pH, which occurs during intermittent feeding of stabled horses. These ulcers can also be induced in experimental studies by intermittent feeding.[1] The pathogenesis of EGGD is not as clear, with gastric acidity being a component but not the entire cause. NSAID administration causes suppression of protective prostaglandin production leading to reduced gastric mucosal blood flow and hence production of protective mucous and bicarbonate in glandular mucosa. The prevalence of ESGD in thoroughbred racehorses in Australia is 82-100%, whereas EGGD prevalence is lower at 47-65% of this population.[2] EGGD is reported to have a higher prevalence in Warmbloods, with stress associated with multiple handlers/riders, individual responses to exogenous ACTH administration and novel stimuli, days of week exercised and trainer effect all increasing the likelihood of occurrence.[3,4]

Clinical signs of ESGD and EGGD traditionally include poor appetite, poor coat, loss of weight and poor performance, girthing pain, low grade colic, windsucking and change in attitude. After starving overnight, diagnosis can be achieved with gastroscopy using a 3m endoscope. Suppression of gastric acid production is the cornerstone of treatment for ESGD and EGGD, with enteric-coated omeprazole the current best option. Improving bioavailability with =administration on an empty stomach is important.[5] Esomeprazole and long-acting intramuscular omeprazole formulations are currently not registered but may be options for the future. Enteric-coated granules are another new option at 2mg/kg dose.[6] EGGD requires a longer duration of treatment. Sucralfate and misoprostol are also used. Sucralfate is a mucosal adherent that also stimulates prostaglandin (PG) and mucous production. Misoprostol is a PG receptor agonist that improves blood flow and hence mucous and bicarbonate production. Management changes such as continuous access to food, avoiding NSAID administration, small forage meals before exercise, constant feeding order and stress reduction are also important.

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Notes

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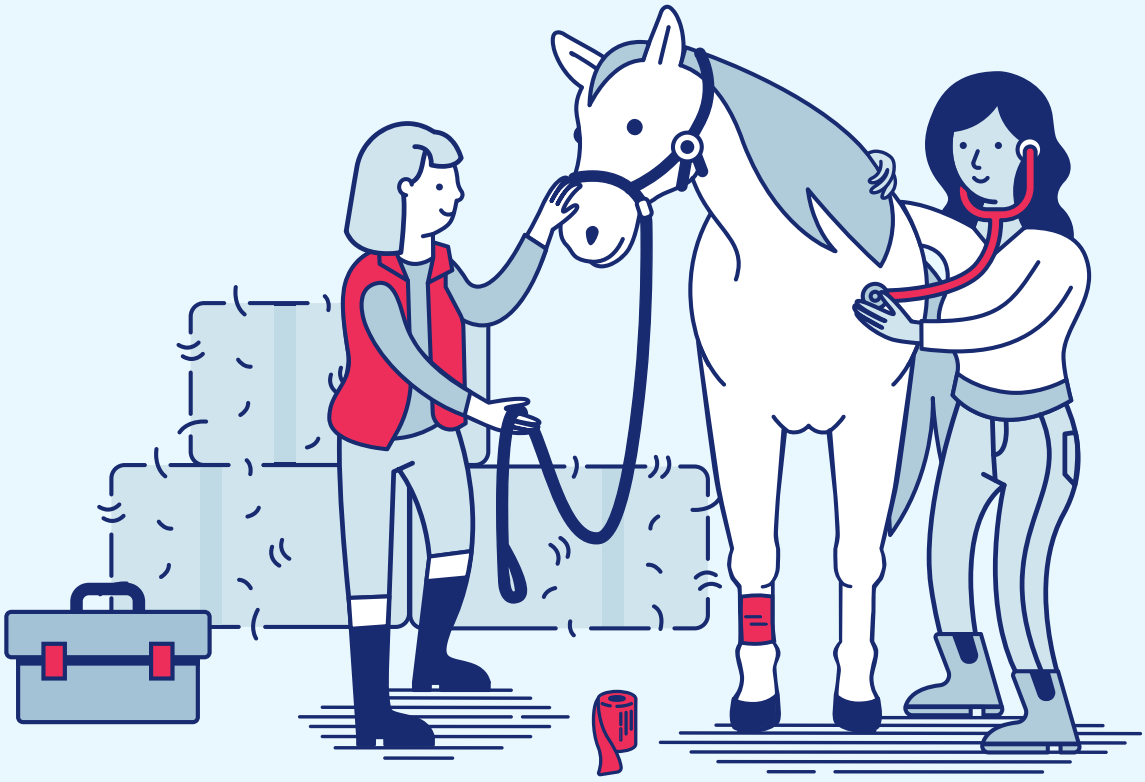

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