



I. Conferencias magistrales

Fetal sexing in early and advanced gestation: more than just a genital tubercle

Dr Stefania Bucca, EVMC, PO Box 5825, Doha, Qatar

Introduction

Fetal gender determination in the mare provides a useful management tool to breeders, by allowing a pre-delivery estimation of the value of offspring. Knowing fetal gender in advance of delivery allows for commercial strategies to be implemented, as the value of stock at sales time is often determined by the gender of the offspring.

Diagnostic windows for fetal gender determination

First stage diagnosis: early gestation

There are two different stages when fetal sex diagnosis can be made by ultrasonography. The first stage is between 57 and 70 days gestation and involves the identification of the genital tubercle by trans-rectal ultrasonography. The genital tubercle is the precursor of the penis in the male and the clitoris in the female and, around day 55 gestation, appears as a hyperechoic equal sign (=), located between the fetal hindlimbs, at an approximately equal distance between the tail and the umbilicus. As gestation progresses, the genital tubercle migrates towards the tail in the female fetus and towards the umbilical cord in the male. The shape of the genital tubercle may change in time into tri-lobed or conical. Disadvantages: Small diagnostic window, a single diagnostic parameter and service required usually at peak time in the breeding season.

Second stage diagnosis: advanced gestation

The second stage for fetal sex diagnosis avails of a much wider diagnostic window between 100 and 260 days gestation and multiple parameters to validate diagnosis (fetal primary sex organs), but may require a combination of trans-rectal and trans-abdominal ultrasound scanning. Furthermore, fetal gender determination in

advanced gestation can be carried out during summer, fall or early winter, at a more convenient time of the year for the busy equine reproduction clinician.

Techniques

Trans-rectal sonographic viewing of the equine fetus requires standard rectal palpation skills, as per routine ultrasound (US) examination of the mare's reproductive tract. Thorough skin preparation of the mare's abdomen is necessary for diagnostic percutaneous US evaluation. Mares are best examined in stocks and although tranquillisation is not usually required, sedation of the mare in advanced gestation reduces fetal activity and lowers the fetus towards the ventral abdomen, enhancing trans-abdominal imaging. Sedation is contraindicated when a trans-rectal approach is adopted.

Cranio-caudal and dorso-ventral orientation of the fetus should be initially established. Gender determination is then made by scanning of the caudal fetal abdomen, hindquarters, and buttocks to identify the position of the genital tubercle or the anatomical structure of the primary sex organs. Frontal, cross-sectional and oblique scanning planes may all be required to obtain adequate visualization of diagnostic parameters, particularly during early fetal gender determination.

Diagnostic parameters in advanced gestation

Fetal gonads are identified within the caudal abdomen as two symmetrical oval structures, ventral to the kidneys, with an oblique orientation of their long axis, converging caudally towards the pelvic inlet. The fetal gonads show a distinctive echotexture that differs from male to female. A marked diversity in echotexture can be appreciated in the female gonad between cortex and medulla with intense peripheral color Doppler signal. Male gonads appear uniformly echodense, with a small outer dotted area (the pampiniform plexus) and a hyperechoic longitudinal, central line (medianistinum). Intense color Doppler signal is detected in these two areas, as they correspond respectively to the pampiniform plexus and the testicular vein.

The fetal primary sex organs may be clearly identified on ultrasound as early as 100 days gestation. In the male fetus a fully comprehensive gender diagnosis will include the identification of: penis and prepuce, scrotum/testicular compartments, urethra and gonads. The penis is visualised in the ventro-caudal abdomen, just behind the umbilicus, may be partially/completely encased within the prepuce or appear fully extended and occasionally erect. The urethra can be easily visualised along the ventral shaft of the flaccid or erect penis as a double hyper-echoic line. The fetal scrotum displays a composite echodensity, as the scrotal compartments appear as two symmetrical, oval, less echodense areas. The hypo-echoic appearance of each scrotal compartment relates to the presence of the adjacent gubernaculum testis.

In the female fetus the primary sex organs to be visualized to reach diagnosis include: mammary gland, nipples, vulva/clitoris, and gonads. The fetal mammary gland can be visualised in the pubic region and appears triangular or trapezoidal in shape and uniformly echodense. The nipples emerge from the ventral border of the mammary gland as relatively large hyper-echodense areas. No relevant structures can be visualised over the ventral perineum, as opposed to the male fetus, where the urethra runs the entire length, up to the anus. The fetal clitoris is a hyper-echoic structure that bulges out of the buttocks, high up in the perineum. The vulvar commissure can be seen coursing between the anus and the clitoris, in a cross-oblique section of the fetal buttocks.

Diagnosis

Diagnosis by a single exam per rectum is rapidly attained when the fetus is in posterior presentation, even up to 8 months gestation. The rate of positive diagnosis per rectum reaches 100% between 110 and 130 days gestation, with an estimated time of less than 150 seconds. In transverse presentation gender determination per rectum is easily accomplished when the fetus assumes a ventro-caudal position within the mare's pelvis. In anterior presentation, the fetal hindquarters can be visualised trans-rectally up to 5 months gestation, according to fetal size and location within the uterus. Rotation of the fetus over the long and short axis is commonly observed up to 8 months pregnancy and frequent changes of presentation occur

around 5-6 months. At this time, repeating the exam 5-10 minutes later may find the fetus in a more advantageous position for diagnosis. A trans-abdominal approach is usually necessary for gender determination over 5 months of gestation, when the fetus lies in anterior presentation.

Finally, proper identification of the mare at the time of examination and the provision of a signed certificate of fetal gender diagnosis should be an integral part of the service offered.

Management strategies for the high-risk pregnancy

Dr Stefania Bucca, EVMC, PO Box 5825, Doha, Qatar

Introduction

Most gestational complications in the mare tend to present during the last trimester of pregnancy. These conditions seem to have an initially slow and elusive course and then suddenly precipitate into a life-threatening emergency; clear examples include: placentitis (covered elsewhere), uterine torsion, hydrops of fetal membranes and ventral hernias.

Uterine torsión

The condition usually presents as a mild intermittent colic during mid to late gestation and most mares will continue to pass feces, despite abdominal discomfort. Involvement of the GI tract can generally be ruled out and the mare usually responds well to administration of non-steroidal - antiinflammatory / analgesic drugs. When blood supply is severely compromised or a concurrent involvement of the GI tract is present, colic signs are more severe and prolonged. A uterine torsion of $\leq 180^\circ$ may go asymptomatic and resolve spontaneously, but rectal palpation will identify excessive tension over the broad ligament involved. Torsions $>180^\circ$ are unlikely to spontaneously correct and increase the risk of compromised utero-placental blood flow and subsequently fetal oxygenation.

Rectal palpation is diagnostic for uterine torsion: one broad ligament is stretched horizontally across the top of the uterus, crossing ventrally and laterally across the midline. The broad ligament on the side of the torsion is usually more caudally placed and runs as a vertical band that disappears under the uterus; ie: in a left uterine torsion, the right broad ligament is stretched horizontally across the uterus. Uterine rupture with subsequent septic peritonitis is the leading cause of maternal fatality.

Management of uterine torsion

Three different modalities of correction, include rolling the mare under general anesthesia, standing flank laparotomy or ventral midline celiotomy.

Hydrops of fetal membranes

Hydrops of fetal membranes refers to the overproduction of fluid into either the amniotic or the allantoic compartments (hydramnion or hydrallantois). Both conditions are uncommon, but universally recognized because of their dramatic appearance and consequences. Their etiology remains obscure, although hydramnion has been occasionally associated with fetal cranio-facial deformities, suggesting a potential role of fetal deglutition in the regulation of amniotic fluid volumes. Congenital abnormalities of the fetus, placentitis and twin pregnancies have been associated with hydroallantois and a genetic component cannot be ruled out. Clinical signs associated with hydrops of fetal membranes typically develop in primiparous or multiparous mares in their last trimester of gestation, after an otherwise uneventful pregnancy. Hydroallantois affected mares present with rapid abdominal enlargement that progresses over a period of a few days up to two weeks. Mares with hydramnion develop less dramatic abdominal distension over a more prolonged time course of weeks to months. Abdominal enlargement is accompanied by clinical signs that occur as a consequence of uterine distension: anorexia, decreased fecal output, depression, reluctance to move or lie down, dyspnea, abdominal discomfort and marked ventral edema, as a result of lymphatic obstruction. In advanced cases ventral abdominal hernia or prepubic tendon rupture will frequently develop.

Spontaneous abortion may occur. Complications associated with delivery are very common and include uterine inertia, delayed uterine involution, puerperal fluid pooling, laminitis and shock. Shock is believed to occur as a result of pooling of fluid in splanchnic vessels, when the abdominal pressure is released.

Diagnosis is made by rectal palpation of the uterus, which will be grossly fluid-filled, with little space for the examining arm and generally no fetus to be felt. The condition is confirmed by transrectal and transabdominal ultrasonography, showing massive amounts of fetal fluids and a growth retarded fetus, which, due to its small size and the expanded uterine environment, will frequently change presentation even passed nine months gestation.

The prognosis for hydrops of the fetal membranes is guarded to poor with respect to a favourable pregnancy outcome or even a full-term gestation. Prognosis for survival of the mare is good if measures are taken to prevent or treat shock and other complications. The prognosis for future use of the patient as a brood-mare is favorable and affected mares can be rebred to produce normal foals.

Management of hydropsical conditions

Maintaining a hydropsical condition is risky due to the danger of further trauma to the mare's uterine and abdominal walls. Therefore induction of abortion or delivery is usually recommended, particularly when the mare shows distress and foaling is not imminent. Reports of successful management of the condition are rare, especially if the mare is not near to term and will require frequent fetal and maternal monitoring, abdominal and limb support bandaging, a laxative diet, NSAIDs and progestagens administration. Controlled drainage of fetal fluids has been attempted in hospitalized patients, by chronic catheterization of fetal fluid compartments, but the technique is fraught with complications. Controlled drainage of fluid through the cervix should precede induction of parturition, as sudden expulsion of large volumes of fetal fluids, sometimes in excess of 100-200L, results in hypotensive shock in the mare.

Ventral ruptures/hernias

Breakdowns of the ventral abdominal wall during pregnancy are most common in older mares of any breed, but it has been suggested that draft mares maybe predisposed to ruptures. Extraordinary uterine weight due to twins or hydrops, direct trauma or severe ventral edema may result in rupture of the prepubic tendon, rupture of the abdominal wall or abdominal hernia. Mares with ruptured ventral abdominal structures present with abdominal discomfort and reluctance to rise or walk in late gestation. The condition may rapidly progress to severe distress, internal haemorrhage, shock and death. A large ventral edematous plaque is invariably observed extending cranially from the udder and often involving the udder itself. Due to the complete lack of ventral support, mares with ruptured prepubic tendon present with an elevated tail head and ischial tuberosity, resulting in lordosis and a "saw-horse" stance and the mammary glands may displace forward. Mammary secretions may be bloody.

Diagnosis of prepubic tendon rupture, and ventral body wall hernia may be difficult to confirm by rectal palpation, as the presence of the fetus makes deep palpation of the ventral abdomen very difficult. In addition, evaluation of the defect by transabdominal palpation is hampered by the extensive edema surrounding the area. Ultrasonography is helpful in identifying disruption of the body wall, hematoma formation, herniation of intestine, but the extent of the damage may not be clearly defined until after parturition, when most of the edema is resolved. Prognosis for survival of the mare is guarded, as some cases may evolve rapidly fatal.

Management of Ventral Ruptures/Hernias

Conservative management strategies that avoid induction of parturition or elective cesarean section and allow for natural parturition have recently been proposed as the treatment of choice, particularly in acute cases. Delivery must be attended since considerable assistance is usually required due to uterine inertia and the lack of an effective abdominal press by the mare.

Successful management of abdominal wall ruptures/hernias may be very challenging and symptomatic treatment will include pain control, diet manipulation, restriction of exercise and abdominal wall support. If the mare suffers of a concurrent predisposing condition such as hydrops, every effort should be made to prevent further abdominal wall deterioration.

Peri-partum hemorrhage

Peri-partum hemorrhage is a serious and often life-threatening condition, thought to be one of the most common causes of death in term and puerperal mares. External and internal reproductive bleeding may occur. External hemorrhage occurs when post-partum bleeding is confined within the uterine lumen, after rupture or laceration of one of the mural vessels; this condition rarely becomes life-threatening, unless the mare experiences other forms of post-partum hemorrhage or a deranged clotting profile. Rupture of the external iliac artery, utero-ovarian artery and uterine artery, regardless of the site of rupture, may lead to direct hemorrhage into the peritoneal cavity, with rapid and profound blood loss, which can result in hypovolemic shock and death. Alternatively, the hemorrhage may be confined to the broad ligament or the serosal layer of the uterus, with hematoma formation. This latter condition carries a more favourable prognosis and hematoma formation in these regions may be an incidental finding during reproductive post-partum evaluation. Large haematomas may cause extensive compression necrosis of pelvic structures, abscess formation and result in chronic peritonitis when abscess fistulization occurs inwardly.

The most common clinical signs are those associated with acute abdominal pain and generalised discomfort, cold sweating, curling of the upper lip, pale mucous membranes and signs of cardio-vascular shock, with tachycardia and tachypnea and prolonged capillary refill time. Some mares will not show any prodromal signs other than peracute death. In suspected cases the mare should be kept quiet and confined to her stall. Analgesia and adequate protection to the newborn should be provided when signs of acute pain are evident. Sedatives, hemostatic agents, volume replacement, resuscitation and surgery are the common therapeutic interventions aiming at preventing further blood loss and maintain adequate perfusion to all vital organs.

Placental pathology: ¿where does it all start?

Dr Stefania Bucca, EVMC, PO Box 5825, Doha, Qatar

Introduction

Placental pathology represents the most common cause of preterm delivery, fetal death, stillbirth and neonatal disease worldwide in the horse ¹. Inflammatory, degenerative, toxic and purely vascular conditions may affect placental tissues and function with variable outcomes. Some pathologies may be inconsequential for the normal development of the foal, while many are fatal and lead to abortion or stillbirth. In between there is a spectrum of less severe pathologies that can result in the birth of a live foal, but the disease process, or its effects, may continue into the post-natal period and beyond. Challenging gestational conditions activate the close interaction between the three pregnancy compartments (maternal, fetal and placental) with resulting compensatory mechanisms, ultimately aiming at maintaining pregnancy. Under these circumstances, identifying the primary insult and pathogenesis can be rather difficult, prompting the question: ¿where does it all start?

Incidence

The incidence of placental pathology is currently based on survey studies of equine abortion. These studies give some indication of general trends and geographical variation on the causes of abortion and foal death. However, direct comparison between studies of different causes of abortion is difficult due to the use of different classifications of disease, different levels of diagnostic investigation and the inclusion or not of neonatal death as well as abortion. Nowadays, umbilical cord torsion, often associated with a long umbilical cord, has been reported as the most common cause of abortion in the UK ², but in US surveys, bacterial infectious placentitis represents the most common cause of abortion ³. It is not clear if these differences

relate to different diagnostic procedures and interpretation or to climatic/environmental or management differences, influencing the risk of placentitis or risk factors affecting cord length and a predisposition to torsion. A more comprehensive assessment of the real incidence of placental pathology should include those conditions affecting the placental functional area, leading to intrauterine growth restriction and a group of placental disorders loosely termed as "placentopathies", associated with premature placental separation. Improving diagnostic techniques may enable us, in the near future, to detect and monitor dynamically placental pathological conditions in order to implement preventive treatment and long-term follow up studies. However, at present we have to rely on placental examination to give historical insight on life in utero.

Pathogenesis

The pathogenesis of a dysfunctional placenta may recognize multiple contributing factors, originating from the fetal or maternal compartments, or more specifically, from a direct insult to the placental tissues. In particular, toxin insult to placental function can potentially take many forms. Key biological components of placental development and function, susceptible to toxic insult, may induce profound changes in placental metabolism. Such changes may result in altered placental development, direct cytotoxic effects on maternal and fetal placental tissue, induction of apoptotic cell death, endocrine disruptors, vasoactive effects on either the maternal or fetal cardiovascular system, altered placental responsiveness to normal physiologic demands (altered homeostasis) and loss of immune-modulation enabling maternal rejection. And the question arises again: where does it all start?...

A large number of infective agents has been implicated in the pathogenesis of placentitis, many being opportunistic or environmental invaders ⁴. Bacterial agents commonly associated with the occurrence of placentitis include *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* as well as *Leptospira* spp and *Nocardioform* bacteria (*Crossiella equi*, *Amycolatopsis* spp and others). Annual variations in numbers and types of placentitis reported in different geographical areas suggest the possible role of environmental factors in the incidence of the condition (i.e: MRLS in Kentucky 2001-2002).

Placentitis

Placentitis refers to inflammation of the chorioallantois, often caused or complicated by infective agents and frequently extending to the amnion (amnionitis) and the umbilical cord (funisitis). The inflammatory response may vary in intensity from slight to severe and show a variable distribution from localized to diffuse. In general, the location of placentitis reflects the route by which the infection entered the uterus ⁴. Four different types of equine placentitis have been described according to the morphological lesions and suggested pathogenesis, namely ascending, focal mucoid (nocardioform), diffuse (haematogenous) and, less commonly, multifocal. Overall, ascending placentitis is the most prevalent type of placentitis.

Lesions distribution

Ascending placentitis

Ascending placentitis in mares is reported to be most commonly caused by microorganisms ascending through the vagina and breaching the cervical barrier. An active role of the cervix in the pathogenesis of the condition is therefore implicit. If there are bacteria and/or fungi on the cervix and the cervical seal is compromised, they can enter the uterus and cause placentitis. Anatomical, hormonal or even neurological factors contributing to cervical incompetence and/or enhanced perineal or vaginal contamination will increase the risk of ascending placentitis. Cervical inefficiency due to critical cervical shortening in mid gestation has been extensively described in women as a leading cause of preterm delivery, with no convincing evidence as to the possible cause. Progesterone administration and cervical cerclage have been used as effective means to correct the condition in the absence of infection ⁵. A similar condition has been recently reported in pregnant mares and a comparable therapeutic approach appears to be highly beneficial ⁶. Ascending placentitis tends to recur in some mares, where regular monitoring of US parameters of the cervix and cervical pole CTUP throughout gestation allows timely implementation of preventive strategies.

Ascending placentitis generally develops and progresses slowly during the course of weeks or months before clinical signs become apparent. The more chronic the infection the more extensive or pronounced the lesions of chorionic thickening and fetal growth restriction are likely to be. Depletion of chorionic villi, thickening and discoloration are commonly observed at the cervical pole of the affected placenta, often associated with a fibro-necrotic exudate. Thickening at the cervical star can prevent it rupturing at birth or abortion, so that the chorion tears across the rostral body. In about 12% of cases, infection will rapidly spread to the fetus causing septicaemia and abortion, before placentitis has become grossly evident. A variety of bacteria can be associated with ascending placentitis, but most commonly *Streptococcus equi* subspecies *zooepidemicus* and *Escherichia coli*.

Diffuse or multifocal placentitis

Less commonly diagnosed, is associated with haematogenous spread of microorganisms to the uterus of the mare with subsequent infection of the placenta. Occasionally mares may show pyrexia and signs of septicemia prior to abortion. This form is usually associated with infection by viral agents (EHV, EVA) and some bacterial microorganisms in the genera *Salmonella*, *Histoplasma* and *Candida*. In several countries an increasingly recognised example of abortion and stillbirth due to a diffuse placental villitis is that caused by a number of different leptospiral serovars.

Focal mucoid placentitis

Also known as nocardioform placentitis, a chronic-active placentitis that occurs at the base of one or both uterine horns or rostral body. Gram-positive "nocardioform" filamentous branching microorganisms (*Crossiella equi*/ *Amycolatopsis* spp/*Streptomyces* spp) have been associated with the distinctive lesions, where sites with villous loss are often coated in brownish, muddy, mucoid exudates. The infection, although limited to the chorionic surface of the placenta, causes late abortion, stillbirths or premature births and has emerged as the most commonly diagnosed type of placentitis during recent years. The pathogenesis of this form of placentitis is presently unknown, and an experimental model to reproduce the disease recently proposed by Canisso *et al* (2015), failed to induce nocardioform placentitis in challenged mares.

Diagnosis

Diagnosis of placentitis during gestation is often difficult, as most mares show no outward signs of infection. Effective treatment of placentitis requires early diagnosis, ideally prior to the appearance of clinical signs (premature mammary development and lactation and vaginal discharge). Currently, ultrasound evaluation of the placenta is used to detect early cases of placentitis⁹ and to implement treatment to prevent abortion and delay premature labor. While this practice has allowed more effective treatment and has improved the outcome in many cases, it is often impractical to ultrasound every mare repeatedly during mid-late gestation. In addition, early stages of placentitis can be missed during ultrasound examination and the technique is also prone to false positive diagnosis, resulting in unnecessary treatment, with long term risk of developing widespread bacterial resistance against antibiotics and the development of "super bugs".

Endocrine monitoring of affected mares can be a useful method to identify those individuals at risk for abortion or premature delivery, since the placenta synthesizes/metabolizes a wide variety of hormonal substances, critical for pregnancy maintenance and well-being. Repeated measurements are required.

In addition, measurement of acute phase proteins has recently emerged as a useful biomarker in mares with experimentally placentitis. Serum amyloid A (SAA) has been reported to have a rapid and dramatic elevation, as early as 2 days post intracervical inoculation. Although SAA appears to be a very sensitive indicator for acute bacterial placentitis, it is a very non specific indicator, as many other acute inflammatory conditions may result in its elevation. It appears likely that more than one biomarker maybe required for accurate and early detection of placentitis in the mare. Following delivery/birth, gross examination and histopathology of fetal membranes should be carried out to confirm diagnosis.

Outcome

Placentitis results in several outcomes. In addition to abortion, stillbirth and pre-term birth, the mare may produce small weak foals, small normal and normal foals. Small, weak neonates represent a special management and medical challenge, as they carry an increased risk of developing sepsis and orthopaedic problems and suffer a degree of prematurity. Small normal neonates usually result from mares displaying clinical signs for quite sometimes. These foals usually do well, as they have completed their fetal maturation stage in preparation of birth¹⁰. They still carry a risk for sepsis and orthopaedic complications.

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Treatment and management of cervical incompetence in the pregnant and non-pregnant mare

Dr Stefania Bucca, EVMC, PO Box 5825, Doha, Qatar

Introduction

Identification of cervical abnormalities is important because these defects are invariably associated with sub-fertility or even sterility. Congenital abnormalities and traumatic injuries to the cervix may preclude

timely dilation and/or closure and prevent its role as a selective valve, leading to cervical incompetence. The inability of the cervix to relax appropriately during estrus has been addressed elsewhere in these proceedings. Cervical incompetence is commonly associated with difficulty conceiving or carrying a foal to term. In humans, cervical incompetence is of major concern ¹ and can result in late term abortions, similar to observations in mares. A variety of techniques have been employed in human medicine including medical therapy with progestagens, cervical cerclage ² and the placement of a cervical pessary ³, some of which may be applicable in the mare.

Anatomical cervical incompetence

Cervical pathology is widely recognized as a major cause of pregnancy failure in the mare. Traumatic, anatomical disruption of the cervical canal may occur at foaling, mostly during uncomplicated births. Cervical incompetence will usually result from severe and/or repeated traumatic insults to the cervix and the inability to form an adequate seal may compromise fertility. Surgical cervical repair is often long-term ineffective, as more severe tears may result from subsequent deliveries. A compromised cervical seal is a predisposing factor to chronic endometritis and ascending placentitis, and supports conditions as pneumometra, urometra and infectious endometritis, particularly when associated with a defective caudal reproductive tract.

Diagnosis of cervical lacerations may be challenging. Most cervical lacerations are best evaluated in diestrus, as the increased cervical tone facilitates assessment of the apposition of cervical folds and damage to the muscular layer. Digital palpation is essential for accurate diagnosis and aims at identifying both longitudinal and circumferential defects of the cervical canal. Complete and incomplete defects are recognized, according to the mucosal layer involvement. Multiple cervical lesions are uncommon and no predisposing site for tearing can be identified.

Surgical repair

Not all cervical lacerations require surgical repair ^{4,5}. Under the influence of progesterone the cervix reduces in size, while smooth muscular tone increases. As a result, small tears may be of little clinical significance. Brown *et al* (1984) ⁶ suggested that only tears involving 50% or more of the vaginal cervix require surgical correction. Mare's reproductive history and clinician's experience are two additional factors to be taken into account. In addition, the rest of the reproductive tract should be critically evaluated to exclude other potential causes of infertility, including evaluation of endometrial biopsies in mares that have been barren for some time. Mares that sustain complete overstretching of the cervical canal, with extensive disruption of the fibromuscular layer are not good candidates for surgical repair.

Surgical repair should be carried out in diestrus and at least 30 days after recent cervical injury. Four to six weeks of postoperative sexual rest should follow, to allow for complete healing and contraction. On alternative, surgery may be carried out after breeding, 2-3 days after ovulation.

Surgical technique

Cervical repair is usually carried out in the sedated, standing mare, restrained in stocks, with the aid of local anesthesia, infused deeply into the vaginal tissue, around the cervical site to be repaired. Epidural anesthesia may be required in mares straining excessively and occasionally cervical surgery will be performed under general anesthesia. Mares are usually treated peri-operatively with systemic antimicrobials, non-steroidal anti-inflammatory drugs (NSAID) and tetanus prophylaxis. The perineum is aseptically prepared and the tail bandaged. To obtain adequate exposure modified Finochietto retractors are used, with elongated blades (>25cm), to reach cranially over the vestibulo-vaginal junction. The cervix is retracted with Knowles forceps or stay sutures (#2 polyglactin 910), and Allis forceps. Long-handled instruments are nonetheless required, as the surgery is performed in the depth of the tubular tract and a good light source is essential.

Three techniques have been described for closure of cervical defects: three-, two- and one-layer closure⁷. The one-layer closure is technically less demanding and can be accomplished more quickly and used at any time, according to McKinnon (personal communication). It involves a simple continuous pattern passed through the outer cervical mucosa and into the muscular layer.

Occasionally cervical damage is beyond repair. Under these circumstances the placement of a cervical cerclage suture may be the only alternative to restore cervical competence and maintain pregnancy to term.

Functional cervical incompetence

Identification of untimely cervical relaxation through serial vaginoscopy or intravaginal palpation poses serious risks to the integrity of the pregnant cervical seal and transrectal palpation, although safe, is less informative. Sonographic cervical length measurement has emerged as an effective prognosticator of preterm delivery in women and extensive research has been made in the past 15 years on the ultrasonographic (US) assessment of the pregnant cervix⁸. A recent study on the US assessment of the pregnant equine cervix under physiological conditions demonstrated progressive changes occurring throughout gestation⁹. The study reported on the safety and non-invasiveness of ultrasonography to assess cervical relaxation, suggesting very little change in cervical size, tone and echotexture occurring up to nine months gestation in normal equine pregnancies. Cervical changes indicating progressive relaxation were consistently observed during the last two months gestation, with gradual palpable shortening and softening of the cervix.

Management of functional cervical incompetence

Detection of signs of untimely cervical relaxation, with or without allantochorial changes, allows for early therapeutic intervention and resolution of clinical signs. Mares carrying a history of ascending placentitis are good candidates for elective US examination of the cervix, as the condition may recur. Transrectal scans should take place at 3-4 week intervals, throughout gestation. Mares suffering from chronic medical conditions, endocrine imbalances and toxic disorders have been observed to show US evidence of premature cervical relaxation, even under progestagen supplementation (personal communication). Cervical cerclage represents an effective means of maintaining cervical closure during pregnancy¹⁰, when adequate cervical tone cannot be achieved by progestagen administration alone. The application of a purse string suture around the cervix is carried out, on the sedated mare, restrained in stocks, up to eight months gestation.

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Ultrasound evaluation of the equine pregnancy and identification of “high risk” conditions

Dr Stefania Bucca, EVMC, PO Box 5825, Doha, Qatar

Introduction

Multiple routine and emergency circumstances in equine practice may require an in-depth evaluation of the pregnancy. Ultrasonography (US) offers safe and continuous viewing of fetal life in the mare, from completion of fetal organogenesis (day 40) to term. The combination of transrectal and transabdominal scanning techniques provides extensive investigation of fetal growth and development and monitoring of the fetal environment. Doppler ultrasonography represents an additional tool to assess fetal viability, by characterizing blood flow through maternal, fetal and placental circulations. Clinical applications of Doppler blood flow velocimetry to the pregnant mare are currently being investigated, showing great promise as a novel diagnostic instruments in the evaluation of fetal health.

Sonographic profile of the equine fetus

The “sonographic” profile of the equine feto-placental unit requires the establishment of a minimum database to ensure adequate fetal growth and development and demonstrate appropriate levels of activity and responsiveness, within an adequate environment.

Fetal growth and development

Several parameters can be measured to estimate fetal size. Orbital diameters/eye volume, aortic diameter, bi-parietal diameter and to a lesser extent fetal chest and femur length have all been reported as useful indicators of fetal growth. The aortic diameter correlates to fetal size more efficiently than any other anatomical structure and measurement should be taken in systole, on a longitudinal scan of the dorsal left hemithorax, in close proximity to the spinal cord of the fetus.

Fetal activity and responsive patterns

Fetal activity and tone reflect central nervous system (CNS) function and development, with decreased activity and declining muscular strength resulting from depressed CNS function. Activity is required to ensure satisfactory muscular development and skeletal joint function, allowing for successful postnatal adaptation. Dormant (inactive) phases are observed at all stages of pregnancy, but are more common and prolonged in late gestation, where they can last up to 60 minutes or longer on occasion. Lack of fetal movements and sudden bouts of excessive activity followed by abrupt cessation have both been associated with a negative outcome. Rhythmical breathing movements may be observed in all fetuses in advanced gestation (from 7 months), when the diaphragm is visualised. Nevertheless, fetal breathing is intermittent in nature and cannot be consistently evaluated.

Fetal Heart Rate (FHR)

FHR and FHR reactivity represent the most sensitive indicators of fetal well-being. Cardiac frequency, obtained by M-mode echocardiography and automatically estimated by the cardiac calculation software, declines as gestation progresses and increases during activity, with accelerations of 25 to 40 beats per minute (bpm) of approximately 30 seconds duration. Sustained tachycardia or a large range of FHRs may indicate fetal distress, but could be brought on by painful maternal systemic problems or excitement. Sustained bradycardia or inappropriate FHR for gestational age, or lack of heart rate reactivity suggests CNS

depression, usually attributable to hypoxia and may indicate impending fetal demise. Fetal cardiac rhythm is usually regular, and cardiac arrhythmias are commonly associated with a negative outcome. Cardiac activity may also be estimated by assessment of peripheral pulses, particularly by the fetal carotid pulse, easily accessible by US per rectum in the fetus in anterior presentation.

Adequate environment

Evaluation of fetal environment includes assessment of fetal orientation, volume and quality of fetal fluids, combined thickness and contiguity of the utero-placental unit, cervical relaxation and, of course, should confirm the presence of a single fetus.

Fetal orientation: presentation

Abnormal presentation causes dystocia and early detection may prevent a serious perinatal crisis, by implementation of specific strategies at delivery. Under normal circumstances, fetal mobility gradually declines as gestation advances and after nine months rotation along the short axis, allowing changes in presentation, is restricted by fetal body size and the encasing of the fetal hindlimbs within the gravid uterine horn. Detection of an abnormal presentation after nine months gestation should raise concern and be investigated as term approaches to formulate an appropriate plan of action.

Volume and quality of fetal fluids

The equine pregnancy includes an allantoic and an amniotic compartment. The distribution of allantoic fluid is directly related to fetal dynamics and uterine tone, with no preferential area of maximal fluid depth detectable. Amniotic fluid tends to collect more frequently around the cranio-ventral half of the fetus. Minimal and maximal allantoic and amniotic fluid depth values are reported in the literature. Pathological increases in fetal fluids have been reported (hydramnion and hydroallantois). Markedly reduced volumes of amniotic fluid (oligohydroallantois) may be observed in mares suffering from severe systemic illness. An association of the condition with a poor fetal outcome has been reported. Objective assessment of fetal fluid depth requires extensive scanning of the mare's abdomen and is best carried out during phases of fetal quiescence.

Combined thickness and continuity of the utero-placental unit

The literature reports reference values for the combined measurement of the utero-placental unit at different stages of gestation. Both uterus and placenta should present with similar echo-texture up until term, when diffuse sono-lucency of the allantoic layers of the placenta may be observed. Adequate utero-placental contact should also be maintained throughout gestation. An average combined thickness of the utero-placental unit of 1.26 ± 0.33 cm has been reported in mares with normal term pregnancies. Measurements should be taken avoiding areas of compression of utero-placental thickness by the fetus, using the ventral uterine vasculature as landmark.

Cervical parameters

Recent data on cervical size and echotexture in the pregnant mare suggest a high degree of cervical tone maintained up to nine months gestation, followed by progressive cervical relaxation until delivery. A high degree of correlation between cervical size and sonographic appearance was also demonstrated.

Doppler ultrasonography

Doppler ultrasonography represents an additional diagnostic instrument to characterize blood flow in the pregnant mare and provides an insight on fetal (umbilical and carotid arteries), maternal (uterine arteries), and placental circulations (intraplacental vessels). In addition, two distinctive color Doppler signal patterns differentiate male from female fetal gonads, offering an auxiliary tool in the diagnosis of fetal gender.

Doppler ultrasonography has become an important clinical instrument for the assessment of placental performance in healthy and high risk human pregnancies, but applications to the equine pregnancies are still limited, due to the lack of reference values. In normal pregnancies, haemodynamic changes in the uterine

arteries progress from a high resistance/low flow pattern during the first half of gestation to a low resistance/high flow system in the second half. Doppler velocimetry indices of the umbilical vasculature and carotid artery are currently being investigated, in order to establish fetal hemodynamic patterns, throughout gestation. Signs of circulatory derangement indicating fetal hypoxia and intra-uterine-growth-restriction (IUGR) could then be identified, as routinely done in the US evaluation of the human pregnancy.

Epigenética gestacional en la yegua: implicaciones para el conocimiento y manejo de la gestación y la cría. Impacto potencial en los potrillos

Epigenetics of pregnancy in the mare. Implications for the knowledge and management of gestation and foals

Luis Losinno, MV, PhD

Laboratorio de Producción Equina, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Argentina

llosinno@gmail.com

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"Si nuestro mundo tuviera que ser entendido sobre la base de los sistemas dinámicos estables, no tendría nada en común con el mundo que nos rodea: sería un mundo estático y predecible, pero no estaríamos allí para formular las predicciones. En el mundo que es nuestro descubrimos fluctuaciones, bifurcaciones e inestabilidades en todos los niveles. Los sistemas estables conducentes a certidumbres corresponden a idealizaciones, aproximaciones". Ilya Prigogine (1917-2003). El fin de las certidumbres (1996). Premio Nobel de Química (1977)

Summary

The environmental effects during gestation epigenetically modulate a series of adaptive physiological events in the conceptus whose effects (beneficial or deleterious) can manifest clinical or subclinical from birth to adulthood and trans generationally to the progeny for several generations. Nutritional levels (deficits or excess), environmental contaminants (air, water, transcutaneous), socio-cultural environment, in vitro manipulation of gametes and embryos can leave their epigenetic marks by various mechanisms, some reversible and self-controlled and others persist most of the time without being detected or perceived generating clinical problems of etiologies often unknown or poorly attributed. In a species such as horses, generally intended for supra-physiological and artificial efforts, these effects are increasingly important.

Introducción

El veloz avance en el conocimiento en nuestra época nos muestra cada día cuanto nos alejamos del dogma conceptual de disciplinas "cerradas" en particular en el campo de las ciencias. El impacto de la biología molecular, la físico-química (en particular cuántica), la genómica -entre otras-, sobre la medicina (fisiopatología, diagnóstico, terapéutica) hace que constantemente debamos revisar y actualizar los conceptos no solo aprendidos en la Universidad sino también los que provienen de la propia experiencia, a la luz de nuevos puntos de vista y evidencias que cuestionan nuestro "espacio de confort" del conocimiento personal.

La relación entre ambiente y genoma y sus efectos potenciales inmediatos y a largo plazo (transgeneracionales) es una de las áreas de mayor interés y estudio en la actualidad relacionada al cáncer, a las dietas, a los efectos de los contaminantes ambientales y sus impactos sobre la salud humana. Los animales domésticos y en particular los caballos no escapan a esto y el objetivo general de este trabajo es realizar una revisión sintética y subjetiva del tema y su impacto potencial en problemas cotidianos de la clínica y

la producción. Los objetivos específicos son: 1) Plantear una hipótesis teórica con sustento experimental relacionada a un concepto de la vida real productiva; 2) desarrollar resumidamente el marco teórico y experimental de la hipótesis y 3) asociar el estado actual del conocimiento al problema concreto de la gestación en la yegua

Concepto de epigenética

El término epigenética (del griego **epi**: sobre; **génesis**: generación, origen, creación- en referencia a la hipótesis de la epigénesis propuesta por Aristóteles-) fue sugerido por primera vez por el biólogo Conrad Waddington de la Universidad de Edimburgo, a raíz de sus experimentos sobre el control génico durante el desarrollo embrionario (Waddington, 1942) básicamente relacionados a un fenómeno de caracteres fenotípicos adquiridos que se mantenían genéticamente estables dentro de la población y que denominó *asimilación genética* (Waddington, 1953; 1956; 1957).

Si bien la postulación atribuida a Lamarck (1809) sobre la herencia de los caracteres adquiridos fue rechazada por la elite de la comunidad científica de la época y durante más de 150 años, sus conceptos permanecieron inquietando a ciertas disciplinas biológicas, en particular la microbiología, botánica e inmunología. A partir de los trabajos pioneros en biología molecular a principios del siglo pasado (Luria, Dellbrück-ambos premios Nobel de Fisiología en 1969-), nuevamente comienzan a considerarse a la luz de resultados experimentales y al nacimiento de nuevas corrientes dentro de la biología evolutiva que cuestionan seriamente los conceptos Darwinianos y Mendelianos clásicos no solo de la herencia sino también de la evolución de las especies (Jablonka y Lamb, 2008; Jablonka y Raz, 2009, Van Soom *et al.*, 2014). Para una introducción a estos conceptos desde una perspectiva biológica recomiendo los libros *Epigenetics. How environment shapes our genes* (Francis, 2011), *Domesticated. Evolution in a man made world* (Francis, 2016) y *Evolución en cuatro dimensiones. Genética, epigenética, comportamiento y variación simbólica en la historia de la vida* (Jablonka y Lamb, 2013).

La hipótesis de Waddington fue publicada en los comienzos de la biología molecular y la genética aplicada (1942) casi en oposición al dogma aceptado y, -como suele ocurrir-, permaneció casi ignorada durante 40 años. El crecimiento en el estudio de la disciplina ha sido exponencial en los últimos años en casi todos los campos de la biología y medicina. Si rastreamos la palabra *epigenetics* en algunas bases de datos científicas, *PubMed* revela 19.316 citas y *Science Direct* 85.660, de las cuales 14.543 y 44.914 publicaciones respectivamente corresponden a los últimos 5 años.

Sin embargo, la definición del término aun es motivo de controversia y está en constante evolución en sí mismo. De todos modos, se acepta a la **epigenética** como la disciplina que estudia los cambios en la expresión génica, potencialmente heredables, que NO involucran cambios en la secuencia del ADN (genoma), generando un **epigenoma** particular y específico de cada individuo y consecuentemente un **fenotipo** (o **fenoma**, según las nuevas denominaciones) (Figura 1). Son múltiples los mecanismos moleculares por los cuales se producen los cambios epigenéticos, pero los más frecuentes y estudiados hasta el presente son: 1) metilación del ADN; 2) modificación de las histonas y 3) modificaciones del ARN no codificante. Para una lectura más detallada de los procesos y mecanismos moleculares recomiendo los trabajos de Dindot y Cohen (2013), Van Soom *et al.* (2014), Fraser y Lin (2016), Ho Shuk-Mei (2017), Grealley (2018).

Gestación-ambiente-progenie

A fines de 1944, al inicio de un invierno muy crudo en lo que sería el último año de la Segunda Guerra Mundial, jerarcas del ejército alemán en Holanda, frente a acciones armadas de la resistencia deciden tomar represalias aislando poblaciones enteras de los suministros de alimentos y destruyendo las fuentes productivas en las granjas de la región occidental del país. En poco tiempo la ingesta de los habitantes descendió a 1.000 calorías diarias (significativamente menor a las 2.300 y 2.900 calculadas para una mujer y un hombre activos respectivamente). En febrero de 1945 la ingesta se redujo a menos de 580 calorías diarias por persona. Para el mes de mayo, cuando Holanda fue liberada por los aliados, se estima que habían muerto 22.000 personas a consecuencia de lo que luego se denominó "*la hambruna holandesa*"

("Dutch famine"). Los mayores efectos fueron sufridos por la población urbana y suburbana de menores recursos. Este hecho es considerado único en epidemiología clínica humana porque tiene una fecha de inicio y finalización exactas y se mantuvieron registros de salud precisos y meticulosos, a tal punto que los sobrevivientes y sus progenies conforman lo que se denomina *Dutch famine birth control cohort study* (Kyle y Pitchard, 2006) (Figura 2).

Clement Smith miembro de un grupo internacional de médicos asignados al lugar, fue el primero que reconoció (y estudió) los efectos de la malnutrición gestacional en esa población a través de los pesos al nacimiento y salud de esos bebés (Smith, 1947) marcando una línea de investigación de esa cohorte de personas cuyos registros y análisis continúan hasta el presente. En las décadas de los '60 y '70 del siglo pasado (más de 20 años después de ocurrido el hecho) investigadores de diversas procedencias encontraron que las personas que habían sido expuestas a la hambruna de sus madres mientras cursaban el segundo y tercer trimestre del embarazo tuvieron un peso al nacimiento significativamente menor que los del primero y presentaban significativamente mayores (más del doble) índices de obesidad que los no expuestos, además de otros problemas médicos en la vida adulta como esquizofrenia, depresión, problemas renales, pulmonares, resistencia a la insulina (Stein y Susser, 1972; Stein *et al.*, 1995). Contrariamente, las personas expuestas durante el primer trimestre del embarazo, evidenciaron mayor peso al nacimiento que los del segundo y tercero y, en la vida adulta, mayores índices de hipertensión, enfermedad coronaria, diabetes tipo II y cáncer de mamas (Stein y Ravelli, 1995; Roseboom *et al.*, 2001; Roseboom *et al.*, 2006). Estudios longitudinales subsecuentes en poblaciones humanas que sufrieron efectos parecidos (restricción alimentaria severa en especial durante el primer trimestre del embarazo) en África, India y China confirmaron en cientos de miles de casos documentados (Painter *et al.*, 2005; Dover, 2009) lo que hoy se conoce como la *hipótesis de Barker* del origen de enfermedades de la vida adulta durante el desarrollo gestacional -*Developmental origin of adult disease*- (Barker *et al.*, 1989; Barker *et al.*, 2002; Barker, 2003)

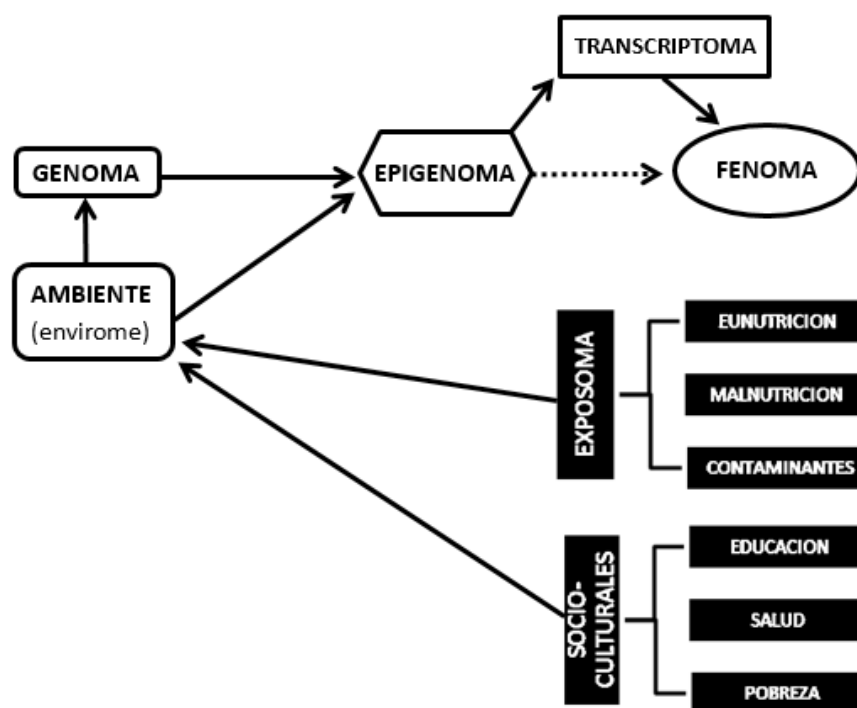


Figura 1. Esquema (simplificado) de la cadena de efectos ambientales sobre la expresión del genoma.

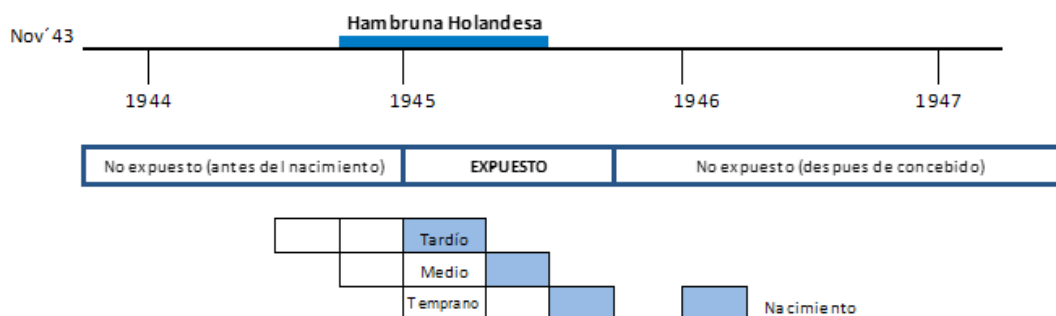


Figura 2. Esquema de la exposición a la restricción nutricional durante la gestación en el periodo de la *Dutch famine* (Holanda, 1945). (Adaptado de Painter *et al.*, 2005).

Epigenética en animales domésticos de producción

Basados en estos hechos documentados a partir de los estudios epidemiológicos en humanos y experimentales en animales de laboratorio, comenzaron las investigaciones en animales domésticos y en particular de producción (bovinos, porcinos, ovinos y equinos) orientados básicamente a desarrollar modelos biológicos aplicables a humanos y también a los potenciales efectos negativos de la dieta (y otros factores ambientales como contaminantes, stress) sobre su salud (y subsecuente producción) y la progenie (Geraghty *et al.*, 2015; González-Recio *et al.*, 2015).

El crecimiento de las publicaciones ha sido exponencial en los últimos 10 años (18.952/19.740 citas en <https://www.ncbi.nlm.nih.gov/pubmed>) y en particular los últimos cinco en lo referido a bovinos de leche, ovinos y caballos (Sinclair *et al.*, 2016; Chavatte-Palmer *et al.*, 2016).

Un caso paradigmático y que generó múltiples estudios que más tarde han sido de enorme importancia en reproducción asistida animal y humana fue el del *Large Offspring Syndrome* o *Abnormal Offspring Syndrome* referido a un síndrome de crecimiento y desarrollo exacerbado al nacimiento en terneros y corderos generados *in vitro* (Van Soom *et al.*, 1994) expuestos a ciertas condiciones de cultivo (Young *et al.*, 1998) y que posteriormente fue demostrado que obedecía a eventos epigenéticos ambientales (co-cultivo, suero fetal) generados durante un periodo crítico del desarrollo embrionario *in vitro* (Young *et al.*, 2001; Farin *et al.*, 2010; Van Soom *et al.*, 2013).

Los efectos de determinados nutrientes (nutrigenómica) y contaminantes ambientales sobre la expresión génica es una de las áreas de gran desarrollo en humanos y animales domésticos (Loores *et al.*, 2015; Rescigno *et al.*, 2017) por el enorme impacto sobre la salud y la producción, pero lo más inquietante son los efectos trans-generacionales de las modificaciones de la expresión del genoma. En estudios recientes se ha demostrado el significativo efecto negativo de la exposición a un agroquímico de uso frecuente en fruticultura (vinclozolin) durante una ventana crítica del desarrollo embrionario sobre la disminución significativa de la fertilidad en cuatro generaciones sucesivas en ratones (Anway *et al.*, 2005). Es difícil medir estos efectos (tanto de la dieta como de tóxicos) en grandes animales con un intervalo generacional muy largo, pero hay decenas de estudios en desarrollo en la actualidad. En vacas de leche, las de más alta producción (coincidente con un periodo crítico de desarrollo embrionario) generan mayor impacto negativo epigenético sobre la embriogénesis de su progenie (González-Recio *et al.*, 2012) con el consecuente perjuicio económico potencial (proveniente de las “mejores” vacas).

Otra de las áreas de investigación en animales está orientada hacia los efectos de la manipulación gamética y embrionaria *in vitro* (OPU, ICSI, IVM, IVC, criopreservación) sobre la expresión génica (y eventualmente sobre el fenoma) de la progenie en directa relación a la reproducción asistida en humanos y animales (Hall *et al.*, 2013; Vrooman y Bartolomei, 2016; Daxinger y Bartolomei, 2016; Fraser y Lin, 2016).

Está claro, de acuerdo a numerosos estudios epidemiológicos en humanos y experimentales en animales, que los individuos de menor peso al nacimiento y/o baja tasa de crecimiento post natal reflejan al menos en parte un desarrollo fetal sub-óptimo lo que incrementa el riesgo de padecer enfermedades (responsables, en humanos del 68% de las muertes en el mundo de acuerdo a datos de la OMS) (*Global status on non-communicable diseases 2014-WHO, 2015; Peugnet et al., 2016*). Los efectos ambientales sobre ambos padres en periodos peri-concepción y gestacionales, incluyendo la lactación, puede inducir procesos adaptativos fisiológicos en el *conceptus* que persisten en la vida post-natal y en algunos casos trans-generacionalmente. En las especies de animales domésticos de producción, estos efectos tienen un impacto negativo sobre caracteres productivos como las tasas de supervivencia post parto, de crecimiento, ganancia diaria de peso, enfermedades ortopédicas del desarrollo, producción de leche, etc. (Chavatte-Palmer *et al.*, 2015). Revisiones exhaustivas sobre el tema han sido publicadas recientemente (Gardner *et al.*, 2009; Kenyon y Blair, 2014; Chavatte-Palmer *et al.*, 2016).

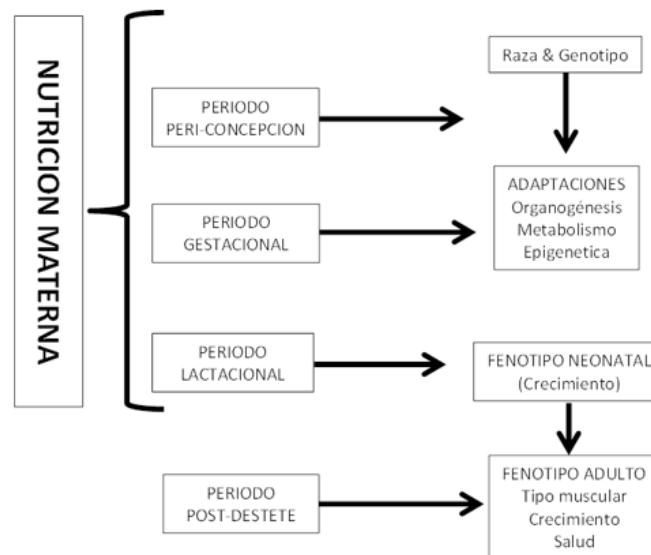


Figura 3. Principios del *Developmental Origin of Health and Disease –DOHaD*. Adaptado de Chavatte-Palmer *et al.*, 2015

Epigenética en equinos

Los caballos, en su doble rol potencial de mascotas o animales de recreación y atletas de competencias, ambos ligados íntimamente al ser humano, están expuestos a muchos efectos ambientales comunes y también a sistemas de reproducción, producción y entrenamiento artificiales manejados en general arbitrariamente, en particular la alimentación.

Las biotecnologías reproductivas (inseminación artificial –IA-, transferencia embrionaria–TE-, aspiración folicular –OPU-, Inyección intracitoplasmática de espermatozoide –ICSI-, clonación–SCNT-) tienen un alto impacto y utilización en sistemas de producción equina desde hace más de 30 años con miles de potrillos nacidos cada año, uno de cuyos puntos críticos es la disponibilidad de yeguas receptoras (IETS, 2013; Lascombes, 2015; Losinno y Urosevic, 2015).

Durante años se ha discutido en el ambiente de la producción equina si la manipulación embrionaria, las receptoras -efecto receptora- y la alimentación (entre otros factores) ejercen algún efecto (directo o indirecto) sobre los potrillos nacidos por TE. Debido a que en general, al menos en Argentina, se utilizan receptoras de un biotipo similar a las donantes y la alimentación es esencialmente pastoril sin (o muy poca) suplementación con concentrados, los potenciales efectos del ambiente mencionado no son, al menos clínicamente, muy evidentes. De todos modos, en los últimos 5 años todos estos factores han comenzado a

estudiarse de manera sistemática y hoy podemos acceder a parte de la información generada y son motivo de discusión en los congresos internacionales sobre el tema (ISEET, 2016; ISER 2018).

Me referiré resumidamente a dos puntos ambientales críticos de efectos potenciales sobre la progenie en equinos: alimentación-dieta en la yegua preñada y efecto receptora en Transferencia Embrionaria.

Los procesos por los cuales determinadas alteraciones en la vida intrauterina temprana generan cambios permanentes en la vida adulta se conocen con diferentes nombres de acuerdo al grupo que los estudia y el enfoque que dan a sus ensayos: *Nutritional Programing of Intrauterine Development* (Barker, 2001; Fowden *et al.*, 2013); *Intrauterine Growth Retardation* -IUGR- (Wu *et al.*, 2006; Coverdale *et al.*, 2015); *Developmental Origin of Health and Disease* -DOHaD- (Barker, 1992; Chavatte-Palmer *et al.*, 2015) pero casi todos relacionan estos cambios principalmente a la provisión de nutrientes, oxígeno y posiblemente muchas otras señales que desconocemos a través de la placenta (Pozharny *et al.*, 2010). Esto puede deberse a un déficit (o exceso) de los nutrientes en la sangre materna por enfermedades específicas (problemas metabólicos) o por la dieta y/o a alteraciones del fenotipo y función placentaria (una de las más conocidas en equinos es la placentitis) entre otras causas (Fowden *et al.*, 2006; Cleal y Lewis, 2015).

Los efectos pueden ser directos, o mediados endocrina y epigeneticamente produciendo cambios estructurales y funcionales en una gran variedad de tejidos y sistemas con potenciales consecuencias en la vida post-natal, que, en especies como los caballos, de vida larga, pueden -algunos- ser compensados durante la juventud pero que se manifiestan con mayor frecuencia cuando comienzan a ocurrir las des-regulaciones propias de la vida adulta y la vejez (Secombe y Lester, 2012; Rescigno *et al.*, 2017). Estos procesos fisiológicos neuroendocrinos y epigenéticos han sido profundamente estudiados en humanos (Felix *et al.*, 2017; Langley-Evans y Muhlhausler, 2018) y varias especies de animales domésticos utilizando modelos de sub y sobre nutrición y recientemente revisados en la yegua por Abby Fowden y su grupo de la Universidad de Cambridge (UK) y Pascale Chavatte-Palmer y su grupo del INRA (Francia) (Fowden *et al.*, 2013; Jellyman *et al.*, 2015; Chavatte-Palmer *et al.*, 2015).

Si bien la subnutrición y/o las dietas desequilibradas en proteínas y micronutrientes son fuentes de problemas potencialmente severos, la sobrealimentación (obesidad) en la población equina ha sido reportada en niveles tan altos como 50% (Stephenson *et al.*, 2011) y en mujeres embarazadas se han reportado tasas de obesidad de 30% (Flegal *et al.*, 2012). Este es uno de los problemas más frecuentes en las yeguas de cría que son suplementadas durante la gestación con altas cantidades de hidratos de carbono solubles de alta digestibilidad. La sobre nutrición (*overfeeding*) en especial en periodos críticos de la preñez puede ocasionar profundos efectos tanto en la madre como el feto en la regulación del metabolismo de la insulina, calidad y volumen del calostro, reducción en el desarrollo del musculo esquelético y susceptibilidad a patologías específicas como síndrome metabólico (Symmonds *et al.*, 2007; Frank *et al.*, 2010; Howell y Powell, 2017).

Si las alteraciones ocurren en el primer tercio de la preñez, en particular durante el desarrollo embrionario temprano (pre-implantacional), el impacto es principalmente sobre la proliferación celular lo que resulta en un menor tamaño de algunos órganos clave (cerebro, riñón, hígado) (Barker, 1997). Pero durante el periodo de desarrollo fetal, que en los caballos es tardío y muy dependiente de la provisión de glúcidos maternos a través de la placenta (Fowden, 1997; Fowden *et al.*, 2013) -lo que los hace más susceptibles a los efectos adversos de la mala nutrición materna-, las alteraciones producen cambios en la diferenciación celular, lo que impacta negativamente en la funcionalidad orgánica (Coverdale *et al.*, 2015; Jellyman *et al.*, 2015). En animales cuya principal función y objetivos de cría luego de la revolución industrial son las actividades deportivas y muchas de ellas de alta demanda física, todos estos efectos pueden actuar sinérgicamente de manera negativa en la expresión del máximo potencial genético y también limitando su performance e incrementando sus probabilidades de lesiones durante el entrenamiento y competencia. No es casual, además, que menos del 50% de los caballos deportivos que nacen pasen el periodo de doma y entren en competencia (Wilsher *et al.*, 2006; Montañez, 2016)

Desde los pioneros trabajos a principios del siglo pasado de Walton y Hammond inseminando yeguas ponys con semen de padrillos Shire (y viceversa) y evaluando las tasas de problemas de parto (ninguno) y de crecimiento de las crías hasta el destete (Walton y Hammond, 1938), el efecto materno durante la gestación ha sido un área de interés y de estudio en la yegua en particular debido en parte a sus implicancias económicas y desde lo biológico a sus marcadas diferencias con el resto de los animales domésticos en cuanto a la espectacular regulación del tamaño fetal por parte del ambiente materno (Figura 3).

Desde principios de los 80's, los elegantes e impactantes experimentos desarrollados en el Equine Fertility Unit de Cambridge (UK) por Twink Allen y sus discípulos y más tarde por Marion Tischner en Cracovia, nuevamente pusieron énfasis en el desarrollo experimental de estos efectos utilizando la TE y la bipartición de mórulas generando gemelos idénticos demostrando de manera categórica el impacto sobre (al menos) las tasas biométricas al nacimiento y que se mantenían hasta los 24 meses en embriones transferidos a receptoras de diferentes razas y tamaños (Allen y Pashen, 1984; Tischner y Kilmczak, 1989; Tishner M, 2000; Allen *et al.*, 2002; Allen *et al.*, 2004).

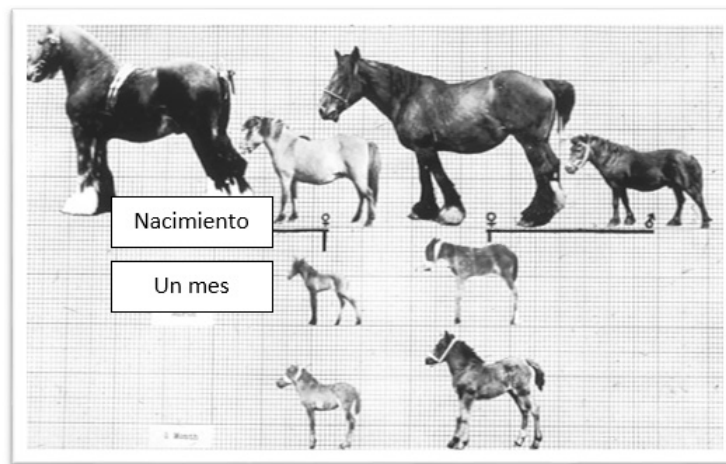


Figura 3. Cruzamientos (por IA) entre padrillos Shire y yeguas Shetland y viceversa y su progenie al nacimiento y al mes de edad. Tomado y adaptado de Walton y Hammod (1938).

Muchos años después, una nueva serie de experimentos utilizando modelos semejantes focalizó sus objetivos en demostrar los efectos metabólicos adaptativos de las preñeces en diferentes biotipos de receptoras (Figura 5) sobre los potrillos al nacimiento y seguidos de manera continua hasta el destete, el año y en algunos casos hasta su carrera deportiva.

Los resultados experimentales, hasta el momento, demuestran que las modificaciones del crecimiento fetal originadas por transferencia de embriones de razas pequeñas en receptoras de mayor tamaño y viceversa generan efectos que impactan en su metabolismo pre y post natal hasta mas allá del destete y los dos años (Peugnet *et al.*, 2016).

Sin embargo, un reporte muy reciente (Vazquez *et al.*, 2018) muestra resultados de un programa comercial de Transferencia embrionaria, donde se transfirieron pares ($n=56$) de embriones (mellizos) provenientes de un mismo lavaje en receptoras de diferentes alturas (Grupo 1: <5 cm de diferencia-media= 2.1 cm-); Grupo 2: $5-10$ cm-media= 7.1 cm; Grupo 3: >10 cm-media= 7.1 cm) y se midieron los potrillos al nacimiento, 6 meses, uno, dos y tres años de edad. Para los potrillos del grupo 1 , hubo diferencias significativas en la alzada al año de edad, para los del grupo 2, a los dos años de edad y para los del grupo 3, al nacimiento, 6 meses y un año. De todos modos, los autores concluyen que cuando la diferencia entre receptoras es de $10-16$ cm o menos, no hay diferencias en las alzadas de los potrillos a los 2 años de edad en la población en estudio.

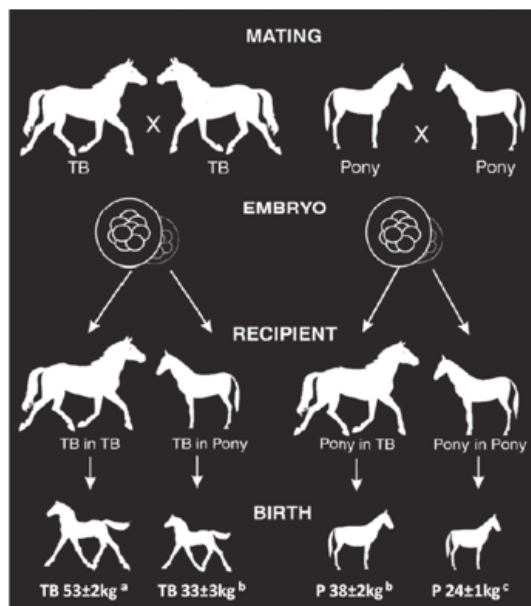


Figura 4. Embriones de SPC y ponies transferidos a receptoras de razas cruzadas y controles. Se expresan los pesos al nacimiento de las crías. Tomado y Adaptado de Allen *et al*, 2002.

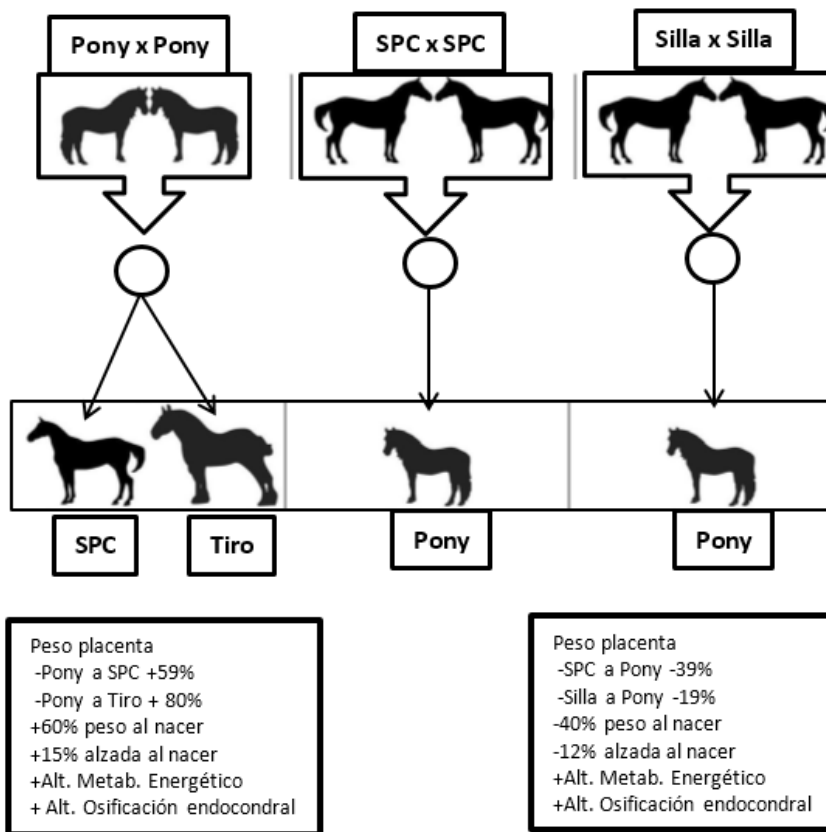


Figura 5. Esquema de apareamientos selectivos y transferencia embrionaria a receptoras de diferentes tamaños para evaluar el efecto materno sobre la progenie (Tomado y adaptado de Peugnet *et al.*, 2014).

En mi opinion siempre es conveniente y saludable que estudios experimentales se contrasten con datos de campo, pero ya sabemos que es mas dificil medir o controlar y sobre todo estandarizar la gran cantidad de variables en estos programas (raza, genetica de progenitores, alimentacion, manejo, sanidad, condiciones ambientales, etc) que potencialmente podrian afectar los resultados y necesitamos mas estudios de este tipo en el futuro antes de hacer afirmaciones categoricas y cualquiera de los casos.

En los caballos, la prevalencia de enfermedades como resistencia a la insulina, sindrome metabolico, laminitis y osteocondrosis ha crecido exponencialmente en los ultimos años (van der Heyden *et al.*, 2008; van Heyden *et al.*, 2013; Wylie, 2011) posiblemente debido a los cambios en las condiciones de cria y entrenamiento y consecuentemente en la alimentacion, de pastoril y semi-extensiva a basada en concentrados y en confinamiento ("efecto feed-lot") y se han asociado a alteraciones en el metabolismo de los glucidos entre otros factores endocrinos de importancia (Fowden *et al.*, 2013). En los potrillos esto es muy dependiente del ambiente materno, aun despues del destete. En la serie de trabajos de Peugnet *et al.* (2016), potrillos Pony x Pony nacidos de receptoras de tiro pesado mayores indices de crecimiento, glucemias mas bajas y concentraciones de NEFA's (*non sterified fatt acids*) mas altas que los controles, desde el dia 180 al 540 de edad. Del mismo modo, potrillos Saddlebred x Saddlebred (de silla) nacidos de receptoras Pony tuvieron retraso del crecimiento (incluyendo al nacimiento), hiperglucemia y menores concentraciones de NEFA que los controles hasta el dia 540 de edad (Peugnet *et al.*, 2016).

Como comentario adicional, los que trabajamos en equidos, conocemos un ejemplo de fenomeno epigenetico ancestral y que desde Aristoteles ha desvelado a biologos, que es el del *genomic imprinting* de los hibridos (mula-burdegano), no solo con manifestaciones fenotipicas conformacionales evidentes sino de **comportamiento** asociadas a estos mecanismos moleculares (Powledge 2011)

Conclusiones

Los efectos ambientales durante la gestación modulan epigeneticamente una serie de eventos fisiológicos adaptativos en el *conceptus* cuyos efectos (benéficos o deletéreos) pueden manifestarse clínica o sub-clínicamente desde el nacimiento hasta la vida adulta y trans-generacionalmente a la progenie por varias generaciones. Los niveles nutricionales (déficit o exceso), contaminantes ambientales (aire, agua, transcutaneos), el ambiente socio-cultural, la manipulación *in vitro* de gametas y embriones pueden dejar sus marcas epigeneticas por diversos mecanismos, algunos reversibles y autocontrolables y otros que persistan la mayor parte de las veces sin ser detectados ni percibidos generando problemas clínicos de etiologías muchas veces desconocidas o mal atribuidas. En una especie como los caballos, destinada en general a esfuerzos supra-fisiológicos y artificiales, estos efectos cobran cada día mayor importancia.

"Como en otro siglo, cuando el telescopio abrió nuevos horizontes para los astrónomos y el microscopio reveló un mundo nuevo a los biólogos, los descubrimientos de la biología molecular todavía no pueden ajustarse a la perfección a los marcos de pensamiento vigentes. Tales revelaciones no vuelven más compleja a la genética tradicional, sino que en cambio ponen de manifiesto todos los supuestos simplistas que se adoptaron en el pasado y muestran áreas enormes de complejidad inesperada. Los genes y la genética ya no son lo que eran."
Eva Jablonka y Marion Lamb. *Evolución en cuatro dimensiones.* Ed. Capital Intelectual, 2013.

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Programas comerciales de transferencia embrionaria: consideraciones para mejorar la eficiencia

Luis Losinno¹, MV, PhD; Melina Pietrani^{1,2}, MV, MSc

¹ Laboratorio de Producción Equina y Cátedras de Producción Equina I y II; Departamento de Producción Animal; Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto. Río Cuarto, Córdoba, Argentina; ² Facultad de Ciencias Veterinarias, Universidad Nacional de La Rioja, La Rioja, Argentina. llosinno@gmail.com

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Introducción

Actualmente, la experiencia acumulada, tanto en el conocimiento empírico como científico- tecnológico marca ciertos *standards* y resultados esperados en programas comerciales que, en general, son conocidos por los clientes-que en definitiva son los que los financian- y por lo tanto son los que exigen a los profesionales proveedores de estos servicios biotecnológicos.

Por otro lado, ahora desde el punto de vista del Veterinario, estos *standards* y resultados marcan los niveles de eficiencia que debería tener su propio sistema, para estar a la altura de los programas de TE a nivel mundial, ser comercialmente competitivos y económicamente viables.

En programas comerciales de TE es una práctica estandarizada registrar las actividades (datos de los ciclos de cada yegua, medicaciones, inseminaciones, tratamientos, flushings, transferencias, etc.), pero resulta difícil intentar emprender un proceso de análisis de eficiencia de un **sistema complejo** como es un programa de TE si no tenemos datos objetivos, numéricos, y en cambio solo tenemos impresiones, opiniones, intuiciones, que si bien tienen su importancia relativa, no resultan suficientes como para realizar diagnósticos de sistemas y sacar conclusiones de posibles fallas y puntos críticos a mejorar. Afortunadamente hoy existen herramientas tecnológicas que nos facilitan el ordenamiento y procesamiento de los datos en tiempo real, desde la carga

de los mismos a la elaboración de informes y consultas *on line*, lo que nos permite posicionarnos profesionalmente en una relación amable y productiva con la tecnología disponible. Toda esta información es de vital importancia para posicionarnos en nuestro sistema y determinar los puntos sobre los cuales trabajar para comenzar a mejorar la eficiencia del programa de TE.

Considerando los sistemas en general, se evidencia que, a pesar de haber incrementado desde los '80 lentamente los valores de eficiencia de la TE trasladada a los sistemas productivos comerciales, son aún muy grandes las pérdidas en términos económicos, tiempo, genética, ciclos de las donantes, semen, expectativas de los clientes, etc.

Aunque los índices con valores medios esperados de resultados de preñez post-transferencia son dependientes de factores tales como: tipo de programa de TE, raza, categoría de yeguas en el sistema, calidad del semen utilizado, alimentación y manejo, calidad y cantidad de receptoras, entrenamiento del personal, etc., es posible establecer valores medios esperados para programas comerciales, tanto de tasas de recuperación embrionaria (TRE) como de preñez (TP) y de muerte embrionaria temprana (MET) hasta el día 60 post ovulación.

En términos generales, en programas comerciales es considerada aceptable una eficiencia global igual o superior a 50%, es decir una tasa de recuperación (TR) por ciclo superior a 70% y una tasa de preñez post-transferencia (TP) superior a 70% (Eficiencia= $TR \times TP = 0,7 \times 0,7 = 0,49$), lo que en términos prácticos se traduce a dos lavajes por preñez como valor teórico esperado. A partir de allí, los incrementos (posibles) resultan de un diagnóstico y control sistemático de pequeños detalles operativos.

De todos modos, hay programas comerciales de gran escala, estabilizados, con eficiencias superiores a 65% en promedio (aproximadamente 1,2-1,5 lavajes por preñez).

Basados en lo anterior, si consideramos en promedio de programas y categorías una tasa de recuperación embrionaria de 70%, el 30% de los ciclos en que hemos inseminado las donantes y preparado las receptoras (semen, insumos, alimento, tiempo, trabajo) han sido **improductivos** (lavaje negativo) y los costos deben ser "absorbidos" por los ciclos productivos de la misma yegua (o por algo o alguien más). Si, además, consideramos una tasa de preñez promedio de 70%, asumimos que 30% de los embriones que recuperamos, vimos en las placas y transferimos a receptoras, NO generaron una preñez (que esperábamos). Las tasas reportadas de pérdidas embrionarias promedio en programas de TE hasta el día 60 varían entre 8 y 15%. Si consideramos un promedio de 10%, una de cada 10 preñeces que vemos ecográficamente en los chequeos entre día 12-14 y 60, estadísticamente se perderán (pero no sabemos cuál de ellas).

No se hará referencia directa en este artículo a los aspectos históricos, científicos, de procedimientos y metodologías de la transferencia embrionaria en detalle, que ya han sido extensamente tratados en revisiones recientes (Stout 2006; Losinno 2014; Losinno y Urosevic 2015; McCue 2016; McCue y Ferris, 2017). El objetivo del mismo es plantear algunos puntos críticos de la práctica de la transferencia embrionaria equina y como pueden impactar respecto a la eficiencia. Deliberadamente no consideraremos en el análisis factores muy críticos e impactantes sobre la eficiencia de los programas de TE como la calidad del semen utilizado, el manejo del laboratorio, etc. y solo nos focalizaremos en algunos puntos específicos sobre los cuales hay publicaciones científicas y experiencias recientes.

Donante

Inducción de ovulación

Dentro de los factores de manejo, la inducción de la ovulación en el momento y con la droga apropiada, pueden condicionar las tasas de ovulación (simple o múltiple) y con ello, modificar significativamente las tasas de recuperación embrionaria.

Tabla 1. Tasa de ovulación en yeguas jóvenes raza Polo Argentino donantes de embriones (Losinno *et al*, 2008)

Tratamiento	Ciclos (n)	Tasa Ovulación (%)	
		Simple	Múltiple
hCG	171	63,2 ^a	36,8 ^d
LAD	390	56,9 ^b	43,1 ^e
Control	849	76,1 ^c	23,9 ^f

ab-c; de-f p<0.001. hCG: Ovusyn®, Syntex SA; LAD: BET LADes®, USA

Tabla 2. Tasa de ovulación en yeguas viejas raza Polo Argentino donantes de embriones (Losinno *et al*, 2008)

Tratamiento	Ciclos (n)	Tasa Ovulación (%)	
		Simple	Múltiple
hCG	48	58,3 ^a	41,7 ^d
LAD	266	50,7 ^b	49,2 ^e
Control	327	68,8 ^c	31,2 ^f

ab-c; de-f p<0.001. hCG: Ovusyn®, Syntex SA; LAD: BET LADes®, USA

Tabla 3. Adaptada de: Panarace M, Pellegrini R.O, Basualdo M.O, Belé M, Ursino D.A, Cisterna R, Desim- one G, Rodríguez E, Medina M.J. *First field results on the use of stallion sex-sorted semen in a large-scale embryo transfer program*. Theriogenology 81 (2014) 520–525.

Ovulaciones/ciclo	Ciclos n	Embriones/ciclo (n, %)
1	699	348/698 (49.8)
2	137	144/137 (105.1)

En las yeguas donantes con ovulaciones dobles espontáneas o inducidas hemos reportado tasas de recuperación de embriones significativamente mayores 106% vs. 53% y 160% vs. 87% (Losinno y Aguilar, 2000).

Intervalo Tratamiento Ovulación (ITO)

Pietrani *et al.* (2016), determinaron que el intervalo de tiempo transcurrido entre el tratamiento con $\text{PgF}_{2\alpha}$ y la ovulación tiene un efecto significativo sobre la tasa de recuperación embrionaria (si el ITO es ≥ 6 días, es mayor la tasa de recuperación embrionaria-TRE- esperada). Esto concuerda con los estudios realizados por Pycok en 2007, Cuervo-Arango y Newcombe en 2010, 2015 y 2017, en los que se determinó un efecto negativo de los ITO cortos (<6 días) sobre las tasas de preñez y las tasas de recuperación embrionaria en diferentes razas y programas reproductivos. Conociendo que la fertilidad se ve reducida en yeguas que responden con una ovulación rápidamente luego del tratamiento con $\text{PgF}_{2\alpha}$, tal vez lo indicado sería esperar a que el folículo regrese o a que la yegua entre en celo espontáneamente. La ventaja de acortar el ciclo con $\text{PgF}_{2\alpha}$ de manera "automática" o sin verificar el estado folicular en estas yeguas se transforma en una desventaja conociendo que la TRE en estas yeguas con ITO corto es baja. En estas yeguas se obtendrá un menor número de ciclos de lavajes por temporada pero probablemente el mismo número de embriones/temporada ahorrando trabajo veterinario, materiales y el costo del semen.

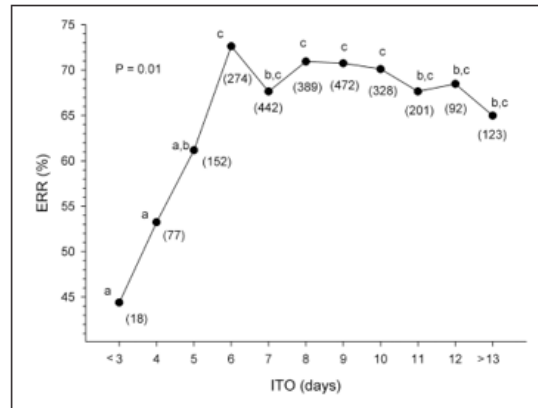


Figura 1. TRE para diferentes ITO (adaptado de Pietrani, 2017).

Recuperación embrionaria

La tasa de recuperación de embriones por cada intento de colecta varía entre 26 y 160% de acuerdo a múltiples reportes durante más de 30 años. La gran variabilidad de estas tasas se debe, entre otras, a la cantidad y calidad del semen utilizado, la edad y estado reproductivo de la donante, el día post-ovulación en el que se intente obtenerlo, el número de ovulaciones, la técnica de lavaje utilizada y el entrenamiento del operador. En estudios controlados, las tasas de recuperación de embriones para yeguas viejas subfértiles (31%) fueron aproximadamente la mitad de las correspondientes a yeguas jóvenes fértiles (71%). Además, las yeguas subfértiles producían una alta proporción de embriones anormales versus los producidos por las yeguas normales (Marinone *et al*, 2016).

Las recolecciones pueden ser hechas en la misma yegua donante, en promedio, cada 14 días durante la temporada de reproducción fisiológica (octubre-abril en Argentina). Es importante considerar que las tasas de recuperación embrionaria pueden ser expresadas considerando: a) número de embriones recuperados/ciclo y b) número de embriones recuperados/ovulación. Ambos valores difieren significativamente en yeguas de polo donde la tasa de ovulaciones múltiples es de aproximadamente 30% y la repetibilidad del evento en la misma yegua, del 70%. El segundo es el índice más preciso y el mejor indicador de eficiencia o pericia del operario, pero es el menos utilizado en la práctica.

Día de lavaje uterino

La mayoría de los embriones llegan al útero luego del transporte a través del oviducto al día 5.5- 6 post-ovulación-PO- (156 horas en promedio es la forma más correcta de considerarlo, Battut *et. al*, 1998). Los intentos para recuperar los embriones en el día 6 resultan en tasas de recuperación ligeramente más bajas comparadas con las del día 7, 8 ó 9. En opinión de los autores, esta tasa consistentemente reportada es posible que no refleje la realidad, debido a que hay dos factores que pueden contribuir a considerar como negativos los resultados de los lavajes tempranos: 1) la falta de entrenamiento de los Veterinarios para la búsqueda de embriones menores a 200 micras, ya que están muy habituados a buscar (y encontrar) embriones de días 7-9 PO en general mayores a 600-800 micras y muchas veces visibles a simple vista, y 2) el extendido uso de estereomicroscopios de muy baja calidad óptica y resolución. Los embriones de día 10 son más difíciles de manejar y requieren de entrenamiento y materiales apropiados, de lo contrario resultan en menores tasas de éxito post-transferencia, aunque recientemente han sido reportadas tasas de recuperación y preñez utilizando estos embriones comparables a los de día 7-8 PO (Wilsher, 2010; García del Gaiso, 2018-comunicación personal-). Considerando todo esto, el día de preferencia de recuperación de embriones es el día 7,5 PO; el día 8 es la segunda alternativa, pero también hay que considerar que en las yeguas viejas puede ser recomendable realizar el lavaje al día 9 o 10 PO debido a que posiblemente su tiempo de tránsito tubárico sea mayor y la tasa de crecimiento del embrión, menor.

Tabla 4. Diámetro relativo del embrión (μm) en relación al día del lavaje (Adaptado de McCue y Squires, 2015).

<i>Día de colecta</i>	<i>Embriones (n)</i>	<i>Media $\pm$ D.E. (μm)</i>	<i>Rango (μm)</i>
6.5	20	191.8 $\pm$ 13.2	150 a 325
7	183	354.0 $\pm$ 13.9	150 a 900
8	35	623.9 $\pm$ 72.9	150 a 2,500

Ha sido reportado y es una percepción generalizada entre los veterinarios, que el uso de semen congelado en programas de TE influencia la tasa de recuperación (menor que con semen fresco o refrigerado) y el tamaño del embrión recuperado (menor que lo esperado para el día del lavaje considerado) (Tabla 5). Es por esto que una práctica corriente es considerar demorar medio día más el lavaje en yeguas inseminadas con semen congelado (días 8-9). No ha sido determinada aun la causa de este fenómeno, pero se hipotetiza que podría deberse a un retraso en la fertilización y/o en el transporte tubárico (McCue y Squires, 2015).

Tabla 5. Diámetro relativo del embrión a días 7 u 8 post ovulación de yeguas inseminadas con semen refrigerado o congelado (McCue y Squires, 2015)

<i>Semen</i>	<i>Día 7 (m)</i>	<i>Día 8 (m)</i>
Refrigerado	401.9	716.9 104.9 ^c
	19.6 ^a	
Congelado	258.2 33.3 ^b	383.5 54.9 ^d

a,b p<0.05; c,d p=0.0553

Relavado

Se ha reportado que 60-70% de los embriones se recuperan en la primera ronda de lavajes (primer litro), pero en caso de que el lavaje (en general utilizando 2 o 3 litros en total) sea negativo y tanto las características de la donante y su ciclo anterior como del semen sean aceptables, es recomendable realizar una nueva ronda (utilizando 20 UI de oxitocina IV) y las tasas de recuperación esperadas en el segundo intento son entre 5 y 15% lo que es muy positivo económica y comercialmente. Una segunda (y posiblemente complementaria) opción es lavar a la yegua 6-12 hs posteriores al primer intento negativo. Si bien los efluentes son más turbios y con mayor cantidad de detritus, se han reportado lavajes positivos en 9.7% de los intentos.

Datos clínicos retrospectivos indican que entre 10 y 30% de las yeguas a las que se les realizan lavajes y no se recupera un embrión y tampoco se les aplica PgF₂ α , son detectadas preñadas, lo que se correlaciona con los datos expresados anteriormente (McCue 2015) y de alguna manera avala o justifica el hecho de considerar el relavado como una práctica a implementar.

Receptora

Las yeguas que se seleccionan como receptoras deberían idealmente ser jóvenes (3-12 años), no ser primerizas, estar en excelente estado de salud, poseer buena condición corporal 3/5 (no obesas), tener buena aptitud reproductiva y mansedumbre. Como condición esencial para nosotros, tener una biopsia endometrial grado 1 o 2A (máximo) según la escala propuesta por Kenney (1978). Resultados de trabajos realizados en nuestro Laboratorio en los últimos años, han demostrado que la diferencia en tasas de pérdida embrionaria en yeguas receptoras seleccionadas utilizando, además de los parámetros mencionados, la biopsia endometrial, es significativamente menor (tabla3) (Losinno, 2005; Castañeira, 2008).

Tabla 6. Tasas de preñez y pérdida embrionaria en yeguas receptoras de embriones. Adaptado de C. Castañeira, C. Alonso, A. Vollenweider, M. H. Miragaya and L. Losinno. *Uterine biopsy score and pregnancy loss rates in embryo recipient mares*. 7th International Symposium on Equine Embryo Transfer, Cambridge, UK, 2008.

Yeguas	N	TP (%)		PE (%)	
		Día 14	Días 15-130	Días 31-60	Total
Con Biopsia	1194	72,5	5,6 ^a	1,6 ^c	7,2 ^f
Sin biopsia	926	71,8	8,2 ^b	5,4 ^d	13,2 ^g
Total	2129	72,2	6,8	3,2	9,8

a-b; cd; ef p<0,001. TP: Tasa de preñez; PE: Pérdida embrionaria.

Sincronización con la yegua donante

El manejo reproductivo de la yegua receptora de embriones ha sido considerado un factor crítico que afecta las tasas de preñez en centros de Transferencia Embrionaria, principalmente el grado de sincronía entre la ovulación de la donante y la receptora. En general se considera que las mejores tasas de preñez se obtienen cuando los embriones son transferidos a yeguas que ovularon desde 1 día (-1) antes a 3 días (+3) después de la donante (Stout, 2003).

Wilsher y col (2008) reportaron que embriones de día 10 posovulación pueden ser transferidos a receptoras ovuladas -2 a +5 días en relación a la donante sin disminución significativa en los índices de preñez. Estudios recientes (Jacob et al., 2012) realizados sobre un importante número de yeguas demostraron una tasa de preñez aceptable (70%) en yeguas receptoras que ovularon 4 o 5 días después de la donante. Esto amplía el rango de aceptación de receptoras, facilitando el manejo. Gibson et al. (2017) fueron más profundo en el análisis de la sincronía entre la donante y la receptora y determinaron que embriones transferidos a receptoras con un grado de asincronía importante con respecto a la donante (+5), sufrían un retardo en el desarrollo, pero no se veían desregulados. Esto se debe probablemente a que los embriones equinos son más tolerantes a la asincronía entre donante y receptora que otras especies.

Recientemente, Oliveira Neto y colaboradores (2018) reportan tasas de preñez aceptables, no significativamente diferentes, en yeguas receptoras (Tabla 7) en diferentes estados reproductivos (anovulatorias profundas, anovulatorias transicionales, estro temprano, estro tardío, diestro tardío y diestro temprano tratadas con PgF2alfa), tratadas con altrenogest inyectable de larga acción (300mg/ml, IM) 4 días antes de la transferencia, el mismo día de la transferencia y luego mantenidas hasta el día 120 de preñez con progesterona inyectable de larga acción. Para cualquier programa de pequeña o gran escala esto podría significar una práctica a considerar, en cuanto a que disminuiría el tiempo de revisión de receptoras y el número total de receptoras requeridas para establecer las sincronías clásicas con métodos convencionales.

Tabla 7. Tasas de preñez post-transferencia embrionaria en yeguas receptoras sincronizadas y tratadas con al- trenogest de larga acción (Adaptado de Oliveira Neto et al., 2018).

Grupo	% Preñez (preñeces/embriones transferidos)
Control	75 (15/20)
Anestro	90 (18/20)
Transicionales (primavera)	90 (18/20)
Estro temprano	75 (15/20)
Estro tardío	75 (15/20)
Diestro tardío	65 (13/20)
Diestro temprano	40 (8/20)
Diestro temprano + PgF2alfa	75 (15/20)

Días de estro de la receptora

En muchas ocasiones las yeguas receptoras solo son monitoreadas para detectar la ovulación, sin importar el estado del útero, ni las condiciones del ciclo estral previo a la ovulación. Cuervo- Arango y colaboradores (2017), demostraron que la tasa de preñez en yeguas receptoras se relaciona positivamente a los días de duración del edema endometrial en éstas, durante el ciclo estral previo a la TE. De acuerdo a este estudio reciente, la probabilidad de preñez de una receptora con 3 o más días de edema durante el estro anterior a la transferencia del embrión, es significativamente superior (83.1%; 157/189) a receptoras con menos de 3 días de edema previos a la transferencia (63.6%; 77/121). Las receptoras con cero días de edema previos a la TE tuvieron los menores índices de preñez (50%; 11/22). Esto sugiere un rol importante de los estrógenos durante el estro para la receptividad del útero y la supervivencia embrionaria post TE. En base a los estudios más recientes, y probablemente a los próximos que vendrán, sería importante llevar un control más estrecho del ciclo estral de la receptora, en particular los días de edema endometrial, considerando el impacto de estas sobre la tasa de preñez.

Método de transferencia

Inicialmente, a fines de los 70's, los embriones equinos eran transferidos a las yeguas receptoras mediante una técnica quirúrgica a través de una laparotomía por la línea media, y más tarde, en los '80 por el flanco con anestesia local. En la década de los '90 comenzó a utilizarse la técnica de transferencia no quirúrgica transcervical, la cual no tuvo buenos resultados al principio, probablemente debido a una manipulación excesiva del cérvix que llevaba a liberación de prostaglandinas y contaminación uterina. Posteriormente, se reportaron altas tasas de preñez por transferencia no quirúrgica en centros de TE en Argentina y Brasil, lo que indicaba un importante efecto de la experiencia y calidad de procedimiento del operador en los resultados, y este sistema fue rápidamente adoptado por todos los programas comerciales y es el que más se utiliza en la actualidad.

Wilsher y Allen en 2004 desarrollaron una técnica que implica la utilización de un espéculo vaginal trivalvo y una pinza cervical (Pinza de Wilsher), para facilitar el ingreso de la pistola de transferencia a través del cérvix con mínima manipulación del mismo y fundamentalmente sin contacto del instrumental con que se transfiere, con la vagina de la yegua. Aunque se reportaron altas tasas de preñez iniciales con este método, los veterinarios en general, no muy afines a cambios en sus técnicas a pesar de la evidencia científica acumulada, no la asimilaron, a excepción de las generaciones más jóvenes, con menos experiencia y dogmatismo.

Un estudio reciente realizado por Cuervo-Arango y col. en el programa comercial de la Universidad de Utrecht (2018), comparó las tasas de preñez obtenidas mediante transferencia convencional y transferencia con pinza de Wilsher con diferentes operadores y con diferencias de experiencia en transferencias entre ellos, observándose en promedio una tasa de preñez significativamente mayor con esta última (92,3% vs 70,9%). Además, observaron que la influencia del grado de experiencia práctica del operador que realiza la TE, sobre la tasa de preñez parece reducirse significativamente con la técnica de Wilsher en comparación con la no quirúrgica tradicional. Dos especialistas con gran experiencia previa en TE con el método convencional obtuvieron tasas de preñez de 78.8% y 79.7% respectivamente versus tres especialistas con poca o ninguna experiencia con el método de Wilsher que obtuvieron 93.4%, 91.2% y 90.9% de preñez respectivamente (Cuervo Arango *et al.*, 2018).

Conclusiones

Es mucho lo que todavía podemos aprender de los datos generados en programas comerciales y experimentales de TE. A pesar que esta técnica está inserta en los sistemas productivos desde hace más de 40 años, como hemos visto, permanentemente se genera información que potencialmente permite incrementar los índices de eficiencia en programas comerciales. Hay que estar alertas frente a la trampa de considerar que la propia experiencia, por mayor que sea, es la única fuente de conocimiento (algo por desgracia bastante extendido en círculos profesionales "exitosos"). Las ciencias biomédicas y en particular las biotecnologías, constantemente generan nuevo conocimiento que se encuentra disponible, cada vez más fácilmente, para todos lo que tengan la actitud de tomarlo.

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Distocia en condiciones ambulatorias: recomendaciones prácticas

Luis Losinno ¹, MV, PhD

¹ Laboratorio de Producción Equina, Cátedras de Producción Equina I y II, Dpto. de Producción Animal, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina. llosinno@gmail.com

Introducción

Dentro de las emergencias médico-reproductivas que el veterinario clínico de equinos posiblemente deba enfrentar en su vida profesional, la distocia es quizás una de las de menor frecuencia y mayor gravedad. Este evento, en la yegua, tiene características muy particulares y diferenciales respecto a los bovinos, una especie que suele ser utilizada como "modelo" en las (cada vez más infrecuentes) prácticas obstétricas en los cursos de pregrado universitarios. De todos modos, aunque sea en bovinos, sería recomendable realizar entrenamientos en las Facultades (o al menos ver a los profesores hacerlos), de manera que como mínimo sepamos qué NO hacer, en primer lugar y cuáles y como son los procedimientos correctos en segundo lugar, y no esperar a una emergencia donde, sin entrenamiento, es posible que los primeros perjudicados sean el paciente y el cliente.

Hay muchas revisiones escritas sobre distocia (ver bibliografía), por lo que esta presentación solo está focalizada en algunos aspectos prácticos y operativos y no es sustitutiva de lecturas más completas, profundas y especializadas sobre el tema.

A diferencia de un parto normal (**eutócico**), se considera **distócico** a cualquier parto que reduce la viabilidad neonatal, causa injurias o daños a la madre o requiere asistencia. En general, creo que nos imaginamos a la "distocia verdadera" cuando el grado de dificultad del periodo de expulsión es tan grande que compromete severamente la vida del neonato y requiere de maniobras complejas, pero mi opinión es que cualquier parto que requiera, aunque sea una mínima asistencia debe ser considerado distócico. ¿Uno puede imaginarse que hubiera pasado si NO intervenía?, ¿cómo hubiera evolucionado? Además, estas situaciones nunca son reportadas como una distocia, dado que en la mayoría de los casos se resuelven de manera favorable - no hay que derivar-, por lo tanto, los índices que se reportan en la yegua (2-4%), son, en mi opinión, engañosos y están muy por encima de esos valores si incluimos lo anteriormente descrito. Existen dos escenarios muy diferentes en cuanto a la posición del Veterinario frente a una distocia y consecuentemente al pronóstico del caso: 1) si es un parto **controlado** por un profesional y 2) si es una **derivación** a una clínica u hospital o una consulta ambulatoria.

En el primer caso, estamos viendo y controlando en tiempo real un proceso biológico secuencial en el que, al menos en el 95% de los casos, todo ocurre como debe ser, sin complicaciones. Pero si hay alguna alteración, debemos tomar decisiones de inmediato y corregirla de la manera apropiada dado que luego de la ruptura del alantocorion, solo tenemos 45-60 minutos de sobrevivida del potrillo (¡si esta en presentación anterior!). Es decir, es una verdadera **EMERGENCIA** donde los puntos clave son el **TIEMPO**, el **CONOCIMIENTO** y el **ENTRENAMIENTO**, en una perfecta combinación (¡recordar que el potrillo está VIVO!). Cualquier déficit en una de las variables NO es sustituida por la otra y mucho menos por la improvisación ciega. La ignorancia en acción es un arma mortal (en general para los demás). Hay que actuar **rápido y bien**, y es nuestra responsabilidad como profesionales.

En el segundo caso, en condiciones ambulatorias, el escenario cambia completamente. En general el potrillo está **muerto** y, pese a lo que habitualmente nos digan, alguien ya trato de arrancarlo del útero y no pudo, por lo que el foco clínico debe estar en la sobrevivida de la yegua y en su posterior fertilidad.

¿Qué está en riesgo en una distocia? La vida del potrillo, la vida de la yegua, la fertilidad de la yegua. Por lo tanto, deberíamos recordar algunos conceptos que pueden ser de extrema utilidad a la hora de tomar decisiones.

UBICACIÓN DEL FETO (presentación; posición; actitud) y DINÁMICA DEL PARTO EUTOCICO.

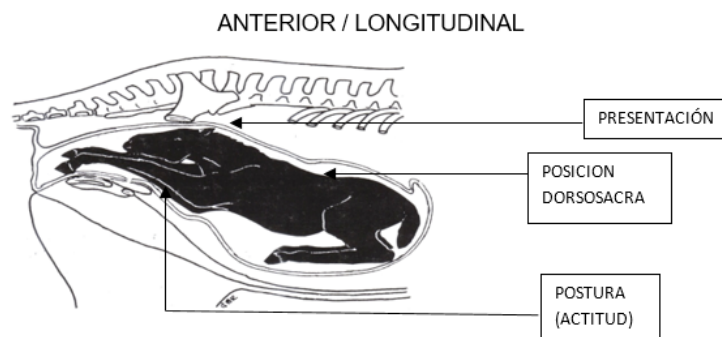


Figura 1. Orientación fetal durante el parto

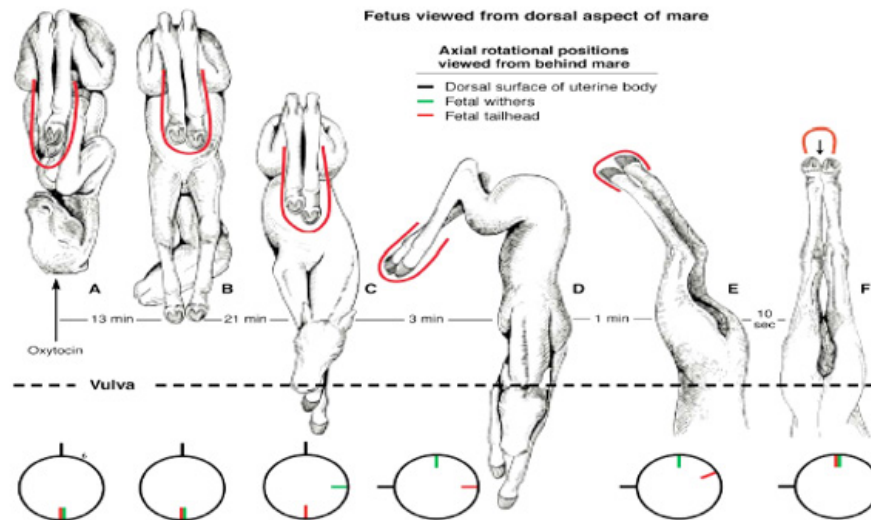


Figura 2. Dinámica temporal del parto normal.

Fuente: Ginther (2005).

Conceptos

1. La distocia siempre es una EMERGENCIA.
2. Las acciones prácticas deben estar sustentadas en: Sólidos conocimientos TEÓRICOS: Mínimas habilidades prácticas, sentido común, confianza en sí mismo, pero no demasiada.
3. A mayor duración, mayor tasa de mortalidad (en la yegua y el potrillo) y menor fertilidad posterior de la yegua.
4. Las **causas** más frecuentes son mal posiciones o actitudes FETALES.
5. Las **complicaciones** más frecuentes son: Desgarros perineales, desgarros cervicales, hemorragias, retención de placenta / endotoxemia / laminitis / shock.
6. Partos eutócicos: Presentación anterior = 98%, presentación posterior o transversa = 2%.
7. Partos distócicos: Presentación anterior = 70%, presentación posterior = 15%, presentación transversa = 15%.
8. Las causas **fetales** de distocia más importantes son: Mala posición / Actitud, anomalías congénitas, mellizos.
9. Las causas **maternas** de distocia más frecuentes son: Pelvis reducida, cirugías vaginales, torsión uterina, inercia uterina.

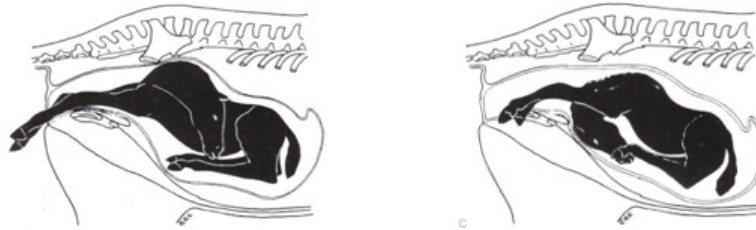


Figura 3. Mala postura fetal más frecuentes relacionadas a la posición del cuello.

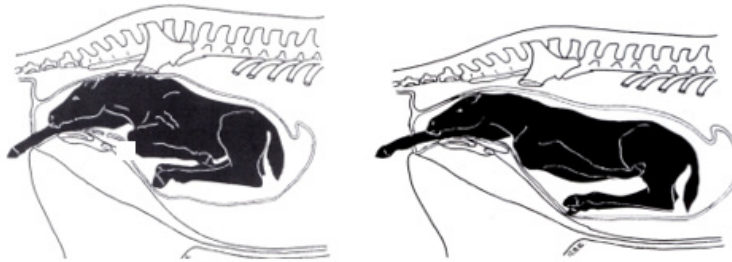


Figura 4. Mala postura fetal más frecuentes relacionadas a la posición de los miembros anteriores.

Objetivos en una distocia

1. Sacar el potrillo.
2. Preservar la vida de la yegua.
3. Preservar la fertilidad de la yegua.

Metodología

1. Prepararse para lo más grave. Sabemos cómo empieza, pero nunca como termina. Visión pesimista inicial muy recomendable.
2. Evaluar el escenario. Como, donde y en qué condiciones deberemos trabajar.
3. Evaluar el estado clínico de la yegua. NO confundir con un cólico. Pueden ser simultáneos. Esto es crítico y no siempre se le da la importancia que tiene debido a la urgencia en “hacer algo” respecto a lo más visible y evidente.

Materiales y equipamiento básico

- Sedación y anestesia: Xilacina; butorfanol; diazepam; ketamina; éter gliceril guayacolato.
- Tocolíticos: Clembuterol.
- Examen obstétrico: guantes, lubricante, antiséptico, sonda nasogástrica y bomba aspirante-impelente, cadenas y sogas obstétricas.
- Fetotomía: fetotomo y accesorios, cable, manijas y ganchos.

Métodos de corrección de distocia

Existen 4 métodos para corregir una distocia. La decisión de cual elegir requiere de: TEORÍA (estudio); SENTIDO COMÚN y EXPERIENCIA y depende mucho del escenario clínico. Para ello es imprescindible: a) una exploración clínica básica, orientada y rápida (2 minutos): frecuencia cardíaca, respiratoria, mucosas, posición, hidratación, sensorio y b) una exploración reproductiva con el objetivo de determinar si el feto está vivo o muerto, presentación, posición y actitud (2 minutos). Mi recomendación es evitar la anamnesis y dedicarse de lleno al paciente dado que, repito, siempre es una emergencia.

Extracción vaginal asistida

Consiste en la corrección manual, asistida, de la dinámica fetal durante un parto distócico con mínima complicación, al menos inicial o aparente. Esta es el método más frecuentemente utilizado, sea por profesionales o "prácticos" con iniciativa. En la mayoría de los partos con dificultad, en general es a lo que primero recurre quien asiste el parto, y no siempre con el conocimiento o entrenamiento para hacerlo. Una mala técnica aquí puede ser fatal, tanto para la yegua como para el potrillo y condiciona en gran medida el éxito de las demás técnicas. La yegua se encuentra en general en estación y puede o no sedarse según criterio profesional. El escenario de trabajo cambia sustancialmente si el parto ha sido controlado y el potrillo está VIVO, donde el canal de parto se encuentra lubricado. En general la respuesta a la maniobra de mutación (repulsión) se dificulta dado que la yegua esta con contracciones severas, para lo que puede utilizarse anestesia epidural, colocación de una sonda nasogástrica y elevación del tren posterior, para facilitar la maniobra, que debe ser rápida y precisa. En general son malas posiciones del cuello (desviaciones laterales o ventral) y/o de uno de los miembros. El pronóstico es favorable en general y no deberíamos demorar más de 15 minutos en corregirlo. Si el potrillo está MUERTO, también la prioridad es sacarlo cuanto antes debido al daño cervical, pero hay que lubricar abundantemente (20 litros) antes de intentar las maniobras. La tracción debe ser controlada y la dirección de las fuerzas guiada por el Veterinario (no más de 2 personas).

Extracción vaginal controlada

Suele ser el segundo paso, tanto si el feto está vivo como muerto y fracaso el intento de la extracción vaginal asistida. Se realiza bajo anestesia general inyectable, en general utilizando el método de triple goteo: xilacina (1,1 mg/kg); ketamina (2,2 mg/kg); diazepam (0,08 mg/kg). Con esta combinación que tiene un alto margen de seguridad, se puede trabajar durante 15 minutos, en general el tiempo suficiente para la corrección. En caso de necesitar más tiempo, se continúa con una solución de éter gliceril guayacolato al 5% en dextrosa 5% vía EV. Se coloca la yegua en decúbito dorsal y se eleva el tren posterior (60-90 cm) utilizando un malacate, lo que posicional el feto hacia craneal, y además se detienen las contracciones. Se realizan las maniobras de corrección de la estática fetal, en un máximo de 15 minutos. Es uno de los sistemas más efectivos no solo en la corrección de las distocias, sino en la preservación de la vida y de la fertilidad posterior de la yegua (menor incidencia de traumas cervicales, vaginales y perineales). Es crítico una buena lubricación previa y ser estricto con los tiempos de trabajo.

Cesárea

Una cirugía sencilla que debe ser indicada rápidamente luego de la evaluación o fracaso de las técnicas anteriores o directamente cuando frente a una distocia el feto está vivo y a término y se tienen las condiciones adecuadas. También está indicada como opción primaria en caso de malformaciones fetales severas, fetos muy grandes, problemas pélvicos en la yegua. Demorar la decisión solo compromete la vida y la fertilidad de la yegua. El pronóstico es muy favorable, aun en condiciones de campo, tanto para la vida como para la fertilidad de la yegua si se contemplan todas las condiciones que requiere esta cirugía abdominal. Insisto en que la peor decisión es ¡no tomar una decisión a tiempo! La descripción de la técnica y sus complicaciones más frecuentes se encuentran detalladas en la bibliografía seleccionada.

Fetotomía

Solo indicada cuando se tiene la certeza que el feto está muerto y si el operador cuenta con los elementos idóneos y tiene entrenamiento y el concepto sobre que se puede y que no se debe hacer en estos casos, por ejemplo, intentar que los cortes (no más de DOS, máximo tres) sean intrauterinos versus intravaginales, en lo posible evitando las laceraciones vaginales. Es una técnica muy recomendable para lograr una rápida resolución del problema con mínimo trauma y excelente pronóstico reproductivo.

Manejo posdistocia

La distocia representa un trauma severo tanto para la yegua como para el potrillo que sobrevive y las primeras 72 hs posteriores deben contemplar una vigilancia clínica y cuidados intensivos en ambos debido al alto riesgo de retención de placenta-metrits-endotoxemia-laminitis; hemorragias; prolapso uterino y crisis

gastrointestinal, además de las laceraciones del tracto reproductivo.

También es una de las mayores causas de pérdida neonatal debido a los problemas que el potrillo sufre durante el proceso, en especial la encefalopatía hipoxio-isquémica y sus complicaciones, por lo que es una de las prioridades a tener en cuenta aun durante el manejo de la distocia, si es posible utilizando el método EXIT (Ex Utero Intra Partum Treatment).

Conclusiones

Recordar que: ***“A mayor duración, mayor tasa de mortalidad (en la yegua y el potrillo) y menor fertilidad posterior de la yegua”***

Una resolución exitosa de una distocia depende de: Tiempo desde la detección del problema a la acción, tener un protocolo de trabajo (mínima improvisación), entrenamiento del Veterinario.

El éxito de una distocia es una yegua preñada y parida nuevamente, es decir fértil.

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Consideraciones para el manejo del semental viejo subfértil

Luis Losinno ¹, MV, PhD

¹ Profesor Asociado, Cátedras de Producción Equina I y II y Director, Laboratorio de Producción Equina. Departamento de Producción Animal, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Córdoba, Argentina. llosinno@gmail.com

Introducción

En los mamíferos superiores, en especial en humanos y primates, existe una marcada diferencia de sexos en cuanto a la duración de la vida reproductiva, donde el excepcional caso de la menopausia femenina, determina un gradual, pero precoz final reproductivo, genéticamente programado, cercano a la mitad de la vida media esperada para la especie. En contraste con estos hechos, en el hombre, si bien existen indicios biológicos concretos referidos genéricamente como **andropausia**, la función reproductiva se extiende hasta como mínimo los 80 años, con disminuciones progresivas en las funciones gonadales (espermatogénesis y esteroideogénesis) y en los parámetros seminales, pero suficientes para mantener la capacidad reproductiva. En términos generales este hecho se repite en los mamíferos superiores (vom Saal *et al.*, 1994).

Senilidad reproductiva en el semental

La mayoría de los estudios comparados sobre senilidad reproductiva en el macho han sido realizados sobre modelos animales de laboratorio (ratón, hámster, conejo, moscas, nematodos) debido a su facilidad operativa de trabajo y sobre todo a su corta vida en relación a los humanos, pero justamente esto cuestiona su valor como modelo biológico comparable a otros animales de mayor longevidad (Ricklefs, 2010). En la mayoría de los sistemas de reproducción, los sementales que han sido seleccionados por su potencial genético, y que desarrollan su actividad en sistemas controlados y en general intensivos, pueden superar, en promedio los 20 años. A través de los registros de las montas o inseminaciones, es posible monitorear aspectos de la fertilidad real en sistemas productivos y en general, la población senil (mayores de 20 años de edad) representa un 10-15% del total de animales en sistemas de producción controlados.

En la actualidad, la mayoría de los sementales utilizados en programas comerciales en el mundo, están de alguna manera relacionados a actividades deportivas o de recreación y es frecuente que sean seleccionados como reproductores de acuerdo a sus características fenotípicas de conformación, su performance individual en alguna disciplina y/o en base a sus ancestros (*pedigree*) y NO de acuerdo a su fertilidad, al menos como primer parámetro (Losinno y Frank, 2008). Como ejemplo, en la raza Pura Sangre de Carrera (Thoroughbreds) la **intensidad de selección**, entendida como la medida de presión que ejercemos para descartar como reproductores los animales de menor mérito genético, es mucho mayor sobre los sementales (6%) que sobre las yeguas (54%). Esto significa que (en promedio) del total de la población de machos disponibles de una generación 94% de ellos y 48% de las hembras respectivamente son descartados como reproductores (Cunningham, 2000).

En el macho, desde la pubertad, la producción de espermatozoides es continua casi hasta el final de su vida (varían obviamente las tasas de producción de acuerdo a muchos parámetros como la estacionalidad, actividad sexual, dieta, aspectos sociales, etc.). Los padrillos, al igual que las yeguas son "ambiente" dependientes en cuanto a la función reproductiva estacional, marcadamente influenciados por el fotoperíodo (y otros factores ambientales y sociales) que afectan el diámetro testicular, los patrones hormonales, la conducta reproductiva y la producción espermática. Estos cambios ocurren gradualmente cada temporada y son un reflejo de la capacidad de adaptación y respuesta del organismo a los estímulos externos que modulan la reproducción. En general, la espermatogénesis es regulada por el eje hipotálamo-hipófisis-testicular (HHT) mediante las gonadotrofinas FSH y LH que actúan sobre el testículo, específicamente en las células de Leydig produciendo andrógenos y estrógenos y en las células de Sertoli produciendo estrógenos e inhibina, generando además de un clásico efecto de retroalimentación endocrino (*feed-back*), un marcado

efecto paracrino y autocrino testicular. Debe quedar claro que esta es una red muy compleja de factores que interactúan de forma permanente regulando un mecanismo que generalmente reducimos en extremo.

Las disfunciones neuroendocrinas, es decir regulatorias y de modulación de procesos fisiológicos como la espermatogénesis son uno de los importantes efectos del envejecimiento que afectan la función reproductiva en el macho (Handelsman, 2006). Estos efectos son de lenta y gradual evolución. Es decir, generalmente sub-clínicos y muchas veces asociados a otros efectos locales sobre las gónadas (neoplasias, degeneración testicular) y sistémicos (Síndrome Metabólico, inmunodepresión, baja libido).

De todos modos, el envejecimiento no es una enfermedad sino un proceso multifactorial generalizado, es decir, que afecta a casi todos los órganos y sistemas simultáneamente dado que son alteraciones celulares, que conducen a una pérdida progresiva de funciones orgánicas, por lo que no deberíamos focalizar la atención del estudio y control de la senilidad solamente en el tracto reproductivo sino, como veremos, en el individuo como unidad biológica (Partridge *et al.*, 2011).

¿Qué es un semental viejo?

De acuerdo con Kirkwood (2000), considero que al igual que en las hembras existe una marcada diferencia entre edad cronológica y edad biológica debido a factores genéticos, epigenéticos ambientales (manejo, alimentación) e individuales que dificultan establecer una edad específica como punto de corte. En humanos, una mutación del gen denominado "Peter Pan" acelera el envejecimiento debido al acortamiento de los telómeros. Se estima que hasta el 7% de la población tiene 2 copias de la misma y parece 8 años mayor que personas de la misma edad (Wolpert, 2013).

Mi criterio para determinar un rango etario a partir del cual considerar un semental como "viejo", incluye un análisis de los factores enunciados, un examen clínico- andrológico individual y fundamentalmente -en caso de ser posible obtenerlos- un análisis de los datos históricos de fertilidad (*breeding records*). En general, y bajo los condicionantes mencionados, podríamos considerar como viejos a padrillos con edades superiores a los 18-20 años, a diferencia de las yeguas donde estos valores se sitúan alrededor de los 15-16 años. En ambos casos, para la categorización etaria de yeguas y padrillos se toma como uno de los parámetros más representativos, además de la edad cronológica, una disminución significativa en los valores poblacionales de fertilidad y de la capacidad homeostática (*ageing phenotype*).

Problemas más frecuentes asociados a la senilidad en el semental

- Disminución gradual y progresiva de la fertilidad (subfertilidad).
- Disminución de la libido.
- Disminución de la capacidad copulatoria (monta-eyaculación).
- Tendencia a la obesidad.
- Incremento de la susceptibilidad a enfermedades infecciosas.
- Incremento de la incidencia de neoplasias testiculares.

Disminución gradual de la fertilidad

Es importante considerar y recordar que la FERTILIDAD, al igual que el éxito, la felicidad, la juventud, etc., **no es** un atributo **permanente** ni **unidimensional**, sino variable, multifactorial y que suele ser cuantificada poblacionalmente. En algunos casos la disminución de los valores estándar esperados (subfertilidad) puede ser reversible y en general la predicción de la fertilidad potencial es difícil (y costosa) de estimar con la agudeza y precisión que suelen demandar los propietarios a los Veterinarios.

Para detectar alteraciones en los índices de fertilidad, que no estén asociados a variables de manejo y de la yegua, entre otros, y que puedan ser atribuidos al padrillo, es necesario como mínimo disponer de datos confiables y ordenados, de lo contrario la disminución de los índices reproductivos será siempre una sorpresa (desagradable) y lo más frecuente es atribuirlo *a priori* a la yegua, al manejo o a cualquier esoterismo circundante.

La causa biológica permanente y progresiva (si descartamos los factores de manejo) más frecuente es la **Degeneración Testicular (DT) Idiopática**, que puede definirse genéricamente como un conjunto de hallazgos clínicos -de origen posiblemente multifactorial desconocido-, progresivos e "irreversibles", que pueden afectar uno o ambos testículos y ser focalizados o generalizados. Estos signos incluyen: 1) disminución de la calidad seminal, 2) de la fertilidad, y 3) del volumen testicular (Turner, 2007). Recientemente se ha asociado este tipo de DT a disfunciones testiculares, más específicamente a nivel de post-receptores gonadotróficos en las células de Sertoli, que afectarían las vías esteroideogénicas (Roser, 2011; Woodward *et al.* 2018).

Si aceptamos que estos hechos tarde o temprano sucederán (es esperable que ocurran en la mayoría de los sementales a partir de los 16-17 años), ¿Cómo podríamos detectarlos? Sencillo (pero infrecuente): **monitoreando a los sementales**. Es decir estableciendo parámetros de control objetivos cuantificables mientras son jóvenes y mantienen tasas de fertilidad aceptables, tanto en los aspectos **clínicos** (evaluaciones seminales periódicas; estimación de la producción diaria espermática [DSO-Daily Sperm Output-] al menos 2 veces por año, durante y fuera de la temporada reproductiva; ecografía testicular de rutina) como **endocrinológicos** (determinación de niveles plasmáticos de al menos FSH, estrógenos y testosterona) (Roser, 2011; Douglas, 2011; Tibary, 2011).

En programas de monta dirigida intensiva donde en general NO se realizan espermogramas de rutina, como en la raza Sangre Pura de Carrera (SPC) y en el caso de padrillos viejos y/o subfértiles, es recomendable tomar periódicamente muestras seminales de uretra post-monta (sobre portaobjetos atemperados) para controlar la presencia de espermatozoides y también de células inflamatorias. Es una técnica simple, barata y al menos permite un cierto control durante el periodo de servicios. Para una lectura más profunda sobre el tema sugiero las excelentes y recientes revisiones de Steiner *et al.*, 2009; Blanchard *et al.*, 2011; y Unphenour *et al.*, 2011. De todos modos, hay que considerar que esta técnica, si bien es práctica, tiene una baja sensibilidad y especificidad por lo que recomiendo ser precavidos con las conclusiones basadas en estas muestras.

Una mención aparte en este punto es la referida al análisis estadístico de los registros y las estimaciones de fertilidad "real" de un padrillo. Hay que considerar que cualquier estimación ("fertilidad observada" o "aparente") es referida inevitablemente a un grupo de yeguas heterogéneo, cuyas fertilidades esperadas (*female true fertility*) son diferentes, pero que en los análisis, en general se asumen como las mismas con la consiguiente sub-estimación de la fertilidad del padrillo. Estos valores, por lo tanto, deberían ser considerados con cierto escepticismo si no se utilizan herramientas de análisis que ponderen los datos. Por ejemplo, la fertilidad estimada de un semental referida a un grupo menor de 25 yeguas tiene un 30% de probabilidad de estar 10% superior o inferior a lo indicado, o sea no representa la realidad. Para una revisión de los análisis de registros y estimación de fertilidad, véase Amman, 2006 y Love, 2006).

Disminución de la libido

La disminución de la libido asociada a la edad puede estar relacionada a:

1. Factores de manejo relacionados a agresiones por parte de las personas que los manejan (padrilleros). Frecuentemente esto es debido a la falta de un entrenamiento básico formal (controlado) o directamente al temor a los animales que se traduce en castigos "preventivos" frente a patrones de conducta normal o en algunos casos agresivos, desmonta activa (forzada) durante los servicios a mano, penalización directa frente a erecciones periódicas espontáneas ("masturbación") (McDonnell, 2011 a).
2. Factores de sumisión frente a la presencia cercana y permanente de otros padrillos dominantes (problemas de manejo y de diseño de las instalaciones), aunque esto es más frecuente en padrillos jóvenes o cuando se los cambia de hábitat. En general suele ser temporario.

3. Cambios de padrilleros y/o escenarios de vida o de manejo (alimentación, frecuencia de servicios, encierro).
4. Exceso de montas, tanto en servicio dirigido como en manadas (*overuse*), algo relativamente frecuente en padrillos comercialmente exitosos y/o mal manejados (*overbooked*).
5. Problemas endocrinos específicos. Los cambios endocrinos más importantes asociados al envejecimiento involucran en primer lugar al páncreas y la tiroides, en segundo lugar a la disminución de DHEA (dehidroepiandrosterona) por parte de las adrenales ("adrenopausia") y en tercer lugar una disminución funcional del eje GH-IGF-1 (Growth Hormone-Insulin Growth Factor-1) (Rajfer, 2003; Lamberts *et al.*, 1997; Douglas, 2011). Estos problemas en los sistemas regulatorios por excelencia del organismo, inevitablemente se traducen en disfunciones multi-orgánicas, al principio subclínicas y muy difíciles de diagnosticar.
6. Problemas musculo-esqueléticos (dolor) o enfermedades sistémicas (insuficiencia cardíaca o respiratoria) que interfieran con la excitación y/o la capacidad copulatoria. Son muy frecuentes, pero en general sencillos de diagnosticar con un exhaustivo examen clínico especializado.
7. Preferencias o aversiones individuales (estado del ciclo de la yegua, pelaje, categoría, altura, padrillero, lugar de la monta, etc.). Casi todos estos factores pueden ser asociados a algún evento pasado y pueden ser revertidos con apropiados sistemas de re-entrenamiento basados en objetivos claros y determinados, mucha paciencia, y aplicación de técnicas específicas (McDonnell, 2011).
8. "Aburrimiento" debido a encierro permanente, bajo nivel de ejercicio, dietas hipercalóricas (obesidad), baja exposición al ambiente (luz natural) y a otros individuos (interacción social) (McDonnell, 2011). También pueden ser revertidos estos efectos con programas de manejo racionales superando el efecto "mascota" humanizante aplicado a las condiciones de vida de los animales.
9. En ausencia de factores físicos u orgánicos que pueden ser diagnosticados por un exhaustivo examen clínico y tratados específicamente (analgésicos, ansiolíticos), la disminución de la libido es una gran limitante en la fertilidad. Además de las pautas de manejo, que deberían ser las primeras herramientas a utilizar, es posible en algunos casos incrementarla con una terapia hormonal adecuada y controlada. Si los niveles plasmáticos de testosterona están por debajo de 0,5-1 ng/ml, la administración de 50 µg de GnRH (SC) 1 o 2 hs antes del servicio puede elevar los niveles a rangos de 2-4 ng/ml, incrementar la libido y no interferir con los mecanismos endocrinos del padrillo (McDonnell, 2011b). Douglas (2011) sugiere 500 µg de GnRH/día, pero es importante considerar que tipo de GnRH se utiliza dado que no solamente varían en su potencia entre análogos, sino que también es muy importante el vehículo en que esta la droga y que determina en parte su acción farmacológica (Douglas, 2011).

La utilización parenteral de soluciones acuosas de testosterona (80-100 mg/450 kpv), IM o SC día por medio ha sido recomendada sin que afecte, en la forma y dosis adecuadas, los niveles endógenos de testosterona. Es esperable una respuesta positiva entre los días 5 y 12 del tratamiento y si la libido se incrementa se recomienda suspenderlo. En general no es necesario repetirlo en la misma temporada, pero hay ocasionalmente padrillos que requieren esa terapia en temporadas sucesivas (Steiner y Umphenhour, 2009).

Disminución de la capacidad copulatoria

Junto con la baja libido, la disminución en las capacidades de montar, penetrar y eyacular, son las mayores causas de baja performance reproductiva en padrillos. Ha sido estimado que las alteraciones musculo-esqueléticas y neurológicas representan al menos la mitad de las fallas copulatorias en padrillos de edad avanzada (Martin *et al.*, 1998). En estos casos (recordar que estos factores pueden ser ADITIVOS a otros descriptos en este artículo) se recomienda (Mc Donnell, 2005):

1. Controlar el dolor, que puede tener efectos adversos sobre el estado general, la libido y la fertilidad.
2. Mantener un buen estado de salud física y entrenamiento (*fitness*) de acuerdo a cada individuo.
3. Cuando este indicado, utilizar medios farmacológicos para incrementar la libido y funciones eyaculatorias.
4. Manejo de las situaciones de monta específicamente (altura de las yeguas, soporte manual del padrillo, piso y condiciones adecuadas, retajeo).
5. Manejo racional del número de yeguas a servir (*book*).
6. Colección de semen *excopula* (sin monta), con o sin inducción farmacológica.

Tendencia a la obesidad

Según datos de la OMS, 1600 millones de personas presentan exceso de peso y al menos 300 millones son clínicamente obesos (WHO, 2006) y la tendencia es creciente en América del Norte (11,7% en 1991 a 30,6% en 2004), Europa, Medio Oriente, Australasia y China, en particular en jóvenes. Si bien la asociación entre obesidad y fertilidad en el hombre ha sido descripta tempranamente por Avicenna (*Ibn-Sina*, Persia, 980-1037 DC), en su monumental (14 volúmenes) *The Canon of Medicine*; es reciente el estudio sistemático, tanto en humanos como en modelos animales (Teerds *et al*, 2011) y su relación con enfermedades crónicas (diabetes, enfermedad coronaria, hipertensión, apnea del sueño, ACV) e infertilidad (Du Plessis, 2010).

Es frecuente observar en sistemas intensivos de producción o estaciones de monta, sementales excedidos de peso o directamente obesos. En parte por falta de un programa individual de ejercicios (voluntarios o forzados) ajustado a cada individuo y también a la oferta de una dieta hipercalórica desbalanceada (efecto mascota) muy frecuentemente asociada al consumo de "alimentos balanceados" para "seniors", algo de lo que recomiendo mantenerse alejados en lo posible, dado que como veremos, hasta ahora nada sustituye el forraje de buena calidad como eje de las dietas para caballos, incluyendo los viejos. Estos factores, asociados a la edad incrementan el riesgo de afectar la fertilidad por diversas vías. Una de las más importantes es el incremento de las enfermedades metabólicas y la disfunción de la Pars Intermedia de la Hipófisis (PPID) con ellas una disminución de la libido y de la calidad seminal (Douglas, 2009 y 2011). La sobrealimentación y falta de ejercicio también aumentan la incidencia de enfermedades digestivas (cólicos), riesgo de laminitis, disminución de la libido, de la longevidad y de alteraciones en la conducta (Unphenour *et al*, 2011; Jackson, 2011).

En términos generales, los objetivos respecto al manejo de la alimentación de un padrillo de edad avanzada podrían ser: 1) mantener una buena condición corporal (5-6 en la escala de Henneke (1983) la más aguda y utilizada en la práctica), que se traduce en PESAR los sementales; 2) Evitar sobrealimentar (el impacto negativo es mayor que el de sub-alimentar); 3) Evitar fluctuaciones en el peso (a partir de los 5 años, no debería tener mayores diferencias aun en actividad), no más de 50 kg de diferencia sobre sus propios valores óptimos durante y fuera de la temporada; 4) La dieta base debería estar compuesta por forraje de buena calidad. De hecho, en la mayoría de los padrillos, si se calculan los requerimientos en base a la última versión de NRC (2007), en general sus requerimientos energéticos, de proteínas y minerales podrían ser cubiertos con 10-15 kg MS/ día de forraje de buena calidad aun en la temporada reproductiva (Jackson, 2011).

Una breve mención a la utilización de aditivos en las raciones de padrillos para "mejorar la fertilidad". Por más de 100 años han sido utilizados en las dietas de sementales, fuentes vegetales de ácidos grasos como aceites de maíz, arroz, lino, soja, canola, para mejorar la calidad del pelo y también como una fuente adicional de energía dado que su digestibilidad es muy alta (mayor al 90%) y su buena palatabilidad (Jackson, 2011). Recientemente, tanto en la literatura científica como en la prensa, se han remarcado los beneficios

del consumo de ácidos grasos omega-3 poli-insaturados de cadena larga (LC-PUFAs), en especial los ácidos docohexanoico (DHA) y eicosapentanoico (EPA) debido a sus efectos sobre el sistema cardiovascular, inmune, osteo-articular, desarrollo cognitivo, etc. y también sobre el sistema reproductivo. Básicamente los estudios iniciales fueron desarrollados en cerdos suplementados con DHA y EPA estableciendo niveles en plasma seminal y membrana plasmática de los espermatozoides y correlacionándolo con la fertilidad y capacidad de crío preservación. En general los resultados fueron positivos demostrando los efectos de estos compuestos en individuos sub-fértiles, al igual que en humanos, pero hasta el momento hay poca evidencia científica respecto a los caballos. Sin embargo, estudios controlados realizados en las Universidades de Texas, Colorado y Arizona utilizando un compuesto formulado específicamente para sementales con fuentes marinas de omega-3, con altos niveles de DHA, EPA y antioxidantes demostraron efectos positivos sobre la capacidad de crío preservación espermática y en algunos casos sobre incrementos en la producción diaria espermática y motilidad progresiva (Brinsko *et al.*, 2005; Harris *et al.*, 2005; Squires, 2011).

Se ha postulado que la mayoría de las raciones equinas estándar podrían tener un impacto negativo sobre la calidad del semen debido a que contienen altas cantidades de precursores para ácidos grasos omega-6 (ácido linoleico del aceite de maíz y grano de soja) y bajos precursores de omega-3, dado que las fuentes vegetales para estos precursores son en general de baja eficiencia de conversión en relación a las fuentes marinas (Squires, 2011). Si bien se considera razonable y potencialmente beneficioso suplementar a los padrillos viejos con fuentes de omega-3, aun se necesitan más estudios controlados específicos en caballos (y no trasladados de otras especies) para afirmar esto contundentemente.

Incremento de la susceptibilidad a enfermedades locales o sistémicas

En humanos, las fallas de respuesta a inmunizaciones en individuos de edad avanzada son tan altas como el 50%, debido que los organismos no tienen la misma capacidad de formación de anticuerpos y de respuestas celulares a los antígenos. Además, presentan una inmunidad de menor duración que los jóvenes incrementando su susceptibilidad a las enfermedades infecciosas (Fagiolo, 1993). Este hecho, es conocido actualmente como **inmunosenescencia** y se caracteriza por cambios en algunos aspectos de la inmunidad celular y humoral, en particular una disminución de la cantidad y de las funciones de las células linfoides (Leech, 1980). Asociado a esto, se presenta otro fenómeno relacionado al incremento de citoquinas pro-inflamatorias y otros mediadores de inflamación, conocido como **"inflammageing"**. En parte debido a estos factores, es más frecuente observar patologías infecciosas en padrillos viejos, en especial en los sistemas de mucosas (respiratorio, digestivo y reproductivo), por lo que es recomendable controlar clínicamente con especial énfasis el funcionamiento de los mismos.

Debido a sus características de longevidad, entre otras, los caballos son utilizados actualmente como modelo de estudio de estos fenómenos y los resultados hasta el momento han generado evidencia que se producen los mismos efectos observados en humanos (Adams, 2009; Horohov, 2010). Por ello es que deberíamos diseñar programas específicos de inmunización y control de padrillos de edad avanzada, muchas veces sometidos a condiciones de stress que incrementan los riesgos de padecer enfermedades tanto sistémicas (respiratorias) como locales (infecciones de heridas, uretritis, vesiculitis).

Incremento de la incidencia de neoplasias testiculares

Las neoplasias testiculares son de baja frecuencia poblacional, pero se incrementan en los sementales de edad avanzada. La mayoría de ellas son benignas y relativamente pequeñas sin producir aumentos en el volumen testicular y se pueden presentar conjuntamente con la degeneración testicular. El problema es que tanto si se presentan solas o en conjunto, ambas disminuyen, por diferentes vías, la producción espermática y consecuentemente la calidad seminal (Turner, 2009). En el caso de tumores muy agresivos como los seminomas, que son los que generalmente producen metástasis o aumentos significativos del volumen testicular, se recomienda -si es unilateral- la hemicastración. De todos modos, la práctica de manejo más recomendable es ecografiar detalladamente al menos una vez por año ambos testículos y epidídimos y registrar los datos dado que es el método no invasivo más utilizado y de mejor capacidad de detección temprana en conjunto con el análisis seminal. La ecografía doppler testicular y la termografía han demos-

trado una mayor sensibilidad diagnóstica frente a estos procesos por lo que deberían ser consideradas ante una sospecha clínica.

Muerte embrionaria, efectos sobre la progenie y disruptores endocrinos

Un aspecto que en mi opinión puede ser importante en el futuro inmediato y del cual no tenemos aún evidencia concluyente en equinos, es el de los efectos de los pesticidas sobre la gametogénesis y sus efectos epigenéticos sobre la fertilidad y sobre la progenie. La evidencia experimental indica que muchos de los contaminantes ambientales afectan de manera irreversible la reproducción, tanto en animales de laboratorio como en domésticos y el hombre, en particular sobre la esteroideogénesis y espermatogénesis produciendo notables efectos sobre la disminución de la libido y la capacidad copulatoria (Veeramacheneni, 2000).

Entendemos por pesticidas a compuestos biológicamente activos elaborados por el hombre para eliminar organismos "específicos". Se estima que alrededor de 2000 pesticidas se encuentran comercialmente disponibles y según datos documentados aun en muy pequeñas cantidades pueden afectar la vida de seres humanos (incremento en las tasas de cáncer) y de su reproducción (disruptores endocrinos que bloquean selectivamente receptores de esteroides, por ejemplo (Gasnier *et al*, 2009). Decenas de reportes, generalmente no citados o invalidados por las compañías productoras de pesticidas e ignorados en muchos casos por organismos de controles nacionales o internacionales, documentan los efectos a mediano y largo plazo de estos compuestos que contaminan el agua y el aire afectando vías metabólicas de manera silenciosa y letal (Veeramachenani, 2007). Uno de los problemas con estos compuestos es que en general la asociación causa-efecto es de mediano a largo plazo por lo que resulta difícil estimar el impacto individual o poblacional, salvo en estudios retrospectivos, cuando ya el daño es irreversible. Recientemente se han desarrollado modelos de cultivos celulares específicos, muchos de ellos provenientes de células madre, para estudiar los efectos de las drogas y/o contaminantes sobre la expresión génica celular (Cordier *et al.*, 2008).

Por último, otro comentario referido a los efectos paternos sobre la muerte embrionaria, fetal y aun sobre la manifestación en la vida adulta de problemas específicos (Carrell *et al.*, 2010). En humanos, y en los países del hemisferio occidental, la edad paterna ha amentado significativamente, con un alto porcentaje cercano a los 50 años y con un incremento significativo de las tasas de pérdidas embrionarias y de nacimientos, en especial en programas de reproducción asistida (Frattarelli *et al.*, 2007; Sartorius *et al.*, 2009; Mendiola, 2009; McGrath *et al*, 2014). Esto ha sido demostrado recientemente asociado a modificaciones epigenéticas espermáticas en relación a modificación de las histonas, cambios en la metilación del ADN y en diferentes tipos de ARN (miRNA; piRNA's; mRNA) y fragmentación del ADN debido a procesos oxidativos (Nannasy y Carrell, 2008; Ball, 2008; Balach, 2010; Gravina, 2010; Teklenburg *et al.*, 2011). Aún restan estudios controlados en equinos que permitan determinar si existen estos patrones, pero es importante que consideremos estos antecedentes como una alerta frente al manejo y análisis de padrillos de edad avanzada.

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Presentación de diferentes casos clínicos únicos que pueden confundir al veterinario en el diagnóstico del mismo

Tulio M. Prado ¹, DVM, MS, Dipl. ACT *et al.*

¹ Associate Professor-Theriogenology, College of Veterinary Medicine, Department of Large Animal Clinical Sciences; Adjunct Associate Professor Institute of Agriculture
Department of Animal Sciences, The University of Tennessee.

Nota del editor: los textos que fueron publicados en otras revistas se deben citar, tal y cómo lo exigen dichas publicaciones. Se incluyen en estas memorias solo con fines de recopilación para los asistentes al evento académico y no deben citarse como si fuesen publicados por la revista CES Medicina Veterinaria y Zootecnia.

Case 1

Sudden infertility in stallion from one breeding season to the other may occur due to age, trauma, or disease; however, when semen collection in a breeding program is performed with a consistent protocol and equipment, sudden infertility is not expected. (A.P. Bouchard, T.M. Prado, C.R. Pinto, S.S. Hayden, B.K Whitlock, Sudden infertility of a stallion collected with a commercial artificial vagina. ABSTRACT Clinical Theriogenology, Official Journal of the Society for Theriogenology/The American College of Theriogenology, 2014; Volume 6 (3): pp 395.

Case 2

Trauma, inguinal herniation, and torsion of the spermatic cord are causes of inflammation of the spermatic cord, epididymis and testis of the stallion. Epididymo-orchitis characterized by coagulative and liquefactive caused by *Salmonella spp.* (A.P. Bouchard, T.M. Prado, J. Schumacher, R.L. Donnell. Necrosis of the spermatic cord, epididymis, and testis of a Friesian Stallion caused by *Salmonella abortus-equi*. ABSTRCT Clinical Theriogenology, Official Journal of the Society for Theriogenology/The American College of Theriogenology, 2014; Volume 6 (3): pp 377.

Case 3

Granulosa cell tumors have been described by ultrasound to vary in shape ranging from uniformly dense masses with one or many large fluid filled cyst like structures, however, this case refer to a single fluid filled Granulosa-Theca Cell Tumor (GTCT) that acutely doubles in size within 48 hours. B.D. Radny, J.L. Klabnik-Bradford, T.M. Prado. Acutely fast grow ovarian sex cord-gonadal stromal tumor in a 13-year-old American Quarter Horse mare. ABSTRCT Clinical Theriogenology, Official Journal of the Society for Theriogenology/The American College of Theriogenology, 2017; Volume 9 (3): pp 495.

Case 4

Hydrosalpinx is a condition that can easily produce infertility specilly if it s bilateal. However, there have been no reports of hydrosalpinx causing increased levels of anti-mullerian hormone (AMH). This case demonstrates a misdiagnosis of granulosa-theca cell tumor based on ultrasound and blood AMH levels, with a true diagnosis of bilateral hydrosalpinx upon abdominal laparoscopy. (J.L. Klabnik-Bradford, B.D. Radny, T.M. Prado. Bilateral hydrosalpinx .with elevated anti-mullerian hormonesin a 5 year-old Belgian mare. ABSTRCT Clinical Theriogenology, Official Journal of the Society for Theriogenology/The American College of Theriogenology, 2017; Volume 8 (3): pp 494.

Case 5

Hemicastration of a Tennessee walking horse stallion with hematocele. Maria A. Hes, Tulio Prado, DVM and Brian Whitlock, DVM. College of Veterinary Medicine, University of Tennessee. Society for Theriogenology (SFT) & American College of Theriogenologists (ACT) Annual Conference. August 2009.

Cirugías reproductivas más comunes para incrementar la fertilidad de las yeguas

Tulio M. Prado ¹, DVM, MS, Dipl. ACT; Jim Schumacher DVM, MS, Dipl. ACVS

¹ Associate Professor-Theriogenology, College of Veterinary Medicine, Department of Large Animal Clinical Sciences; Adjunct Associate Professor Institute of Agriculture
Department of Animal Sciences, The University of Tennessee.

Introduction

Surgical procedures on the mare's reproductive tract to increment fertility are performed primarily to correct urogenital abnormalities that contribute to contamination of the reproductive tract. Contamination results from injuries that occur during parturition or from conformational changes that occur as mares age. We will discuss only the most common surgical procedures performed to reconstruct vulvar, vestibular, and cervical abnormalities. For more detailed descriptions of these and other surgical procedures involving the reproductive tract, the reader should refer to veterinary medical surgical texts.

Mares with conformational changes of the urogenital tract that can be corrected by surgical reconstruction are candidates for surgery, provided that the results of a thorough breeding soundness examination indicate that the procedure has a good chance of restoring the mare's fertility. An exception to the need for a breeding soundness examination is a mare with a rectovestibular laceration or fistula, because these injuries nearly always arise in fertile, young mares during foaling as a result of fetal malposture during delivery. An affected mare has no reason to be infertile after surgical reconstruction of the vestibule and vulva prevents contamination

of the reproductive tract. For other procedures used to surgically correct reproductive abnormalities, however, a breeding soundness examination is indicated to assess the breeding potential of the mare. If the endometrium, cervix, vagina, or vestibule has been severely and irreparably damaged, the owner may elect not to invest time and money into surgery and aftercare. Defects commonly corrected by reconstructive surgery of the reproductive tract include the following:

Pneumovagina

Pneumovagina is a condition characterized by constant contamination of the vestibule and vagina with feces that results from conformational faults that cause a mare to aspirate air into the into the tubular portion of the reproductive tract. The condition is commonly called "wind-sucking," and it usually culminates in ascending infection of the vagina, cervix, and endometrium. Causes of pneumovagina include tearing or stretching of the vulvar seal or the vulvovaginal sphincter and a sunken perineal body, characterized by cranial displacement of the anus, resulting in tipping of the vulva cranially over the brim of the pelvis. The condition is quite common in underweight, aged, pluriparous mares. The most common surgical procedure used to correct pneumovagina is the Caslick operation, sometimes referred to as a vulvoplasty.

To perform a Caslick vulvoplasty it is better if you placement of mare into stock and tranquilize and wrap and tie tail so you can wash mare's perineum. Local anesthetic solution is injected subcutaneously at the dorsal commissure of the and infiltrated along the margin of each labium to slightly below the floor of the ischium. The level to which the margins of the labia are sutured ventrally can be determined by placing firm pressure with the fingers on either side of the vulva and pressing down to locate the ischium. If the labia are not sutured to slightly below the floor of the ischium, the vulva may bounce far enough cranially, as the mare moves, to permit air to be aspirated into the vagina. Care should be taken to ensure that the ventral portion of the vulva remains spacious enough to allow urine to escape during urination, for insertion of the stallion's penis during copulation, or for insertion of a vaginal speculum, if the mare is to be bred by artificial

Lidocaine 2% is injected at the mucocutaneous junction (Junction of pigmented skin and pink vulvar mucosa) in adequate volume to create a bleb (bubble like swelling) from the level of the pubis dorsally to connect with the bleb from the other side of the vulva. Lidocaine is also placed lateral to the lower extend of each bleb for the future placement of the "Breeding Stitch".

The large bleb of lidocaine utilized in this procedure provides analgesia of the area for an extended period of time. The procedure routinely takes less than 15 minutes per animal. A thin band of tissue (2-4 mm) is removed from the area injected with local anesthetic.

OR

An alternative method is to incise the bleb with a surgical scalpel. Either of these methods will expose underlying tissue.

Surgical incisions are sutured in a manner which opposes the tissue from each side of the vulva and closes the upper side of the vulvar opening.

Breeding stitch. If the labia must be opened for breeding, for vaginal examination, or to permit unobstructed foaling, they should be reapposed when a large vulvar opening is no longer required. A breeding stitch is a single, simple interrupted suture sometimes placed at the ventral aspect of the vulvar closure to preclude the need to open the sutured labia of a mare that must be bred. A wide based suture is placed in the subcutaneous tissue and is tied loosely. This suture will prevent the tearing of the sutures or tissue of the vulvoplasty in the event of natural breeding or the future vaginal examination by the veterinarian.

Mares receiving this procedure do not demonstrate any residual, pain, or problems. This procedure can be repeated at least yearly with no residual effects.

Perineal body reconstruction

When the entire vulva of a mare has an extremely sunken anus and perineal body that deviated so far cranially and ventrally that the vulvar cleft assumes a nearly horizontal position over the ischium. A Caslick suture may not correct pneumovagina in such mares and may prevent the mare from adequately expelling urine. To prevent both pneumovagina and urine pooling caused by this extreme conformational abnormality, the perineal body must be reconstructed (i.e., perineoplasty or vestibuloplasty). Often a modified vestibuloplasty, as described by Slusher (1986) is performed.

This vestibuloplasty entails removal of an isosceles triangle of mucosa from the dorsal aspect of the vestibule.

In preparation for reconstruction of the perineal body, as described by Slusher (1986), the mare is placed in a stock and tranquilized, and the perineum is desensitized by a caudal epidural analgesia:

Caudal epidural analgesia is indicated for many of the procedures of the urogenital system. The most common indication is for standing urogenital surgical procedures of the perineum, anus, rectum, vulva, and vagina. It is also used for other non-surgical procedures such as multiple ovulation embryo transfer (MOET) and embryo recovery in horses.

The technique consists of the deposition of a local anesthetic solution between the dura mater and periosteum of the spinal canal. Because of the extremely importance of this procedure in bovine practice and that the veterinarian's experience/performance play a very important role in practice, this procedure is recommended to be performed by each of the students in the advance reproductive training.

Remove hair from the injection site if possible and clean the area well with alcohol or another skin antiseptic.

With the mare in standing restraint, the practitioner should stand alongside its pelvic region. Move the tail up and down while locating the fossa between the last sacral vertebra and the first coccygeal vertebra and then between the first and second coccygeal vertebrae (preferred site for mares).

Insert the needle ventrally and cranially at a 45-degree angle until contact is made with the floor of the spinal canal of the preceding vertebra. The use of a stylet is optional.

If blood appears in the lumen of the needle following insertion, presumably the needle has been inserted too ventrally and has invaded the ventral venous plexus. Two options may be considered: Retract the needle slightly, disregard the blood, and infuse the anesthetic solution (no serious sequelae result). Remove the needle, and discard, reinsert a new sterile needle to the proper depth, and infuse. Attach the syringe with the estimated optimal dose of anesthetic solution to the needle and start infusion. The plunger should go down freely (almost a gravity feeding) if the needle is properly situated. Optimal dose for a 1,000-lb mare: 3 to 4 ml of 2% lidocaine or mepivacaine (1 to 1.25 mL/100 kg), (additional 80 mg of xylazine is added to the lidocaine for better anesthesia). Detach the syringe from the needle; the needle may be retained in the inserted position during surgical for additional infusion of anesthetic solution, if indicated.

This procedure is a very safe procedure for the cow and the mare. The incidence of any complication to this procedure is rare.

The dorsal aspect of the vestibule is exposed by retracting each labium laterally, with a loose suture or Backhaus towel clamp placed through the labium at the juncture of its dorsal one-third and ventral two-thirds, and by retracting the dorsal commissure of the vulva dorsally and caudally, also with a loose suture or towel clamp. A point on the dorsal aspect of the vestibule that lies directly beneath the anus is marked to serve as the apex of a triangle of mucosa to be removed. The distance between this mark and the dorsal commissure of the vulva is measured, and one-half of this distance from the dorsal commissure of the

vulva is marked on the mucocutaneous margin of each labium. A line between these two points on the labia serves as the base of the mucosal triangle to be removed.

The points of the triangle are connected using a scalpel, and the mucosa overlying this area is removed. Two or three, no. 1 or 2 nonabsorbable sutures are placed horizontally in a line from the apex of the triangle to the base.

Small rolls of gauze swabs work well as Stents to prevent the sutures from pulling through the skin. Just enough tension is placed on the sutures to bring the triangular area into a vertical position. Sutures are removed in 5 to 10 days. Excessive tension placed on the sutures causes tissue necrosis. If sutures begin to cause tissue necrosis, they can be removed, one daily, or at alternate-day intervals, to relieve pressure.

This surgical procedure effectively increases the area of the perineal body and returns the vulva to a more vertical position, but the sunken position of the anus remains unchanged. If the procedure is done properly, the vulvar opening is not diminished appreciably, and the mare can be bred by natural service.

Urovagina

Old, pluriparous mares sometimes suffer from reflux of urine into the vagina during urination attributable to conformational changes in the vestibule and vagina that result from progressive descent of these structures into the abdomen from repeated stretching, during pregnancy, of the tissues that suspend the uterus and birth canal. These conformational changes cause the external urethral orifice to be positioned cranial and ventral to the brim of the pelvis and dorsal to the cranial portion of the vagina, leading to pooling of urine in the vaginal fornix. Pooling of urine (i.e., urine pooling) into the vagina is termed urovagina or vesicovaginal reflux. The constant presence of urine is irritating and contributes to inflammation and sometimes infection of the uterus and birth canal (i.e., vaginitis, cervicitis, and endometritis). Severely affected mares dribble urine chronically from the vulva. The skin of the tail, ventral aspect of the vulva, and the inner aspect of the thighs may become chronically irritated causing exudate to accumulate in these areas. Before performing surgery to alleviate urine pooling, the endometrium should be biopsied for histological examination. If the mare has severe, widespread, periglandular endometrial fibrosis, which permanently lowers the mare's ability to conceive and carry a viable foal to term, the owner of the mare may choose not to proceed with surgery. The mare is a more suitable candidate for corrective surgery if the endometrium is not severely and permanently damaged.

Different surgical techniques have been described to correct urovagina in mares, including caudal retraction of the transverse fold, as described by Monin, and the McKinnon and Brown techniques of urethral extension. To correct urovagina, I prefer to use the urethral extension technique described by Prado *et al* (2012). (Prado TM, Schumacher J, Kelly G, Henry R. *Evaluation of a modification of the McKinnon technique to correct urine pooling in mares. Veterinary Record* 2012; 170: 24-621. Published Online First: 4 May 2012). The mare is prepared for surgery by systemic administration of a sedative and desensitization of the perineal region similarly as done for the perineal body reconstruction.

Prado *et al.* (2012) modified the technique of urethroplasty described by McKinnon and Beldon (1988) by transversely splitting the urethral fold and retracting the dorsal half caudally to cover the caudal portion of the urethral extension. This modification was designed to help prevent a defect from forming in the cranial portion of the extension. Using this modification, the dorsal half of the transversely split urethral fold is retracted caudally, under tension, for 4 to 5 cm, to cover the submucosa of the dorsal aspect of the cranial portion of the urethral extension. The dorsal shelf can be retracted approximately 2 cm even further caudally by creating a large, cranially-pointed, V-shaped mucosal incision on the floor of the vagina, cranial to the external urethral orifice. The right and left margins and the central long axis of the retracted dorsal shelf are sutured to the exposed submucosa of the extension. The V-shaped incision on the floor of the vagina is left to heal by second intention.

Prado *et al.* (2012) reported that 10 of 30 mares (33%) that received this technique of urethroplasty developed a fistula in the extension. The fistula of 8 of the 10 mares (26.6 %) was grossly visible and palpable, but the

fistula of 2 mares could be detected only by inserting a dye, under pressure, into the lumen of the extension. The defect in the extension of these 10 mares was, without exception, located in the caudal half of the extension, and caudal to the caudal edge of the retracted dorsal shelf of the urethral fold, where it was considered to be less likely to contribute to vesicovaginal reflux and to be more accessible for repair.

The study by Prado *et al.* (2012) showed that digital palpation alone is often insufficient to detect a fistula. A minute fistula may be detected only by infusing a dye, under pressure, into the extension. These authors speculated that even a minute fistula may contribute to failure of the extension to prevent vesicovaginal reflux, but whether or not a defect so small that it cannot be detected grossly can result in perpetuation of vesicovaginal reflux is not known. None of the mares in that study suffered from vesicovaginal reflux before surgery.

A Caslick's suture is placed in the vulva if the mare also suffers from pneumovagina, a condition that commonly accompanies urovagina.. Some mares may also require vestuloplasty, as described above. Aftercare consists of administration of a broad-spectrum antimicrobial drug for 3 to 5 days. Administering an antimicrobial drug that is eliminated through the urine (e.g., trimethoprim-sulfamethoxazole) may be beneficial. Administering a nonsteroidal, antiinflammatory drug, such as phenylbutazone or flunixin meglumine, for 12 to 24 hours after surgery may help to relieve postoperative pain.

In general, factors that contribute to formation of a fistula in the urethral extension, regardless of the technique used, are the difficulty in apposing the cranial aspects of the mucosal flaps, hydrostatic pressure created during urination, fibrosis from chronic vaginitis or injury suffered during parturition, and a jet-effect dorsal to the urethral opening during urination. Another factor that could contribute to dehiscence of the urethral extension is using a suture that may prematurely lose tensile strength if placed in an alkaline environment. Using polyglactin 910 or polyglycolic acid, therefore, is best avoided in surgeries where this suture contacts the normally alkaline urine of horses.

Retrovestibular laceration and fistulas

A perineal laceration or fistula occurs at parturition when the foal's foot or nose catches the annular fold of the hymen at the vaginovestibular junction. This injury occurs predominately in primiparous mares because their annular fold is more prominent than that of pluriparous mares. A 1st-degree perineal laceration involves only the skin and mucous membrane of the dorsum of the vestibule. A 2nd-degree perineal laceration is characterized by disruption of the constrictor vulvae muscle, compromising the ability of the perineal musculature to constrict the vestibule. A 3rd-degree perineal laceration is characterized by a complete disruption of tissue between the rectum and vestibule, resulting in a common rectal and vestibular vault. A rectovestibular fistula occurs when the tissue between the rectum and vestibule is perforated by the calf, but the malposture of the foal is corrected before the foal is delivered, allowing at least a portion of the perineal body to remain intact. A rectovestibular laceration or fistula allows the mare's vestibule to become contaminated with feces, which results in bacterial infection of the vagina and endometrium. Mares with a 1st-degree perineal laceration can be treated with a Caslick's vulvoplasty, but mares with a 2nd-degree perineal laceration require a vestibuloplasty because the constrictor vulvae muscle is disrupted, causing the perineum to sink, predisposing the mare to pneumovagina and urovagina.

An attempt to repair a perineal laceration immediately after injury is usually unsuccessful because the lacerated tissue soon becomes inflamed and contaminated with feces and other debris. Before repairing a rectovestibular laceration or fistula, the mare's reproductive tract should be palpated *per vagina* and *per rectum* to determine if the mare has also incurred a cervical laceration, has uterine adhesions or pyometra, or is pregnant. Histologic evaluation of the endometrium may be indicated if the mare has gone through more than one reproductive season. Even though the vagina is constantly contaminated with feces, the uterus is unlikely to be permanently damaged, provided that repair was not neglected beyond several reproductive seasons.

A 2nd-degree perineal laceration or fistula is usually repaired with the mare sedated and standing after administering epidural anesthesia. The procedure to repair a rectovestibular laceration is performed in a manner similar to that described for mares with a laceration and is composed of 2 stages - rectovestibular reconstruction and an anoperineal reconstruction. Both stages can be performed during the same operation, or the anoperineal reconstruction can be completed 3 weeks or more after rectovestibular reconstruction. Note: When performing the rectovestibular stage of repair, I prefer to use a six-bite suture pattern. Dissection between the rectal and the vaginal submucosa is extended for several inches cranial to the tear to enable placement of sutures that invert vaginal submucosa and mucosa into the vaginal lumen and rectal submucosa into the rectal lumen, thereby relieving tension on more caudally placed sutures that oppose the rectal and vestibular shelves, which in turn, decreases the likelihood of a fistula forming at the cranial aspect of the repair.

Postoperative treatment of a mare after repair of a 3rd-degree perineal injury usually includes administering antimicrobial non-steroidal, anti-inflammatory therapy for several days. The mare's stool should be kept soft and scanty for at least 8 days. The integrity of the repair should not be evaluated before the 8th day to avoid disrupting the repair. Most mares are capable of eliminating bacteria from the endometrium within 1 estrous cycle. Natural breeding should not be allowed for at least 6 weeks, but mares may be bred by artificial insemination within 2 weeks after repair.

Rectovaginal fistulas

A rectovestibular fistula should be converted into a laceration only if it is exceptionally large (i.e., greater than 3 fingers in diameter). A fistula 3 fingers or less in diameter can be repaired using the Forssell technique, which spares complete disruption of the intact perineal body. Using this technique, the skin of the perineum is incised in a frontal plane, midway between the ventral aspect of the anus and the dorsal commissure of the vulva. The incision is extended cranially through the perineal body to 3 to 4 cm beyond the fistula, separating the rectovestibular defect into a dorsal, rectal hole and a ventral, vestibular hole. The rectal hole is closed in a transverse plane (because the musculature of the rectum is primarily circular, and sutures placed perpendicular to the muscle fibers are subject to less stress than are sutures placed parallel to the direction of the muscle fibers with no. 00 or 0 absorbable, monofilament suture placed in an interrupted or Lembert or Halsted suture pattern. Preplacing all sutures and then tying the sutures from the center outward may allow the sutures to be placed more uniformly. Care must be taken to place all sutures into submucosal tissue to avoid tearing tissue when sutures are tightened. The vestibular hole is closed in a sagittal plane (because its muscle fibers are primarily longitudinal) with no. 00 or 0 absorbable, monofilament suture placed in an interrupted or Lembert or Halsted suture pattern.

Closing the openings of the rectum and vestibule at right angles to each other reduces the likelihood of rectal contents leaking into the repair site. The dead space remaining between the rectum and vestibule is then closed using simple interrupted sutures of no. 0 or 1 absorbable suture material. The incised skin of the perineal body is closed with interrupted, nonabsorbable, monofilament sutures, which are removed at 10 to 14 days. The frontal plane of dissection is difficult to close and so may be left unsutured to heal by second intention.

Postoperative treatment of the mare following repair of a rectovestibular laceration or fistula is similar to the one for RV tears.

Repair of third-degree perineal lacerations or rectovaginal fistulas are often accompanied by complications:

Dehiscence of the repair, the most common of which is dehiscence, regardless of the method of repair.

Complications caused by failure to recognize a concurrent abnormality. A complication of surgery to correct a third-degree perineal injury is failure to recognize a concurrent abnormality or to recognize that the mare is pregnant.

Preoperative considerations to avoid failure of repair. A mare with an acute, third-degree perineal injury should receive tetanus prophylaxis, broad-spectrum antimicrobial therapy until the wound begins to fill with granulation tissue, a nonsteroidal anti-inflammatory drug for at least several days to relieve discomfort, and a stool softener.

Cervical lacerations

A cervical laceration is incurred during parturition and is often not detected until it is discovered during routine postpartum examination or during examination to determine the cause of infertility, abortion, or repeated uterine infection. Most cervical lacerations are longitudinal and are best identified by palpating the wall of the cervix between an index finger (or thumb) inserted into the lumen of the cervix and a thumb (or index finger) placed on the vaginal aspect of the cervix. Repair of a torn cervix may not be necessary if the cervix remains competent.

Competency can be evaluated in the mare the best when the mare is in diestrus because during this stage of the cycle, a competent cervix must be dilated to allow insertion of a finger into the lumen of the uterus. The result of cervical incompetency is failure to conceive or eventual loss of the conceptus early or late in gestation. Opinions vary about the length of laceration that causes the cervix to be incapable of maintaining a seal. LeBlanc (2006) and Embertson (2009) recommended repairing those lacerations that extend more than one-third of the length of the *portio vaginalis* (i.e., that part of the cervix extending cranially from the external os to the junction of the cervix and vagina), whereas Brown *et al.* (1984) advocated repairing lacerations that extend more than 50% of the length of the *portio vaginalis*. O'Leary (2009) and Pollock and Russell (2011) advised repairing only those lacerations that extend the entire length of the *portio vaginalis*. Regardless of its length, a cervical laceration that interferes with the competency of the cervix must be repaired to restore fertility.

The cervix should be repaired while the mares are in diestrus. Before the cervix is repaired, the owner should be warned that the cervix is often lacerated during the subsequent parturition because fibrosis at the site of repair may render the cervix incapable of dilating completely.

The cervix is usually repaired in two or three layers. The mare should receive sexual rest for one month. Complications include dehiscence of the repair and formation of luminal adhesions, which can result in pyometria. Preparation for repair of a cervical defect is similar to that for repair of a third-degree perineal laceration (i.e., sedation, epidural anesthesia, tail wrap and tie, etc.).

The inner mucosal layer (i.e., toward the cervical lumen) is sutured first with no. 0 or 00 absorbable suture placed in an inverting (i.e., into the cervical lumen) continuous horizontal mattress pattern. Suturing begins at the cranial end of the defect and continues caudally to the external os.

The middle, muscular layer is sutured with no. 0 absorbable suture inserted in a simple continuous pattern (F). This is the critical layer of closure, and so, sufficient tissue must be procured to ensure that the layer remains intact after healing. Thickness can be checked periodically during insertion of this suture line by inserting a finger into the cervical lumen.

The outer mucosal layer (i.e., toward the vaginal lumen) is sutured cranially to caudally in an everting manner (i.e., into the vaginal lumen) using no. 0 or 00 absorbable suture material inserted in a continuous horizontal mattress pattern.

The retention sutures are removed, and the vagina and the external cervical mucosa are covered with an oily, antimicrobial preparation. The mare should receive a Caslick vulvoplasty, if necessary. A suitable, broad-spectrum, antimicrobial drug can be administered to the mare for 3 to 5 days if infection is a concern. The mare should receive sexual rest for one month, and its cervix should be examined for competency and patency before the mare is bred.

The greatest difficulty in repairing a lacerated cervix is poor access to the cervix. A serious complication of repair of a cervical laceration is development of : Intra-luminal and peri-cervical adhesions, septic peritonitis.

Endometritis, failure to conceive or to produce a live foal or if a cervical Wedge Resection for Treatment for Pyometra.

Other reproductive invasive surgeries

The following surgeries are invasive and will require expensive equipment to be able to do them, However they are another way to solve some of the problems when mares fail to get pregnant because the uterus is too low and has difficulties cleaning up and there are obstruction of the oviducts. I will explain the surgeries on the power point presentation, but the references for these surgeries are the following articles:

Brink P, Schumacher J, Schumacher J. Elevating the uterus (uteropexy) of five mares by laparoscopically imbricating the mesometrium. *Equine Vet J.* 2010 Nov; 42 (8): 675-9.

Woods J, Rigby S, Brinsko S, Stephens R, Varner D, Blanchard T. Effect of intrauterine treatment with prostaglandin E2 prior to insemination of mares in the uterine horn or body. *Theriogenology.* 2000 Jun; 53 (9): 1827-36.

Inoue Y, Sekiguchi M¹. Clinical application of hysteroscopic hydrotubation for unexplained infertility in the mare. *Equine Vet J.* 2018 Jul; 50 (4): 470-47.

Inoue Y. Hysteroscopic hydrotubation of the equine oviduct. *Equine Vet J.* 2013 Nov; 45 (6): 761-5.

Colpotomy for twin reduction and others tasks

The vaginal celiotomy is often referred to as a colpotomy. It can be performed using laparoscopic techniques, including hand-assisted laparoscopic techniques, and can be performed with the mare anesthetized, using any of these approaches, or with the mare standing, through a vaginal or flank celiotomy. The mare must be placed in the Trendelenburg position when colpotomy is performed with the mare anesthetized, and this positioning increases the risk for cardiovascular anesthetic complications.

The temperament of the mare is an important factor when deciding whether to do it with the mare standing or anesthetized. Surgeries through a vaginal approach avoids a cutaneous scar and provides the most rapid return to function of all the approaches. The scar left by a paramedian, oblique paramedian, or ventral mid-line approach is inconspicuous, but the time between surgery and the mare's return to function is much longer than when surgery is performed using a vaginal approach. The vaginal approach also allows access to both ovaries and uterus through a single incision and, therefore, is most often used for spaying mares, removal of momified fetus or twin reduction after 60 days of pregnancy. The colpotomy is generally left unsutured, making vaginal approach a rapid procedure. If an ovariectomy needs to be performed with the mare standing, through a flank or vaginal celiotomy is less expensive than other methods of ovariectomy, because it avoids the expense of general anesthesia. Vaginal ovariectomy, however, requires the use of a specialized instrument, the écraseur.

The approach selected is generally based on the reasons for what type of surgery is going to be performing, facilities and equipment available, financial constraints imposed by the owner, temperament of the mare, and experience and expertise of the surgeon. Each approach can be accompanied by complications, some of which are inherent to the approach, such as accidental transection of the circumflex iliac artery, when an ovary is removed through a flank celiotomy. for example.

I will explain the surgeries on the power point presentation, but the references for these surgery is the following article: Tulio M. Prado, Jim Schumacher. How to perform ovariectomy through a colpotomy. *Equine Veterinary Education*, August 24, 2017.

Estudio del sexo del feto con relación al tiempo de inseminación y la ovulación

Tulio M. Prado ¹, DVM, MS, Dipl. ACT

¹ Associate Professor-Theriogenology, College of Veterinary Medicine, Department of Large Animal Clinical Sciences; Adjunct Associate Professor Institute of Agriculture
Department of Animal Sciences, The University of Tennessee.

Nota del editor: los textos que fueron publicados en otras revistas se deben citar, tal y cómo lo exigen dichas publicaciones. Se incluyen en estas memorias solo con fines de recopilación para los asistentes al evento académico y no deben citarse como si fuesen publicados por la revista CES Medicina Veterinaria y Zootecnia.

Abstract

Oocyte fertilization can occur in most mammalian species by sperm recently ejaculated or a few days old. It has been reported that the timing of artificial insemination (AI) in human, cattle, sheep, deer, and mice relative to the time of ovulation influences the sex ratio of embryos. An study was done to determine the effects of timing of insemination relative to ovulation on the embryonic sex ratio in mares. I will explain the surgeries on the power poit presentation, but the references for these surgery is the folowing article: A.P. Drexler, T.M. Prado, L.L. Amelse, B.K. Whitlock. Effects of time of insemination relative to time of ovulation on embryonic sex ratio in mares. ABSTRCT "Clinical Theriogenology, Official Journal of the Society for Theriogenology/The American College of Theriogenology, Volume 8, Number 3, September 2016. pp 357".

Diferentes técnicas prácticas para reducir quistes endometriales

Tulio M. Prado ¹, DVM, MS, Dipl. ACT *et al.*

¹ Associate Professor-Theriogenology, College of Veterinary Medicine, Department of Large Animal Clinical Sciences; Adjunct Associate Professor Institute of Agriculture
Department of Animal Sciences, The University of Tennessee.

Abstract

Uterine or endometrial cysts vary in shape and size on ultrasound and can be seen as single or multiple cyst. The presence of endometrial cysts are important because they can be mistaken for pregnancy by resembling an embryo on ultrasound or palpation, interfering with embryo mobility and inducing pregnancy lost. The risk of these consequences are greater when the collective volume of the cyst(s) is higher.

Uterine/endometrial cysts. Vary in shape and size and are present as single or multiple cysts. Hey can be mistaken for a pregnancy and will Interfere with embryo mobility and induce pregnancy loss. The risk of these consequences are greater when the collective volume of the cyst(s) is higher. The most common techniques to ablate uterine cysts in the mare are:

- CO2:YAG laser ablation technique. Removes a small portion of the cyst wall keeping the cyst from refilling.
- Removal of entire cyst. Will create scarring that may interfere with pregnancy.
- Draining of cyst: May refill.
- Cyst Rupture: Will create scarring that may interfere with pregnancy.
- Partial removal of the cyst wall via uterine alligator forceps. We developed a novel technique would prevent the cyst from refilling with minimal scaring similar to the CO2 laser.

How the novel technique work: we removed part of the wall of the cyst via alligator forceps. After performing this technique we infused the uterus with antibiotics (2gm Ampicillin diluted with 14cc of sterile water).

After that on the following recheck the cyst was no longer appreciated on US. All mares that were treated were bred and became pregnant and this technique was used on each mare without complication. Cysts were removed while the mares were in estrus and bred the following day when animal has good edema and follicle over 35mm and later treated with Oxytocin 4-5 hours later. Mares are recommended to be bred next day and using this method and technique all treated mares have become and maintained pregnancy. In conclusion, this novel technique is very similar to laser and can be done anywhere (field) helping to decreased cost with a minimal and less expensive equipment. It also has the benefit that if you do it in same estrus cycle you are going to breed, the cervix is easier to dilate and the mare doesn't have to be transported multiple times.

Reference

1. Endometrial cyst ablation using uterine alligator forceps in three broodmares. B.D. Radny, J.L. Klabnik-Bradford, T.M. Prado. ABSTRACT Clinical Theriogenology, Official Journal of the Society for Theriogenology/The American College of Theriogenology, 2018; Volume 9 (3): pp 330.

Cirugías del tracto reproductivo del macho, salvando su capacidad reproductiva

Tulio M. Prado ¹, DVM, MS, Dipl. ACT; Jim Schumacher DVM, MS, Dipl. ACVS

¹ Associate Professor-Theriogenology, College of Veterinary Medicine, Department of Large Animal Clinical Sciences; Adjunct Associate Professor Institute of Agriculture
Department of Animal Sciences, The University of Tennessee.

Abstract

Reproductive surgical techniques are considered by practitioners of theriogenology to be the most beneficial method of managing infertility-causing conditions or diseases of the stallion. Injury or diseases of the reproductive tract may cause abnormalities that may result in infertility of the stallion. The surgeon must be adept at selecting the surgical procedure most likely to resolve a particular reproductive problem. Often, the most cost-effective method of dealing with reproductive conditions or diseases of the stallion is to castrate. However, the surgeon should be aware of the risks of the reproductive surgical procedure selected and should be able to recognize and resolve an intraoperative or postoperative complication when it arises. Each type of reproductive surgery has predictable risks, and by knowing these risks, the surgeon is better able to avoid intraoperative complications and reduce postoperative morbidity. The surgical procedures described herein are some of the commonly used methods to restore fertility in stallions suffering from injury or diseases of the reproductive tract.

Inguinal herniation

A stallion experiences inguinal herniation when intestine, usually the ileum or distal portion of the jejunum, enters the vaginal sac or cavity (i.e., the inguinal canal) through the vaginal ring. This hernia is sometimes referred to as scrotal, rather than inguinal, when the intestine extends into the scrotum. Inguinal hernias of stallions are sometimes improperly called "indirect hernias," a term used to describe a somewhat similar condition in men.

Ruptured inguinal (scrotal) herniation occurs when the viscera within the hernia protrude through a rent in the vaginal sac into the subcutaneous tissue of the scrotum. Inguinal rupture is protrusion of viscera into the subcutaneous tissue of the inguinal canal or scrotum through a rent in the peritoneum and musculature adjacent to the vaginal ring.

Inguinal hernias of foals are usually congenital and are considered to be hereditary. They occur when the vaginal ring is so large that it permits viscera to enter the vaginal sac. Congenital inguinal hernias of foals may occur unilaterally (usually on the left side) or bilaterally and may occur more often in Standardbreds, Tennessee Walking Horses and American Saddlebreds. Ruptured inguinal hernias occur most commonly

in new-born foals and may be caused by the high abdominal pressure generated during parturition. Inguinal hernias of adult stallions are generally considered to be acquired, but the underlying cause may be a congenitally enlarged vaginal ring.

Ruptured inguinal hernias, and inguinal ruptures cause a noticeable increase in the size of the scrotum. Palpation of the scrotum of an affected horse may elicit a sensation of crepitus, and peristalsis of entrapped intestine may cause movement of scrotal skin. Viscera outside the abdomen can be identified during transscrotal, ultrasonographic examination. Congenital inguinal hernias of foals, because of the relatively large size of the affected vaginal ring, are rarely strangulated and reduce easily. Rupture of a congenital inguinal hernia should be suspected if the viscera cannot be reduced, if the scrotum is cold and edematous, or if signs of colic accompany the hernia.

Acquired inguinal herniation is usually first recognized when the stallion begins to show signs of severe colic caused by strangulation of the herniated intestine. Scrotal and testicular edema usually accompany an acquired inguinal hernia because the vasculature of the spermatic cord becomes compressed. Intestine entering a vaginal cavity through a vaginal ring can be palpated per rectum. Omentum may also enter the vaginal cavity independently or with intestine.

Foals with a congenital inguinal hernia should be monitored regularly for signs of strangulation of the hernial contents. The hernia often resolves spontaneously by the time the foal is 6 months old, but the mechanism by which this occurs is not well understood. Application of a truss may hasten resolution. The truss is applied with the foal in dorsal recumbency after the hernia has been manually reduced. Care should be taken to avoid interfering with urination by compressing the penis. The truss is usually changed at 3- to 5-day intervals, and the hernia is often corrected within 1 to 2 weeks after application of the truss. Surgical correction of congenital herniation is necessary only if intestinal strangulation is detected or if the hernia fails to resolve.

To surgically reduce inguinally incarcerated intestine, the stallion should be anesthetized, positioned in dorsal recumbency, and prepared for inguinal exploration and for celiotomy at the ventral midline. The scrotal skin and subcutaneous tissue are incised, and the parietal (common vaginal) tunic and its contents are exposed by blunt dissection. The parietal tunic is incised to expose the testis and its spermatic cord and the incarcerated intestine.

The incarcerated intestine should be reduced through the vaginal ring into the abdomen. Reduction is most easily accomplished by placing traction on the incarcerated intestine through a small ventral midline or suprapubic paramedian celiotomy. Often, the vaginal ring is so constricting that replacement of the intestine is impossible without first enlarging the ring. The ring is most easily enlarged by cutting it with a curved bistoury. Devitalized intestine can be resected and anastomosed at the inguinal incision, but resection and anastomosis are usually more easily accomplished after the intestine is exteriorized through the celiotomy.

Attempting to save the testis is impractical unless it and its vasculature appear undamaged. To remove the testis, the parietal tunic and its contents are isolated, and the scrotal ligament, which attaches the tail of the epididymis to the caudal aspect of the scrotum, is severed. The spermatic cord is transected using an emasculator or is ligated with heavy absorbable suture and severed distal to the ligature. The parietal tunic and overlying cremaster muscle can be excised with scissors or the emasculator, taking care not to damage intestine that has not yet been reduced into the abdomen.

The superficial inguinal ring is closed with heavy, absorbable suture material using a continuous or interrupted pattern. Inguinal fascia and skin can be sutured or left unsutured to heal by second intention. The remaining testis often hypertrophies within a few months after the affected testis has been excised, and fertility is usually maintained.

To preserve a viable testis while preventing escape of intestine from the inguinal canal, the cranial half of the superficial inguinal ring can be sutured toward the caudally located spermatic cord. Another method of salvaging the testis, while preventing intestine from escaping through the vaginal ring, is to laparoscopically implant a mesh over the deep inguinal ring, with the horse anesthetized and in dorsal recumbency. Another laparoscopic technique used to salvage the testis is to insert a coiled mesh through the vaginal ring, with the horse standing, and stapling the mesh to the ring. Fibrous reaction to the mesh obliterates the vaginal ring. These laparoscopic procedures are performed after the horse has recovered from previous surgery to reduce the hernia.

Torsion of the spermatic cord

Torsion of the spermatic cord occurs when the spermatic cord rotates around the vertical axis of the testis. Torsion of 180 degrees or less seems to cause no discomfort to stallions and is often considered to be an incidental finding. Torsion of 180-degrees, however, may have a detrimental effect on testicular function, even in the absence of clinical signs of substantial vascular compromise. On occasion examination may identify rotation of one or both testicles no more than 180 degrees and is believed to occur in light breeds in about 15% of Paso fino stallions. Torsion of 360 degrees or more causes acute occlusion of the testicular blood supply, and if it is not corrected quickly, the testis and spermatic cord distal to the torsion become gangrenous. Torsion of the spermatic cord in the stallion apparently occurs intravaginally.

Signs of torsion of the spermatic cord in stallions include scrotal swelling and signs of colic. Other diseases that involve the testis and associated structures, such as inguinal herniation, orchitis, and epididymitis, produce similar signs, but these diseases can usually be excluded by palpation of the contents of the scrotum, by palpating the vaginal rings per rectum, and by ultrasonographic examination of the scrotum and its contents. Horses with gangrenous necrosis of an abdominal testis caused by torsion of the spermatic cord may display no clinical signs of torsion.

A 360-degree torsion of the spermatic cord usually necessitates removal of the affected testis. If the testis is salvageable, orchiopexy, using nonabsorbable suture material, can be performed to permanently fix the testis in its proper position. One suture can be placed at the cranial aspect of the testis and one at its caudal aspect. The effect of transient torsion of the spermatic cord on the production of antisperm antibodies or the release of other factors that may affect fertility of stallions is not known.

Hydrocele

A hydrocele is a pathologic accumulation of serous fluid between the visceral and parietal layers of the vaginal tunic. Because of the insulating effect of the fluid, temperature-induced dysfunction of spermatogenesis of both testes may occur, the outcome of which can be poor semen quality causing subfertility. The vaginal tunic secretes fluid, and this fluid is resorbed through the lymphatic vessels and veins of the spermatic cord. Hydrocele results when production of fluid is increased or its resorption decreased. A hydrocele can accompany testicular neoplasia or scrotal trauma, or it may be idiopathic. Idiopathic hydrocele may occur during hot weather and resolve when the ambient temperature drops. Because the vaginal cavity communicates with the peritoneal cavity, a hydrocele may form as a result of passage of abdominal fluid through the inguinal canal. Migration of parasites into the vaginal cavity and associated structures has been implicated as a cause of hydrocele.

A hydrocele appears as a painless, fluid-filled scrotal enlargement. It may occur bilaterally or unilaterally and may develop acutely or insidiously. If development is chronic, the testis within the affected tunic is usually smaller than normal as a result of atrophy. A hydrocele can usually be differentiated from other diseases that cause scrotal enlargement through by palpating the contents of the scrotum, by transscrotal ultrasonography, and by palpating the vaginal rings per rectum. During ultrasonographic examination of a hydrocele, anechoic to semiechoic fluid is seen surrounding the testis. Diagnosis is verified by aseptic aspiration of a serous, amber transudate from the vaginal cavity.

Exercise may cause a hydrocele to temporarily decrease in size, and occasionally, a hydrocele may resolve spontaneously. Aspirating fluid from a hydrocele usually provides only transient relief because the fluid soon reforms. Treatment of an affected horse should be focused on removing the cause of the hydrocele, but because the cause can rarely be identified, horses with a persistent, unilateral hydrocele are usually treated by excising the affected testis and vaginal tunic before spermatogenesis of the contralateral testis becomes affected by increased scrotal temperature. Prognosis for fertility is poor if a bilateral hydrocele persists, but reestablishment of fertility is likely if both hydroceles resolve. Sclerotherapy, using tetracycline or polidocanol injected into the vaginal cavity or plication or removal of redundant portions of the vaginal tunic, has been used to treat men affected with hydrocele, but these treatments have not been evaluated in affected stallions.

Hematocele

A hematocele resembles a hydrocele but is a collection of hemorrhagic fluid within the vaginal cavity. Scrotal swelling associated with hematocele can be quite pronounced. A hematocele is usually caused by trauma to the scrotum or its contents, but because the peritoneal and vaginal cavities of the horse communicate, a hematocele can also occur as an extension of hemoperitoneum.

A hematocele caused by acute trauma to the scrotal contents is usually associated with signs of pain. Ultrasonography may help differentiate hematocele from hydrocele and other causes of scrotal enlargement; some causes of hematocele, such as rupture of the tunica albuginea of the testis, can sometimes be detected by ultrasonographic examination. Diagnosis is confirmed by aseptic aspiration of blood or sanguineous fluid from the vaginal cavity.

A small hematocele may cause no problem with fertility and may dissipate without treatment, but a large hematocele may insulate the testes, causing interference with spermatogenesis. Clotting of blood and formation of fibrin in the hematocele may result in thick adhesions between parietal and visceral tunics. If the hematocele is large, the hemorrhage should be evacuated from the vaginal cavity, and the testis and epididymis should be carefully inspected to identify the source of hemorrhage. A tear in the tunica albuginea should be sutured. Orchiectomy is indicated if the testis or epididymis is badly damaged. The effect of testicular trauma on formation of antisperm antibodies and secondary subfertility in stallions is not known. Removal of the affected testis may be indicated to minimize the likelihood of such a complication and to prevent depression of spermatogenesis of the contralateral testis from increased temperature caused by inflammation of the damaged testis.

Testicular neoplasia

Testicular neoplasms of the horse are rarely reported, probably because most horses are castrated at an early age, and perhaps because testes removed from apparently normal stallions are seldom examined closely for the presence of neoplasia. Only primary testicular neoplasms (i.e., those that originate within the testis) have been reported, and those can be divided into germinal and nongerminal types. Germinal neoplasms arise from the germ cells of the seminiferous epithelium and are the most common type of testicular neoplasm. Germinal testicular neoplasms reported to occur in the horse include the seminoma, teratoma, teratocarcinoma, and embryonal carcinoma. The seminoma is the most commonly reported testicular neoplasm of the horse. Nongerminal testicular neoplasms arise from testicular stromal cells and include the Leydig cell tumor and Sertoli cell tumor. Nongerminal testicular neoplasms of the horse are less commonly reported than are germinal testicular neoplasms. Although cryptorchidism has been shown by epidemiologic studies to increase the incidence of some forms of primary testicular neoplasia in men and dogs, the influence of cryptorchidism on the development testicular neoplasia of horses has not been established definitively. A large percentage of testicular teratomas seem to found in abdominally retained testes, but failure of descent of a testis, rather than being a predisposing factor in the formation of a teratoma, may more likely be a result of the teratoma. Whether cryptorchidism influences the development

of Sertoli-cell or Leydig-cell neoplasms in horses is difficult to discern from the small number of reports, but the few Leydig-cell neoplasms of horses that have been reported have been found predominately in retained testes.

When a horse with a suspected testicular neoplasm is examined, the contralateral testis should be used for comparison, keeping in mind that testicular neoplasia can occur bilaterally. The neoplastic testis is often heavier than its normal counterpart. A neoplastic testis is usually painless when compressed and usually remains freely movable within the scrotum. Scrotal enlargement caused by neoplasia must be differentiated from other causes of scrotal enlargement, such as torsion of the spermatic cord, orchitis, epididymitis, hydrocele, hematocele, and inguinal herniation or rupture. Careful external palpation and ultrasonographic examination of the contents of the scrotum and palpation of the vaginal rings per rectum can be used to differentiate these conditions from testicular neoplasia. Painless, scrotal enlargement that develops insidiously is more likely to be caused by testicular neoplasia than by inflammation or ischemia.

Ultrasonographic examination may be helpful in determining if a testis is neoplastic. Normal testicular parenchyma is homogeneously echogenic, but a neoplastic testis usually contains areas of decreased echogenicity. Affected testes may contain single or multiple tumors. Testicular neoplasia can be confirmed by cytologic examination of a needle aspirate or by histologic examination of a specimen obtained with a punch or incisional biopsy of the testis. Although testicular biopsy has been performed without noticeable side-effects in normal stallions, the long-term effects of testicular biopsy have not been well studied. If testicular neoplasia is strongly suspected, the affected testis should be excised. Before a neoplastic testis is removed, the sublumbar lymph nodes should be examined by palpation per rectum for enlargement caused by metastatic spread of the tumor.

Penile and preputial injuries

Penile and preputial injuries, such as lacerations and hematomas, are usually caused by kicks, especially to the erect penis; mounting of stationary objects; masturbation; attempting to breed a mare across a fence; severe bending of the penile shaft caused by sudden movement of the mare during coitus; and improperly fitted or maintained stallion rings. Damage to the penile shaft, including the urethra, can be inflicted during castration performed by an inexperienced surgeon. Deep lacerations that extend into a corporeal body may result in impotence, and those that extend into the urethra may result severe necrosis of tissue from escape of urine.

Even superficial lacerations can result in severe penile damage if left untreated. An untreated laceration to the penile epithelium may result in cellulitis and preputial edema, which, in turn, lead to prolapse of the penis and internal preputial lamina from the preputial cavity. Prolapse of the penis and prepuce may lead to penile paralysis from damage to the penile nerves or to further damage to the exposed penile and preputial epithelium. Puncture or laceration of the glans penis may lead to severe hemorrhage during breeding when the corpus spongiosum becomes fully engorged.

Fresh lacerations to the penile and preputial epithelium should be sutured with soft absorbable or nonabsorbable suture material. An infected or heavily contaminated laceration should be left open to heal completely by second intention, or when it shows no signs of inflammation, it can be sutured. If the wound is left open, it should be dressed often with a nonirritating, antimicrobial ointment. If the laceration is accompanied by severe preputial edema, the penis and prepuce should be retained within the preputial cavity by using a retainer bottle, nylon netting, or nylon hosiery suspended at the preputial orifice with a crupper and surcingle made of rubber tubing. The penis can be restrained within the preputial cavity for several days with sutures placed across the preputial orifice, but sutures can exacerbate the preputial trauma. If the penis cannot be retained in the preputial cavity, an enclosing abdominal support bandage can be used in an attempt to decrease dependent edema sufficiently within a few days to allow the penis to return of to the preputial cavity.

Penile hematomas that continue to expand should be explored to determine if the origin of the hemorrhage is a rent in the tunica albuginea. A stallion whose prepuce or penis has been traumatized, should be isolated from mares until the wound has healed.

Priapism

Priapism, or persistent erection without sexual arousal, occurs when detumescence of the engorged corpus cavernosum penis (CCP) fails because of disturbances of arterial inflow or venous outflow. Priapism occurs uncommonly in horses but is economically devastating when a valuable breeding stallion is affected; impotence is the usual outcome, and phallectomy may be required.

Priapism of stallions, is primarily caused by administration of a phenothiazine-derivative tranquilizer that blocks the sympathetic impulses that initiate detumescence. When detumescence fails, blood in the CCP stagnates, and partial pressure of CO₂ in the stagnant blood rises, causing erythrocytes to sickle. The sickled erythrocytes obstruct venous outflow from the CCP, and the collecting veins eventually become irreversibly occluded. Arterial supply to the CCP is still patent in the early stages of priapism, but if priapism persists, it too becomes irreversibly occluded. Eventually, the trabeculae of the cavernosal tissue become fibrotic and lose their expansile capacity necessary for normal erection. In addition to damaging erectile tissue, prolonged erection may also result in penile paralysis by damaging the pudendal nerves, perhaps by compressing the nerves against the ischium. The weight of the pendant penis may also damage the retractor penis muscles.

Horses with priapism have been treated empirically by administering diuretic and corticosteroid drugs, general or regional anesthesia, penile and preputial massage, application of an emollient dressing, and slings. Although such treatments usually fail to resolve priapism, some, such as massage and application of an emollient dressing, are beneficial because they prevent damage to the integument of the exposed organ. Benztropine mesylate, a cholinergic blocker administered systemically or 1% phenylephrine HCl, a sympathomimetic drug aseptically injected directly into the CCP (2 to 10 mg), have been used successfully to bring about detumescence in horses affected with priapism. These drugs should not be given if the horse has ventricular tachycardia or high blood pressure, and the horse's heart rate should be monitored after administration.

When the horse does not respond to medical treatment, its CCP should be irrigated to evacuate sludged blood. Heparinized, physiological saline solution (10 U heparin/mL physiological saline solution) is injected through a 14- or 12-gauge needle inserted into the erect CCP proximal to the glans penis. Sludged blood and saline solution are evacuated 10 to 15 cm caudal to the scrotum through a small stab incision in the tunica albuginea of the CCP or through one or two 14- or 12-gauge needles inserted into the CCP. The CCP is irrigated until fresh hemorrhage appears in the efflux. If a stab incision is made in the tunica albuginea of the CCP, it should be sutured after irrigation. If arterial blood fails to appear after irrigation, the arteriolar supply to the CCP is probably permanently damaged, and impotence is likely. Failure of erection to subside after irrigation indicates that arteriolar inflow is patent and that venous outflow is still occluded. If erection recurs after irrigation of the CCP, the CCP can be anastomosed to the corpus spongiosum penis (CSP) to create a shunt for blood trapped within the CCP. This shunt is unlikely to interfere with subsequent erection and ejaculation.

Hemospermia

Hemospermia, a cause of infertility in stallions, has been attributed to bacterial and viral urethritis; improperly applied stallion rings; habronemiasis of the urethral process; and wounds to the glans penis. Hemospermia is also commonly caused by a urethral rent of unknown cause. Regardless of the cause of hemospermia, the source of hemorrhage is probably the CSP. Hemorrhage occurs at the end of ejaculation, when contraction of the bulbospongiosus muscles causes pressure within the CSP to increase from 17 to 1000 mm Hg. Blood in the ejaculate, even in amounts that cannot be detected grossly, can contribute to infertility.

Hemospermia may occur more commonly in frequently bred stallions. Affected stallions are sometimes slow to ejaculate, and ejaculation sometimes appears to cause pain. Hemospermia is usually diagnosed by gross examination of semen that has been collected with an artificial vagina. The site of hemorrhage can often be determined by examining the urethra with a sterilized, flexible endoscope that is at least 100 cm long. Endoscopic examination of the urethra of stallions affected with hemospermia often reveals a longitudinal defect, 5 to 10 mm long, on the caudal surface of the urethra at the level of the ischial arch; no gross signs of inflammation surround the defect.

The fertility of stallions that have a slight amount of hemorrhage in the ejaculate can sometimes be preserved by adding an extender to the semen to dilute the concentration, and hence effect, of red blood cells in the ejaculate. Horses infertile or subfertile because of hemospermia have been treated by sexual abstinence and by systemic administration of formalin, methenamine, or antimicrobial drugs. Enforcing sexual abstinence for a protracted time, many months, is often unsuccessful in resolving hemospermia. Horses with hemospermia seem to be most effectively treated by temporary urethrotomy performed at the level of the ischial arch. Urethrotomy is performed with the horse standing, using sedation and epidural anesthesia. To facilitate identification of the urethra during dissection, a urethral catheter or small foal stomach tube is inserted into the urethra and advanced until it is proximal to the ischial arch.

A longitudinal incision, 8 to 10 cm long, centered on the ischial arch, is made on the perineal raphe. The incision extends through skin, retractor penis and bulbospongiosus muscles, CSP, and urethral mucosa to expose the lumen of the urethra. Incising the urethral mucosa may be unnecessary for resolution of hemospermia. Opening the CSP without entering the urethra may reduce the risk of complications associated with urethrotomy, such as urethral fistula or stricture. The wound is allowed to heal by second intention. Daily installation of suppositories composed of an antimicrobial drug and a corticosteroid into the urethral lumen has been advocated but is unnecessary. Stallions should receive sexual rest for at least 3 months after surgery. Horses may bleed at the urethrotomy for more than a week after surgery, especially at the end of urination when the bulbospongiosus muscles contract. The ischial wound generally heals within 3 to 4 weeks.

Incising the CSP at the level of the ischium may decrease cavernosal pressure at the end of urination, and this decreased pressure in the CSP may be responsible for the apparent success of temporary urethrotomy in eliminating hemospermia. When the bladder has emptied, the bulbospongiosus muscles contract to expel urine that remains in the urethra, and these contractions increase pressure within the CSP and may prevent healing of a urethral lesion that communicates with the CSP. The incision into the CSP converts this semiclosed, vascular space into an open space, and during urination, blood flow is diverted from the urethral lesion to the urethrotomy, thus permitting the lesion to heal.

Because the urethral rents are typically located at the caudal surface of the urethra near the ischial arch and are accessible through ischial urethrotomy, primary closure of the defect may be indicated. Urethral endoscopy aids the surgeon in identifying the exact location of the defect. To confirm the location of the defect, a hypodermic needle can be inserted percutaneously into the lumen of the urethra at the level of the ischial arch during endoscopic examination. The urethral rent is sutured in an interrupted pattern using 3-0 absorbable suture. The perineal wound is left unsutured to heal by second intention.

Unilateral necrosis of the spermatic cord, epididymis, and testis of a Friesian Stallion caused by *Salmonella Serotype Hartford*

A.P. Bouchard, T.M. Prado, J. Schumacher, R.L. Donnell.

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Dr., Knoxville, TN 37996, USA.

Resumen

Importance- Trauma, inguinal herniation, and torsion of the spermatic cord are causes of inflammation of the spermatic cord, epididymis and testis of the stallion. Epididymo-orchitis characterized by coagulative and liquefactive caused by *Salmonella* spp. has been reported to occur in humans, rams, and chickens, but we can find no similar reports in the stallion. This case report describes a stallion affected with epididymo-orchitis caused by *Salmonella serotype Hartford*. **Diagnostic approach and treatment-** A 5-year-old Friesian stallion was presented to the University of Tennessee's Veterinary Medical Center because of discomfort and enlargement of the right testis first observed 4 days previously. Examination revealed a thick plaque of edema and an enlarged right testis and spermatic cord. Palpation of the testis did not elicit signs of pain from the horse. Ultrasonographic examination, using Doppler, of the right testis and cord revealed hyperechoic areas in the cord and testis consistent with necrosis. The right testis was moderately enlarged (7.1-cm diameter), and fluid was observed between the visceral and parietal tunics. The distal 3 cm of the spermatic cord had no blood flow. Testicular necrosis caused by torsion of the spermatic cord was suspected, and unilateral orchidectomy was recommended. **Results and discussion-** During intra-operative examination, the right spermatic cord was observed to be oriented properly but its distal 3 cm was necrotic. The cord and testis were edematous and contained areas of fibrosis. Scrotal fascia was thickened, and the parietal tunic was 6 to 7 mm thick and adhered to the testis. The testis and necrotic portion of the spermatic cord were excised using a closed technique of orchidectomy. The scrotal incision was sutured. Culture of foul-smelling exudate contained within the testis produced >500 colonies of *Salmonella serotype Hartford*. Severe, chronic, diffuse, necrosuppurative orchitis and epididymitis, and cellulitis and bacterial rods were observed during histological examination of the testis. The scrotal incision healed by first intention.

Techniques for diagnosis of upper respiratory disease and comparison of findings in resting and exercising endoscopy

Eric J. Parente ¹, DVM Dipl. ACVS

¹ New Bolton Center, Univ. of Pennsylvania, Kennett Square PA.

Abnormal respiratory noise at rest or during exercise is the most common complaint prompting an examination of the larynx. Videoendoscopy provides the best structural and functional assessment of the larynx. Resting videoendoscopy should always be the first step since many of the functional abnormalities will be evident on a thorough resting endoscopic examination. Since many field practitioners still use fiberoptic endoscopy, the differences between the two should be understood; particularly that videoendoscopy has many advantages over fiberoptic endoscopy. Videoendoscopy provides a markedly larger field of view and a stronger light source which allows a much more detailed assessment of the larynx. Videoendoscopy also allows the clinician to better appreciate a functional assessment of the airway by using a water flush to stimulate a swallow. It should be well understood that the functional assessment made standing provides evidence on what is speculated to occur during exercise.

Asymmetric, incomplete abduction of an arytenoid is a common abnormality that can have different etiologies. The specific etiology must be determined and evaluated in the context of the expectation of the horse prior to making decisions on treatment. Recurrent laryngeal neuropathy, chondropathy, and dysplasia often

have structural differences that are endoscopically apparent as well as palpable abnormalities and ultrasonographic differences. These differences can be subtle and multiple diagnostic tools may be necessary to definitively differentiate between the three. Even with a definitive diagnosis, the impact the abnormality on non racehorse performance is sometimes difficult to discern. An exercising examination would seem logical to make that determination, yet many show horses have been reported to improve dramatically after laryngoplasty despite the respiratory dysfunction considered inconsequential.

Other manipulations during a resting endoscopic examination, such as evaluation of the ventral epiglottis, may also provide information that may be useful in determining an exercising abnormality. This can be performed quickly with good help and appropriate instrumentation. After local anesthetic is applied and sedation administered the videoendoscope is passed up one nostril while a grasping forceps is passed up the other to elevate the epiglottis.

Exercising endoscopy should be employed whenever a dynamic abnormality is suspected and a clear diagnosis cannot be confidently made that is consistent with the historical information, or when an abnormal noise or poor performance persists postoperatively. Several abnormalities are seen during exercise that cannot be reliably predicted based on resting endoscopy.

There are several different exercising systems. Considerations when choosing a system that is best suited for your practice include: image quality (which includes the light source), ease of use, patient compliance, recording ability, and cost.

The value of exercising endoscopy is dependent upon not only the equipment, but even more importantly how the test is performed. Early comparisons of treadmill vs. overground exercising endoscopy surprisingly yielded more definitive diagnoses with the treadmill, but that likely had nothing to do with the equipment, but more likely how the test was performed. In early studies people were apprehensive to work the horse as hard with all the equipment required for overground endoscopy, and thus sometimes did not arrive at a diagnosis. Now with smaller more user friendly equipment, that is not the situation. The advantage of overground exercising endoscopy is that the test "can" be performed very similar to the competition conditions. That not only includes speed and distance but also head and neck position. The effect of head and neck flexion has been shown multiple times to have a large impact on the dynamic changes that occur in the airway during exercise, and not just in race horses. In several studies with nonrace sport horses it has been shown that without appropriate head and neck flexion during the exercising exam a diagnosis would not have been found. The position of the head and neck that is created with a rider up is practically impossible to recreate under treadmill testing.

Other less commonly used imaging tools of the larynx include ultrasound, magnetic resonance imaging and computed tomography. Radiography is still used, but very infrequently because it provides very limited information that cannot be already gleaned from other diagnostic techniques. Ultrasound has been used with greater frequency to evaluate the arytenoid cartilages and the intrinsic muscles of the larynx. The ultrasound equipment used is fairly standard equipment, performed with a linear transducer operating at 10 MHz. Differentiating recurrent laryngeal neuropathy vs. arytenoid chondropathy vs. laryngeal dysplasia is its greatest use. Magnetic resonance imaging and computed tomography are in their infancy in evaluation of the upper respiratory tract but have great promise. Magnetic resonance imaging has been employed to define the abnormalities associated with laryngeal dysplasia, and with improvements in technology and capabilities it is likely that computed tomography will be used similarly.

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Abnormalities of the guttural pouch

Eric J. Parente ¹, DVM, Diplomate ACVS

¹ *University of Pennsylvania, Kennett Sq. PA.*

The guttural pouches can be the site of significant abnormalities of the upper respiratory tract and should be evaluated based on any suspicion of a problem within them. Empyema, mycosis, tympany, and temporohyoid osteopathy are the more common reasons to assess the guttural pouch. A resting endoscopic examination can reveal the presence of blood or purulent material at a guttural pouch opening, but this is not conclusive evidence that the discharge is emanating from the pouch. Since the pouches open passively during swallowing it is possible that material in the nasopharynx can be pushed into the opening during a swallow and thus give the appearance that it is originating from the pouch. Only looking into the pouch can provide direct evidence that the discharge is originating from the pouch.

Examination of the guttural pouch can be most easily be achieved with a smaller diameter endoscope (9mm or less) and using a "stylet" passed through the biopsy channel of the endoscope. With experience, both pouches can be accessed easily from one nostril. To do so the endoscope is positioned just in front of the pouch opening. The scope should be rotated as needed so the biopsy channel is closest to the lateral wall of the pharynx. The biopsy instrument is passed into the pouch. Because the Eustachian tube is oriented in a ventral rostral to dorsal caudal, direction the biopsy instrument must be directed dorsally to fully enter the pouch. It is common for clinicians to have difficulty entering the pouch because their biopsy instrument is not directed dorsally enough. Once the instrument is well seated in the pouch, the endoscope is rotated as needed to have the biopsy channel medial, thus prying open the pouch ostium and facilitating passage of the endoscope. The biopsy instrument is slowly withdrawn as the endoscope is passed forward into the pouch to limit the push back from the biopsy instrument if it is wedged in the pouch. While watching the video it should always appear that the scope is moving forward toward an open space.

Familiarity with normal anatomy of the guttural pouch is critical to making valid judgments about any abnormalities. The lining of the pouch is thin and fairly translucent allowing easy identification of underlying structures. The presence of lymphoid hyperplasia is infrequent, but not unusual. The stylohyoid bone divides the pouch into a fairly large medial compartment and a smaller lateral compartment. The external carotid and maxillary arteries are the larger vessels in the lateral compartment. In the medial compartment the internal carotid artery and multiple nerve branches (glossopharyngeal, vagus, accessory, and hypoglossal) can be identified. Infrequently bulging on the medial floor of the pouch will occur with retropharyngeal lymph node swelling.

While we typically think of the guttural pouches as a potential source when the horse has evidence of purulent nasal discharge, the clinical signs of guttural pouch disease are not just nasal discharge. Severe epistaxis as discussed in an earlier presentation can be the result of guttural pouch mycosis and purulent discharge can be associated with empyema. Yet, guttural pouch tympany will have external signs of soft

swelling without nasal discharge, and temporohyoid osteopathy will cause neurologic deficits without nasal discharge. Implicating the guttural pouch as a source of disease should always be made by a combination of the physical exam findings as well as resting endoscopic evaluation of the guttural pouch.

Purulent material from the guttural pouch defines some degree of guttural pouch empyema. Guttural pouch empyema can be a primary infection, or secondary to ruptured lymph nodes into the pouch (possibly secondary to *Streptococcus equi*). If it is secondary to lymph node rupture, there may be some degree of blood, and typically large bulges on the ventral floor of the medial compartment are seen endoscopically. Resolution of the problem can likely be obtained if the purulent material is lavaged with a balanced electrolyte solution from the guttural pouch in the early stages of the disorder. Caution should be exercised about adding anything remotely caustic to lavage solutions since neurologic dysfunction (dysphagia) has been caused by such treatments. Culture for appropriate antimicrobial treatment can be beneficial but is not always necessary. The chronic cases become more difficult to resolve and often require surgical intervention.

Chronic empyema can result in chondroid formation or in the more severe cases, complete filling of the pouch with purulent material. It is easy to identify the chondroids endoscopically as multiple rounded masses on the floor of the guttural pouch and can also be seen on lateral radiographs of the head. The presence of chondroids will prevent resolution of the infection until they are removed but the chondroids do not typically cause any other abnormalities. When the pouch becomes completely filled with inspissated purulent material the dorsal pharyngeal roof will be deviated over the front of the larynx and may cause obstruction of the respiratory tract. It can be difficult to pass the endoscope into the guttural pouch since there is not any available air space in front of the tip of the endoscope to allow visualization. Passing the endoscope by feel and then slowly backing up the tip of the endoscope a small distance will create enough space to allow visualization of the material. Typically there is no space to see anything else but inspissated purulent material. Lateral radiographs will have a homogenous radiodensity filling the guttural pouch without any identifiable air space dorsally.

Resolution of the infection in either situation by flushing alone is impossible. Evacuating all the purulent material and keeping the pouch empty is the goal. With a small number of chondroids, the endoscope can be used with a snare or basket to grab the individual chondroids and remove them. With a large number of chondroids or chondroids of large size, creating an alternative opening has great advantages. With transendoscopic laser surgery a salpingopharyngeal fistula can be created. Not only does this allow easier evacuation of the material from the pouch at the time the opening is created, but it also allows continued passive drainage/evacuation postoperatively when the horse is feeding from the ground. We have taken a similar approach with fungal infection (mycosis) of the guttural pouch in conditions where the horse has NOT had any epistaxis. Several horses have resolved the mycotic infection with just the salpingopharyngeal fistula and no medical treatment.

Tympany is another pouch abnormality, but this is most common in very young foals. It is observed more in fillies than colts and Arabians appear to be overrepresented. The foals will have a soft air filled swelling behind the vertical ramus of the mandible. The disorder is most commonly unilateral despite both sides of the foal's head will have swelling. On close observation it becomes clear that there is a larger affected side. The foal will also have a history of abnormal respiratory noise or possibly milk at the nares after nursing. Endoscopically there is dynamic collapse of the roof of the pharynx. Frequently there will be some fluid in the affected pouch, which is thought to be a secondary change. To obtain resolution of tympany, a patent air passage needs to be created from the affected guttural pouch to the pharynx. There are two ways to obtain this. Traditionally the septum between the two pouches has been cut to allow airflow from the abnormal to normal pouch which then allows normal airflow into the pharynx. More recently we prefer to use a salpingopharyngeal fistula to obtain the same goal. The fistula is easier to create, is done in the standing, sedated animal, and seems less likely to stricture and lead to recurrence.

The final guttural pouch disease to address is temporohyoid disease. This disease affects the joint between the stylohyoid and the base of the skull as well as the stylohyoid itself. Horses will show evidence of vestibular disease and possibly facial nerve deficits. Radiographs can be helpful in making the diagnosis but direct visualization of the stylohyoid itself via endoscopy of the guttural pouch is most sensitive. Large proliferative density is seen around the temporohyoid joint, the bulla, and the stylohyoid bone. Whether the origin of the disease is infectious or primarily just inflammatory is unclear. Regardless, most horse are treated with antimicrobials and anti-inflammatories. Since the temporohyoid joint becomes fused to the base of the skull, the significant "normal" movement of the stylohyoid can cause fracture at the base of the skull and death. The surgical goal for the disease is to disengage the proximal stylohyoid from the rest of the hyoid apparatus, thus removing the risk of skull fracture and potentially decreasing the proliferative change around the joint which compresses the facial nerve. The earlier surgeries describe resecting a central portion of the affected stylohyoid. This a difficult procedure and there are reports of these tissues bridging back together over time. The most recent approach describes removing the ceratohyoid. While these surgeries should prevent fatal fracture, the resolution of the neurologic signs is slow and the amount of resolution is extremely variable.

Treatment and prognosis for laryngeal hemiplegia

Eric J. Parente ¹, DVM, Diplomate ACVS

¹ University of Pennsylvania, Kennett Sq. PA.

Laryngeal hemiplegia (recurrent laryngeal neuropathy) has been recognized for many years and is one of the most common problems of the upper respiratory tract but is still challenging to effectively treat. There are multiple methods of treatment, and the tieback (laryngoplasty) procedure is still the most common method of treatment. Performing a tieback to abduct and lock the left arytenoid in an open position is a simple concept but much more complex than it appears. Part of the complexity is taking what should be a dynamic functional larynx, and making it into a somewhat static structure without complications. Furthermore we are working with tissues (cartilage) that have significant variability to their shape and stiffness and thus the ability to construct and maintain a rigid structure is difficult. This leads to significant variability of success and success that is greatly dependent upon the primary complaint and the expected level of performance for the individual horse. The success rate for treatment of nonracehorses is generally very high (>80%), while the success for a racehorse is reported to be from 25-70% depending on the criteria and methodology used to determine success.

The primary reason for the limited success is the inability to maintain an adequate airway during exercise. Other reasons involve complications as a result of the surgery. Aspiration or coughing is a significant concern but anecdotally appears to be a function of the surgeon's experience and not directly associated with the degree of abduction. The occurrence of seroma formation is <10%, and infection at the surgical site even lower. While many surgical infections with implants require suture removal, if addressed early the laryngoplasty suture does not always need to be removed. Finally a small percentage of horses (particularly those well abducted) can develop granulation tissue on their corniculate without a history of coughing postoperatively. This stresses the importance of a follow up endoscopy at 4 weeks before resuming training.

The first step to success is obtaining an accurate diagnosis. The diagnosis is most often made with resting endoscopy. Several different laryngeal grading systems have been developed for horses examined while standing loosely restrained in a box stall or in stocks, with a twitch applied and without tranquilization or sedation. The majority of grading systems are similar to what is described here with minor variations. Grade I (synchronous and symmetrical abduction) and grade II (symmetrical but asynchronous abduction) are considered normal, in that the horse is capable of obtaining and maintaining complete abduction of the arytenoid cartilages during sustained exercise. Laryngeal grades III and IV are abnormal. Any horse with grade IV laryngeal movements clearly demonstrates progressive and severe axial collapse of the paralyzed

arytenoid cartilage during exercise. Its clinical significance will depend on the level of exercise and how the horse is judged (performance or noise). Most racehorses with grade III (asynchronous and asymmetrical movement) dysfunction will also experience dynamic arytenoid collapse but many show horses will not.

Since laryngeal dysfunction is almost always left sided, any right sided observations should cause the clinician to pause and reevaluate the horse. Right sided dysfunction is most commonly secondary to 4th branchial arch disease or general dysplasia. This should be confirmed prior to considering surgery since structural abnormalities may prohibit the chance of a successful laryngoplasty procedure. It can be confidently diagnosed with ultrasound or MRI. Causes of trauma to the recurrent laryngeal nerve will result in dysfunction but should also be confirmed prior to considerations for surgery.

The first challenge of laryngoplasty surgery is attaining the exact position of the arytenoid desired at the time of surgery. Intraoperative videoendoscopy is essential to more precisely position the arytenoid. Despite videoendoscopy, less than two thirds of the horses had the ideal position of abduction on the morning following surgery in one retrospective study. Furthermore, the most common complication postoperatively is loss of abduction. One study reported a loss of abduction of one grade or more in almost half of the horses over a 6 week period. While initially it is intolerable to think that this could happen it is consistent with all the experimental studies. Critical evaluation of the experimental studies published indicates a significant postoperative loss of abduction. There are likely multiple causes for loss of abduction, and many different causes have been proposed but none have been clearly defined. There is no evidence that the prosthesis material, the horse's age, or the preoperative degree of dysfunction impacts laryngoplasty failure. Thus there is no benefit of waiting until a horse is completely paralyzed before pursuing surgery. There is actually more recent data to show that horses undergoing laryngoplasty that were not completely paralyzed returned to a higher level of performance than horses that were completely paralyzed. It is possible that the inability to consistently maintain abduction is inherent in the technique and the tissues we are working with. This prompted us to further evaluate those possibilities.

Initially we demonstrated the great variability in the cricoid cartilage, which is an important structure in anchoring the laryngoplasty suture. We have also shown significant variability in distraction of the suture anchored in the cricoid. These factors could in part explain the variable loss of abduction over time. To compact these factors we developed a different approach to minimize abduction loss and improve stability with a modified laryngoplasty approach. There are two significant modifications from the standard laryngoplasty. The first is approaching the muscular process caudal to the cricopharyngeus muscle rather than between the crico and thyropharyngeus. The second is transecting the insertion of the cricoarytenoideus dorsalis muscle from the muscular process and entering the cricoarytenoid (CA) joint to debride part of the articular cartilage and facilitate ankylosis of the CA joint. An experimental study demonstrated the effectiveness of this technique. We completed a retrospective study to assess our technique clinically. We evaluated horses over several years and looked at quarterly earnings. Our data indicate that the horses treated by modified laryngoplasty improved significantly relative to performance while suffering from hemiplegia and earned as much over time, and competed as long or longer than their cohorts that previously raced better than them.

While laryngoplasty may never be the perfect procedure, if the goal is to create a stable partially abducted arytenoid, methods as described above to improve the probability of achieving this goal could be employed. The only potential disadvantage of this technique is if the appropriate degree of abduction is not achieved, there will be a limited time of opportunity to change the position of the arytenoid before a joint ankylosis ensues. If several months after surgery the horse exhibits significant aspiration, or on the other extreme a lack of adequate abduction, repeat laryngoplasty is unlikely to be an effective option for surgical correction. An arytenoidectomy would be recommended.

Other potential reasons for limited improvement in performance after laryngoplasty do not just revolve around structural stability but involve dynamic function. There is evidence that the horse with limited resting

abduction after laryngoplasty can often undergo further dynamic collapse of the affected arytenoid, or have other soft tissue structures obstructing the airway during strenuous exercise. If there is not adequate abduction of the left arytenoid it is common that the left aryepiglottic fold and/or the right vocal cord will deviate axially during inspiration and result in partial obstruction and noise. If the arytenoid is poorly abducted it is likely that the left arytenoid will undergo further dynamic collapse during exercise. Resting evaluation of the arytenoid cannot predict stability under exercising conditions accurately. There is also experimental evidence that horses after laryngoplasty may not have gross evidence of aspiration but can have microscopic contamination of their lower airway. The impact of this lower airway contamination on performance is unknown but it is easy to speculate that it could initiate a complex of lower airway inflammation that does impact gas exchange.

Given the challenge of obtaining a positive outcome with laryngoplasty, other alternative treatments of cordectomy, ventriculectomy, arytenoidectomy, or reinnervation have been considered as viable treatment options. These different surgical options are tailored more to the use of the horse and the degree of laryngeal dysfunction. Arytenoidectomy is generally reserved for chondropathies, and reinnervation has limited success but vocal cordectomies still can be successful in select cases of laryngeal dysfunction.

The vocal cordectomy (sometimes in conjunction with the ventriculectomy) is reserved for horses that maintain some abduction capability and that are not racehorses. The goal is to minimize noise production for show horses. It is rarely recommended as a sole treatment for a racehorse. Vocal cordectomy can be performed in the standing horse with transendoscopic laser resection or via laryngotomy (standing or anesthetized). While bilateral ventriculocordectomy is advocated by some clinicians to decrease abnormal noise, and can be performed successfully with traditional surgery, it should not be attempted with transendoscopic laser surgery at one time because it will likely lead to ventral scar/webbing formation in the larynx. The value of a ventriculectomy is arguable. If the vocal cord is completely resected, the ventriculectomy likely has little benefit.

When is nasal discharge more than just nasal discharge

Eric J. Parente ¹, DVM Dipl. ACVS

¹ *New Bolton Center, Univ. of Pennsylvania, Kennett Square PA.*

Nasal discharge can represent something benign, life threatening, or anything in between. Often horses are placed on antimicrobials for any nasal discharge because of limited ability to evaluate the problem and effectively treating the horse, but with advanced diagnostic imaging and newer surgical techniques the effectiveness of treating such patients has improved dramatically.

In most cases nasal discharge is not an emergency, but any horse with nasal hemorrhage should be evaluated immediately. While bilateral epistaxis is most commonly from exercise induced pulmonary hemorrhage and not life threatening, a severe bleed from the guttural pouch could be from one or both nares and is life threatening. Guttural pouch hemorrhage is most often the result of a mycotic infection eroding through major arteries in the pouch lining. A large volume of blood evident on endoscopic examination at the guttural pouch opening is often the only evidence needed prior to referral for surgery. Trying to endoscopically evaluate the inside of the guttural pouch close to the time of a severe bleed is usual futile since the amount of blood within the pouch will obscure visualization. Surgery is aimed at obstructing blood flow through the major vessels in the lining of the guttural pouch. Ligation at the cardiac side as a sole procedure is often ineffective because of retrograde flow, so the vessels must be obstructed or embolized on the cranial side as well. If there is not an opportunity to obstruct vessel cranial to the lesion, ligation at the cardiac side can be effective in a small percentage of horses. The surgery not only prevents further hemorrhage but also causes the mycotic lesions to resolve without any medical therapy. Any evidence of neurologic disease (laryngeal dysfunction or dysphagia) should be determined prior to surgery.

Other causes of epistaxis to consider are trauma, neoplasia, fungal infections, or ethmoid hematomas. All of these are typically of a much lower volume relative to guttural pouch mycosis. Severe trauma can result in avulsion of the longus capitis muscle from the insertion of the basisphenoid bone and moderate bleeding from the guttural pouch. Blood seen from the nasomaxillary opening can be secondary to trauma and bleeding into the sinus without external signs of trauma. Radiographs will often reveal a fluid line. Treatment is not necessary, but antimicrobial treatment is recommended to prevent secondary sinusitis. Neoplasia or fungal infections that cause epistaxis are usually seen easily on endoscopic examination and are often mixed with purulent discharge. Treatment of neoplasia is often unrewarding but fungal infections can be treated effectively with topical anti-fungals if a method to ensure long contact time is instituted.

Progressive ethmoid hematomas causing epistaxis are very common. Unlike guttural pouch mycosis, hematomas typically cause a small volume, intermittent bleed not associated with exercise. It is extremely rare to have facial deformity and the source of bleeding could be within the nasal passage (ethmoid recess), within the sinuses, or both. Both endoscopic and radiographic evaluations are essential to fully appreciate the extent of the lesion, since they can often be in multiple sites. Small lesions can also exist within the sinus without obvious radiographic abnormalities, so caution must be used in interpretation of radiographs.

In many cases therapy may consist of intralesional formalin injections under endoscopic guidance in the standing horse on an outpatient basis. While this technique is very client/patient friendly and effective at abating clinical signs, it is unlikely to provide a "cure." Previous reports of success have been based on telephone follow up and not follow up endoscopy or radiography. Many patients have recurrence after several years and it may be in part because what we see within the nasal passage is just the tip of the lesion and we are not effectively treating the base or origin. Laser ablation may be more effective than intralesional formalin, but would require significantly more expense and repeated treatments with large lesions. For this reason, laser treatment is usually reserved for the smaller lesions. Our current treatment regimen for hematomas within the ethmoid recess is to treat with intralesional formalin at 4 week intervals until they are <1cm in diameter and then perform laser ablation. Hematomas within the sinus are managed differently.

Purulent discharge usually represents response to bacterial infection of the guttural pouch or sinus cavities. Discharge at the opening of the guttural pouch can be pushed into the opening during swallowing and may not represent a guttural pouch infection. Endoscopic examination of the pouch is necessary to refute or confirm the pouch as the origin. Discharge from the sinus cavity can often be seen dripping over the ventral turbinate from the nasomaxillary opening above. Complete examination of the ventral and middle meatus is recommended since infrequently a separate opening into the nasal cavity may present, or the material becomes so inspissated that the material becomes contained entirely within the middle meatus. Primary bacterial infections of the sinus without an underlying cause are common. Many patients will respond favorably to antimicrobial therapy without further diagnostics or treatment. Long term antimicrobials are usually required and if there is not an immediate response, further evaluation should be performed.

Radiography is commonly the next diagnostic procedure performed. If there is soft tissue density not consistent with a fluid line, or abnormalities with the teeth, referral should be considered. If the only radiographic abnormality is the presence of fluid within the sinus, then culture and lavage can be performed in the standing horse. A small trephine hole just rostral and ventral to the eye will allow placement of a teat cannula for aspiration and lavage. The only potential disadvantage is if the surgical site becomes a nidus of infection. Reasons against standing culture and lavage would be any radiographic indications of abnormalities not consistent with a primary sinusitis. Oral examination should be complementary to the endoscopic and radiographic evaluations to determine if any teeth abnormalities are resulting in a secondary sinusitis. When available, computed tomography provides much more information than radiography and more frequently is being used instead of any radiographs.

Evaluation and treatment of sinus disease

Eric J. Parente ¹, DVM Dipl. ACVS

¹ *New Bolton Center, Univ. of Pennsylvania, Kennett Square PA.*

Introduction

Clinical indications of sinus or nasal disease may include nasal discharge, facial deformity, or stertorous breathing with asymmetric airflow. When discharge is unilateral it is a strong indicator of a sinus problem, but infrequently bilateral disease may occur and thus bilateral discharge does not rule out sinus disease. The presence of purulent discharge is often an indicator of a bacterial infectious process, while sanguineous, or purulent/sanguineous discharge usually indicates progressive ethmoid hematoma, neoplastic or fungal disease. Historical information and other physical examination findings are important in arriving at a diagnosis, but quality imaging is essential. That imaging starts with an endoscopic examination.

The source of any nasal discharge can usually be determined by a comprehensive upper airway endoscopy. Discharge emanating from the caudal aspect of the middle meatus is the result of fluid exiting the sinus cavity through the nasomaxillary opening and indicative of either a primary or secondary sinusitis. Infrequently the disease process drains into the nasal cavity further rostrally, and the origin of the discharge can only be seen if the endoscope is passed down the entire middle meatus.

Differentiating between primary or secondary sinusitis can be difficult in the very early stages of disease. A thorough oral examination with quality radiography may be all that is required, but there are incredible advantages to computed tomography when evaluating the head/sinus cavities. Primary sinusitis can often be resolved with long term antimicrobial treatment and sinus lavage. Secondary sinusitis is most often the result of tooth abnormalities/infections and will require removal of the primary problem as well as treatment of the sinus. Under more chronic conditions the purulent material can become inspissated and will require physical debridement to resolve the sinusitis. This can be achieved via sinusotomy or sinuscopy.

Uncommonly, sinusitis can also become severe enough to cause nasal obstruction, and/or facial deformity, but more commonly these distortions are associated with a neoplastic or cystic mass. Ethmoid hematomas can also cause some nasal obstruction but rarely cause facial deformity and will produce small volume epistaxis, not purulent discharge. Cysts usually cause facial or nasal deformity and typically don't cause any nasal discharge unless a secondary sinusitis occurs. Differentiating the cause of the symptoms is important prior to any decisions about treatment since most neoplastic diseases have a poor prognosis. Signalment, history, and diagnostic imaging should provide enough information for the clinician to differentiate the etiology of the disease.

While many sinuses disease require surgical intervention, ethmoid hematomas are the exception. They are common neoplastic-like masses within the sinus or nasal passage. While they are more commonly associated with the ethmoid region within the nasal cavity, they can be concurrently within the sinus cavity or solely within the sinus cavity. Repeated intralesion formalin injections are very effective at reducing their size. Depending upon their location and size, laser or manual debridement is often recommended for a more effective treatment.

Sinus surgery

A strong understanding of anatomy and normal variation is often undervalued to achieving a positive outcome with sinus surgery. Without this knowledge or experience it is more likely normal structures will be damaged; not only leading to more surgical trauma than necessary but also hemorrhage that may inhibit effectively accomplishing the intended goal of the surgery. Preoperative imaging is a significant piece to resolving the puzzle, and computed tomography (CT) has dramatically improved our ability to not only diag-

nose disease more accurately but also to develop a surgical plan. Magnetic resonance imaging may provide more detail about the types of tissue being imaged, but provide less structural detail.

There are three surgical approaches that can be employed to asses or treat sinus disease: sinoscopy, frontonasal flap, maxillary flap. The first (sinoscopy) is always done standing while the two flap techniques can be done standing or under general anesthesia. The approach chosen should be based on the intended goal and the location of the lesion. In other words, many things can be done through a sinoscopy, but you are not going to successfully remove a large firm sinus mass through a sinoscopic portal. If your goal is just to explore the sinus and/or obtain diagnostic biopsy specimens, then sinoscopy is very useful. Sinoscopy is particularly useful at resolving chronic ventral conchal sinus infections that are recalcitrant to just anti-microbial treatment and lavage.

The most common complications of sinus surgery historically are intraoperative hemorrhage or incomplete resolution/recurrence. Both are best avoided by careful purposeful dissection and leaving dissection of the areas that are recognized as more likely to result in hemorrhage for the last part of the surgery. Too often surgeons grab and pull things out of the sinus not recognizing what anatomy is attached and can be damaged in the process. While many sinusotomies can be performed standing, and some surgeons advocate standing surgery because they believe hemorrhage is dramatically diminished in the standing horse, there are several potential disadvantages. It is very difficult to pack the nostril of a standing horse if necessary, it is difficult to complete anesthetize the surgical area, and it may be more difficult to completely and carefully dissect tissues within the sinus cavity in the standing horse. Regardless of how the surgery is performed, collecting blood that is lost and quantifying it accurately will allow the surgeon to more accurately treat the entire patient. Not surprising, large animal surgeons *can* underestimate blood loss.

Similarly resolution of the primary problem is predicated on a well-executed surgical plan. While many tooth abnormalities can be resolved standing, repulsions are still occasionally required and intraoperative imaging is necessary for accurate orientation. It was previously thought nasosinus fistulation was required for a successful sinus surgery, but in reality as long as the normal drainage angle is maintained and foreign material (including inspissated pus) is completely removed, fistulation is not required.

The prognosis for sinus and nasal surgery has significantly improved over the last 15-20 for several reasons. Improved diagnostic techniques have allowed earlier recognition of disease, more accurate diagnoses, and improved surgical planning. There is also a much better understanding of anatomy so less collateral damage occurs and patient management (not just disease management) has further limited the risk of complications.

Treatment of sinus hematomas is dependent upon the size of the hematoma. When moderate or small hematomas are found within the sinus cavity, laser ablation/debridement is performed via a standing sinoscopic procedure. Larger hematomas within the sinus may require extirpation via sinusotomy. Intralesional formalin treatment via trephine has been ineffective. After extirpation, the sinus and nasal passage are packed with sterile gauze and the packing is removed 3 days post operatively. A large opening is left between the sinus and nasal passage which provides an avenue to see within the sinus via standard endoscopy in the future. Since we have seen hematomas recur or arise in different areas with long term follow up, endoscopy and radiographic followup is recommended initially at 6 months and then annually. Any lesions found can then be treated by intralesional formalin or laser ablation before they become substantial in size.

Recent advances of laryngeal surgery

Eric J. Parente¹, DVM Dipl. ACVS

New Bolton Center, Univ. of Pennsylvania, Kennett Square PA.

Most of the advances in laryngeal surgery revolve around improved diagnostic capabilities resulting in better understanding and more targeted treatment. While not every horse needs a dynamic overground endoscopy to make a diagnosis, if there is any uncertainty it is critical. Based on previous experience many dynamic abnormalities can be predicted from the resting endoscopic examination, history and physical exam findings, but not all. Other diagnostic tools, such as MR, CT or ultrasound can be extremely beneficial in understanding the specific anatomical changes that will impact surgical treatment. When dynamic abnormalities are noted, often standing transendoscopic laser surgery can be employed to resolve the abnormality.

Distinguishing the cause of incomplete abduction of an arytenoid is the most common potential problem. Recurrent laryngeal neuropathy vs. chondropathy vs. dysplasia is a distinction that must be made correctly for any chance of performing the appropriate surgery and having a positive outcome. While RLN is a functional abnormality with normal cartilaginous structure, the other two are not and therefore must be approached much differently and because of the variability of each, tailored surgical treatments may not completely resolve the abnormality but can be functionally effective and yield a very positive outcome.

There is also improved understanding of the different types of complications of laryngoplasty. It is now known that horses after surgery for RLN can still make abnormal respiratory noise and a "failed" laryngoplasty is not always the cause. Respiratory flows will vary significantly depending on the degree of abduction and stability of the arytenoid and these flows can cause adjacent soft tissues to cross the glottis. Postoperative aspiration can result in coughing or pneumonia, yet aspiration is likely multifactorial and not just related to the degree of abduction. Furthermore, something like esophageal incompetence can cause coughing during exercise without any indications of a problem at rest. Treatments for these disorders are again tailored to the specific individual. Evolving therapies for RLN including standing laryngoplasty and reinnervation have merit but have not yet shown long term positive outcomes beyond an experienced surgeon performing laryngoplasty.

There is also improved understanding in the importance and challenge of managing epiglottic entrapments. For years entrapments were considered a "racehorse" abnormality. We recognize now it is more commonly seen in racehorses but can be present in any horse and can have devastating consequences if not recognized and managed appropriately. Our recent data shows that epiglottic entrapments can vary greatly in their prognosis and likely require a longer period of convalescence than previously recommended. Postoperative standard endoscopic evaluations do not allow the clinician to see the ventral surface of the post surgical epiglottis and can appear very normal when the ventral surface has not healed. Return to training prior to healing can result in long term negative effects. This is particularly true if resection of soft tissue is necessary. Furthermore, even with resolution of the entrapment, the resulting ventral surface of the epiglottis can impact laryngeal position during exercise. Transendoscopic laser surgery has been extremely useful not only in axial divisions of entrapping tissue but also ablation of recalcitrant ulcerative lesions and resection of cysts. There is greater recognition of the association between entrapments and displacements. Simple entrapments alone do not cause significant airway obstruction but can precipitate intermittent dorsal displacement of the soft palate. With recognition of this association this has prompted more aggressive action earlier to prevent displacement by performing a tie forward surgery.

Magnetic nanoparticles for x sperm separation from equine semen. Field and commercial results

Nanopartículas magnéticas para separación de espermatozoides x en semen equino. Resultados de campo y comerciales

Hernán Ramírez Castex ¹, MV, MSc; Luis Losinno ², MV, PhD

¹Bioteq, Centro de Medicina Reproductiva Equina, Chile; ²Laboratorio de Producción Equina, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Argentina. hernanramirez@bioteq.cl

Summary

Preselection of sex before conception has been one of the objectives pursued by scientists and breeders for many years. Within dairy and meat cattle industries there are productive reasons to calculate the financial benefit of preferring one sex. However, equines are subject to other variables i.e Polo horses where the industry prefers 100% mares (as in dairy cattle).

The only replicable and efficient way up to now in separating the sperms that contain the X chromosome from those that contain the Y chromosome from a semen sample is through the use of cell sorting technique by high flow cytometer developed by Johnson in 1986 (Samper et. al, 2012). However, it has only been used commercially and at big scale in dairy cattle industry. The number of spermatozoa that can be effectively selected per hour in the flow cytometer has been a limitation for its mass application, especially for species that require a high number of sperms to inseminate a female, as it is the equine scenario (Panarace et. al, 2014). And also to consideration the level of damage that spermatozoa suffers during the process and the inability to get an appropriate frozen dose after sex sorting.

The aim of this study was to test a new method of sex sorting spermatozoa that was also field friendly for using, none or less damage for spermatozoa and faster than cytometer. The use of magnetic beads in the nano scale could accomplish these features. The results showed that sperm parameters where mainly not affected comparing to control groups (not exposed to nano particles) and fertility tests went well on our first small scale inseminating mares.

Introducción

La preselección de sexo antes de la concepción ha sido uno de los objetivos de científicos y criadores en programas de producción animal desde hace muchos años. En la industria de la producción de leche y carne bovina existen razones objetivas y productivas para calcular el beneficio económico de desear un sexo o el otro, sin embargo, en equinos este objetivo es más subjetivo y sujeto a otras variables.

En la actualidad, en la industria equina se realiza principalmente la determinación del sexo post concepción. Hasta ahora se han utilizado y probado variadas tecnologías y metodologías en equinos con este fin (para una revisión actualizada ver Aurich *et al.*, 2014). Dentro de estas biotecnologías se puede mencionar la determinación temprana del sexo en embriones equinos a través de la detección del antígeno sexo específico macho H-Y, utilizando anticuerpos fluorescentes (Wood, 1988). Es importante notar que este método no es muy efectivo en embriones que han iniciado la formación del blastocelo y aumentan los falsos negativos (Ramalho *et. al*, 2004). Dado que los embriones equinos entran al útero como mórula tardía ó blastocisto temprano, sólo una pequeña proporción de embriones recuperados podrían ser procesados por este método (Battut, *et. al* 1997; Checure, *et. al* 2016).

Una segunda técnica es la detección de secuencias sexo específicas de ADN. Los embriones equinos son sensibles a la biopsia celular comparado con otras especies, ya que el desarrollo exitoso a nivel uterino depende de una cápsula acelular intacta (Stout *et al.*, 2005). Sin embargo, recientemente han sido repor-

tadas transferencias exitosas de embriones equinos biopsiados (Choi *et al.*, 2010). La PCR utilizada para determinar el sexo embrionario trabaja con genes específicos localizados en el cromosoma Y. La técnica requiere equipo sofisticado así como personal capacitado, por lo tanto, es difícil su aplicación en condiciones de campo ó regiones dónde es requerida esporádicamente (Alonso *et al.*, 2015).

De todos modos, la técnica más utilizada en la actualidad es la determinación del sexo fetal en útero. La identificación y ubicación ultrasonográfica del tubérculo genital en el feto es la primera opción cuando migra hacia la cola en la hembra y hacia el cordón umbilical en el macho, lo que permite la determinación del sexo fetal ecográficamente. El momento óptimo para la determinación del sexo fetal es entre el día 59 y el día 68 de gestación. Las tasas de éxito de la técnica son muy variables y dependen principalmente de la experiencia del examinador pero al ser una técnica sencilla, práctica y rápida actualmente en la mayoría de los programas de sexado fetal temprano con operadores entrenados la eficiencia es en general mayor al 96-98% (Curran y Ginther 1991; Bucca, 2005; Martínez *et al.*, 2016).

La segunda opción ultrasonográfica es la identificación de las gónadas en el feto. A partir del día 100 de gestación, la combinación de la ultrasonografía transabdominal y transrectal permiten el examen visual del feto por completo, con una identificación real de los órganos sexuales primarios completamente desarrollados (Bucca, 2005).

Por último en la gestación avanzada se ha descrito el análisis del sexo fetal desde material fetal colectado de la circulación materna. Este método consiste en la identificación de ADN fetal libre circulante en sangre materna (De León *et al.*, 2012). La ausencia de secuencias de cromosoma Y en plasma materno implica que el feto es hembra, sin embargo, esto también podría significar que es la consecuencia de no detectar ADN fetal libre en presencia de un feto macho. La significancia de esta técnica sería poder implementarla en estadios tempranos de gestación. En el presente no se sabe si células fetales del cinturón coriónico entran a circulación materna al momento de la invasión del endometrio, evento que ocurre al día 37 de gestación (Allen *et al.*, 1975).

Según lo descrito anteriormente, a pesar que existen varias posibilidades para determinar el sexo de un concepto en el caballo, la mayoría de éstas demandan equipos de alto costo, experiencia y entrenamiento. O, por otro lado, son factibles de realizar a etapas de la gestación muy avanzada donde a veces no se puede tomar una decisión a tiempo para descartar un sexo no deseado o no es posible realizarlo bajo condiciones de campo; y si lo es lo hace ineficiente y costoso dado el tiempo invertido de trabajo e insumos previos a la toma de la decisión como ocurre en la determinación del sexo fetal por ultrasonografía a los 60 días de gestación (i.e programas de cría de caballos de polo en Argentina).

En cuanto a las técnicas preconcepción, la única forma reproducible y eficiente hasta hoy de separar espermatozoides que contienen el cromosoma X de los que contienen el cromosoma Y basado en el contenido de ADN desde una muestra de semen por medio del uso de la citometría de alto flujo desarrollada por Johnson en 1986. (Samper *et al.*, 2012).

Sin embargo, sólo ha sido utilizada a nivel comercial y a gran escala en bovinos. El número de espermatozoides que pueden ser eficazmente seleccionados por hora en el citómetro de flujo ha sido una limitante para su aplicación masiva especialmente en especies que requieren de un alto número de espermatozoides para inseminar una hembra cómo es el equino (Panarace *et al.*, 2014). Además se deben considerar las susceptibilidades de estos gametos a los variados procesos que ocurren durante el sexado por citometría de flujo (Garner, 2006).

Por otro lado la aplicación en condiciones de campo de semen congelado y sexado requiere de mayor investigación para producir muestras de semen de mejor calidad que las que existen en el presente para hacerlo comercialmente sustentable. (Clulow *et al.* 2009). Aunque muchas mejoras se han ido implementado con los años para aumentar las tasas de clasificación de espermatozoides y su calidad, aún menos del

50% del total de espermatozoides analizados son clasificados correctamente, con un 25% aproximado de espermatozoides X y 15 a 20% de espermatozoides Y. Consecuentemente el uso de semen sexado requiere de técnicas de inseminación artificial intracornal profunda a bajas dosis (Clulow *et al.*, 2009).

Las tasas de gestación con semen fresco sexado tienen rangos entre 10 y 40%, cuando se ha utilizado entre 5 y 25 millones de espermatozoides vía inseminación profunda. La baja dosis y la alta dilución de la dosis inseminantes tienen por lo general efectos adversos en las tasas de gestación (Clulow *et al.*, 2009). Morris, Hunter y Allen reportaron tasas de fertilidad aceptables utilizando un millón de espermatozoides suspendidos en 100 a 250 microlitros de diluyente junto con inseminación histeroscópica (Morris *et al.*, 2000). En los últimos años, distintos trabajos independientes de Morris, Samper y Lascombes, inseminando entre 13 y 40 millones de espermatozoides y utilizando 15 padrillos diferentes, han resultado en tasas de gestación entre 40 y 60% usando inseminación profunda (Aurich *et al.*, 2014).

Dentro de los métodos para mejorar la eficiencia y la calidad del semen después de haber sido sexado, está el uso de soluciones de amortiguación y coloides de centrifugación. Para tener un impacto en la industria, la muestra de semen de un padrillo debe tener la capacidad de ser transportado a un laboratorio que lo procese para sexado y posteriormente lo envíe al lugar de residencia de la yegua a ser inseminada. (Aurich *et al.*, 2014).

Aunque una tasa de sexado lenta logra mejor clasificación y separación de espermatozoides X e Y, logrando una mayor pureza de la muestra, la eficiencia del proceso es afectada negativamente. Más aún, a mayor tiempo de procesado, menor calidad espermática de la muestra. El sexado por citometría de flujo es tiempo dependiente porque las células son clasificadas a tasas de entre 3000 y 5000 células por segundo. Estas tasas resultan en un tiempo de proceso de 1,5 horas para clasificar solamente 16 millones de células para inseminación o criopreservación (Clulow *et al.*, 2009).

Un área de la medicina y la tecnología que recientemente ha explorado en la infertilidad masculina es la nano-tecnología o nanomedicina. Esta se refiere a la tecnología que trata o utiliza partículas sintetizadas a la escala de nanómetros o nano-escala (1 a 100 nm). Es interesante recordar que un nanómetro es una milmillonésima parte (10^{-9}) de un metro (o una millonésima parte de un milímetro) y una dimensión de 100 nanómetros (el tamaño típico de un virus) es importante para la nanotecnología porque debajo de esta dimensión se pueden observar nuevas propiedades de la materia, en especial debido a las leyes de la física cuántica (Andrada, 2012). Estas nano-partículas pueden ser fabricadas en diferentes tamaños y composiciones y su biocompatibilidad con fluidos biológicos las convierten en un excelente dispositivo o herramienta para interacciones con células marcadas y no marcadas para propósitos que utilicen fluorescencia o magnetismo (Feugang *et al.*, 2015).

Es así como existen las técnicas de nano purificación espermática utilizando estas nano-partículas (NP) con procesos asociados de magnetismo, fluorescencia, inmuno-mediados o con marcadores de superficie de membrana logrando separar espermatozoides vivos de muertos y apoptóticos y así mejorar la infertilidad del semen procesado (Feugang *et al.*, 2015).

La tecnología de separación magnética de células (*Magnetic Activated Cell sorting-MACS*) es una técnica para la preparación de espermatozoides usada recientemente en técnicas de reproducción asistida. Esta tecnología, ha mostrado que puede separar espermatozoides motiles, viables y morfológicamente normales que despliegan una tolerancia significativa a la crio-preservación y tienen un mejor potencial de fertilización.

MACS provee pureza y recuperación óptima con resultados consistentes y fiables. Técnicamente es un procedimiento conveniente con un sistema fácil de aplicar en cualquier laboratorio. Provee de resultados rápidos dado los cortos períodos de incubación y la separación de las partículas después del procedimiento no es necesaria (Said *et al.*, 2005; Makker *et al.*, 2008;).

Otra característica que se ha estudiado es el potencial Zeta o potencial electroquinético de los espermatozoides. Este es el potencial eléctrico que se produce en el plano de desplazamiento del espermatozoide entre su membrana plasmática y el entorno que lo rodea. Los espermatozoides maduros poseen una carga eléctrica entre -16mV y -20mV (Chan *et. al*, 2006).

El potencial Zeta de la fracción que contenía más de 80% de espermatozoides "Y" fue aproximadamente -16mV. En cambio en la fracción con más de 95% de espermatozoides "X" fue de -20mV. En otras palabras, la carga neta negativa en la superficie de las células espermáticas con cromosoma "X" es mayor que la del espermatozoide "Y" (Ishijima *et. al*, 1991).

Se puede resumir que existen y se han estudiado un gran número de tecnologías con el fin de seleccionar y separar espermatozoides en distintas especies, entre ellas el equino, con los objetivos principales de purificación para mejorar las tasas potenciales de fertilización o para manipular el sexo de la progenie desde un momento pre-fertilización. Sin embargo a pesar de la variedad de tecnologías todavía no existe una que reúna características de ser económica, práctica y de fácil implementación y disponibilidad.

Esto generó el objetivo de este estudio para ensayar una técnica accesible y que no afecte los parámetros espermáticos standard, para separar espermatozoides que contengan el cromosoma X de Y en semen equino a través del uso de Nano-partículas magnéticas.

Los objetivos específicos planteados fueron:

1. Determinar la tasa de separación de espermatozoides X de espermatozoides Y post tratamiento.
2. Determinar la tasa de espermatozoides viables después del proceso de sexado.
3. Determinar la fertilidad del semen sexado inseminando yeguas con semen fresco sexado estableciendo el sexo de los fetos por ultrasonografía transrectal a los 125 días de gestación y al parto.

Materiales y métodos

Protocolo de inseminación artificial

Se estableció como dosis inseminante la cantidad de 200 millones de espermatozoides con motilidad progresiva y sexados que contengan el cromosoma X (hembras). Esta dosis es obtenida después del proceso de sexado con nanopartículas magnéticas descrito por Ramírez *et.al* (2017) utilizando un dispositivo magnético específico (Figuras 1 y 2). Y la técnica de inseminación artificial (I.A) empleada ha sido la intra-cornual profunda a bajo volumen.

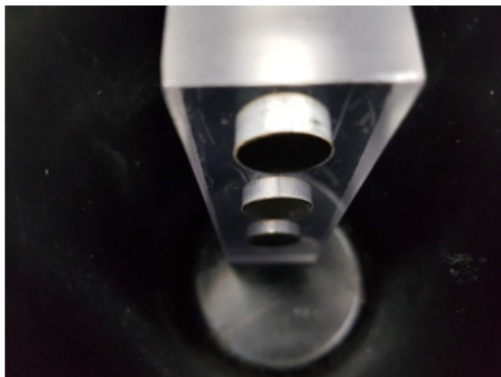


Figura 1: Dispositivo de atracción magnética específico de NP.

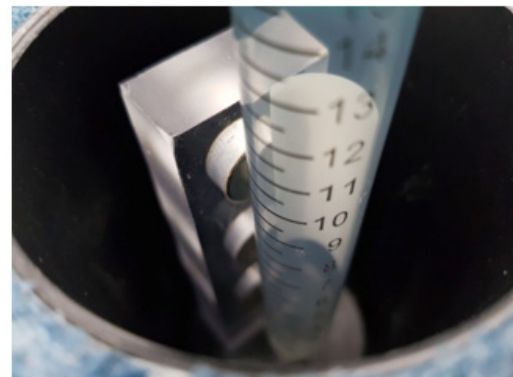


Figura 2. Dispositivo de atracción magnética específico de NP con el tubo de 15ml.

Protocolo de Preparación de la Yegua para I.A

Todas las yeguas se controlaban ecográficamente desde el inicio del celo cada 24 horas hasta que un folículo de mínimo 35mm y presencia de edema endometrial en grado 2 era observado. Ese día la yegua era inducida en la tarde (19 a 21 hrs) con algún análogo de GnRh como Deslorelina Acetato o Histerelina para que al día subsiguiente (36 horas después) se programara la colecta o envío del semen para ser procesado para sexaje y posterior I.A según descrito anteriormente. De esta manera todas las I.A se realizaron con al menos 36 horas de inducidas y la ovulación se controló vía ecográfica durante ese mismo día o el día siguiente. Se inseminaron yeguas con semen fresco, refrigerado y congelado.

Resultados

Desde Octubre 2017 y hasta la fecha de entrega de este resumen se han logrado 108 preñeces en yeguas utilizando semen sexado de padrillos con NP. Se ha utilizado semen fresco, refrigerado y congelado tanto para preñeces directas como en protocolos de transferencia embrionaria (T.E) y vitrificación de embriones. Las razas involucradas a la fecha han sido Chilena, Criolla, Cuarto de Milla, Polo, Holsteiner y Árabe. A continuación se presenta en la tabla N° 1 los datos tabulados por tipo de semen y técnica empleada y sus respectivas tasas de fertilidad. En la tabla N° 2 se muestran las proporciones de gestaciones confirmadas hembra por ecografía a los 60 y/o 125 días de gestación así como al parto según el caso. En la figura 3 la primera potranca nacida en el mundo por I.A con semen sexado con N.P.

Tabla 1. Resultados *in vivo* de tasas de preñez y recuperación embrionaria utilizando semen sexado fresco, refrigerado y congelado en equinos.

	N	%
Preñeces	108	
Sexadas por US	66	
Confirmadas hembra	59	89,3
Nacimientos con semen sexado	26	
Confirmados hembra	25	96,2

sexado para hembras.

Tabla 2. Proporciones de gestaciones hembras controladas por ecografía durante la gestación y al parto en equinos.

Semen	Preñeces		
	IA	TE	
	Preñez/ciclo (%)	Tasa de recuperacion (%)	Tasa de preñez (%)
Fresco	23/28 (82,1)	29/34 (85,2)	22/29 (75,8)
Refrigerado	23/32 (71,9)	44/54 (81,5)	35/44 (79,5)
Congelado	5/9 (55,5)		



Figura 3. Primera potranca nacida por I.A con semen sexado con N.P el 22-11-16. Raza Cuarto de milla.



Figura 4. Primera potranca raza pura chilena (Criolla) nacida por I.A con semen sexado con N.P el 08-04-18.

Conclusiones

La utilización de nano-partículas magnéticas es un método eficaz de separación de espermatozoides que contienen el cromosoma X de los que tienen el cromosoma Y confirmado por la prueba de FISH, Citometría De Flujo y qPCR con promedios sobre 90%.

La viabilidad espermática, no se vio afectada por la utilización de estas NP y su interacción con los espermatozoides equinos durante el proceso de sexado.

La motilidad total y progresiva evaluada por sistema de análisis computarizado AndroVision® no mostró diferencias entre grupo control y sexado tanto antes como después del proceso de congelación.

Los parámetros de Motilidad, VSC, VSL, VAP, Capacitación y PY no mostraron diferencias entre semen control y sexado después de la congelación. El parámetro de fragmentación de ADN sí mostró un mayor nivel de fragmentación en el grupo sexado según la prueba de selección espermática.

En los ensayos preliminares *in vivo*, el semen sexado utilizando NP magnéticas mantuvo su capacidad fertilizante y no generó pérdidas gestacionales tempranas.

La proporción del sexo hembra logrado en las gestaciones se mantiene sobre 90% utilizando las NP magnéticas como medio de sexado de semen equino para separar espermatozoides con cromosoma X.

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II. Resúmenes de trabajos presentados

Cirugía

Standing surgical treatment of chondroids from the guttural pouches of a horse through a modified whitehouse approach: a case report

Tratamiento quirúrgico de condroides en las bolsas guturales de un equino mediante abordaje whitehouse modificado: Reporte de caso clínico

Diego Cárdenas Rojas ^{1,2*}, Diego Duque Betancur ².

**MV, MSc, Grupo Investigación en Ciencias Agropecuarias - GlSCA, Facultad de Medicina Veterinaria y Zootecnia, Fundación Universitaria Autónoma de las Américas, Tv 42#62-72 Medellín, Colombia. diego.cardenas@uam.edu.co; ² MV, MSc, Facultad de Medicina Veterinaria y Zootecnia, Grupo de Investigación en Ciencias Animales - INCA-CES, Universidad CES, Cl. 10ª#22-04, Medellín, Colombia.*

Resumen

A una clínica veterinaria de la ciudad de Medellín, Colombia, llegó remitido un equino, hembra de raza Caballo Criollo Colombiano (CCC), con 34 meses de edad, para la extracción quirúrgica de condroides en las bolsas guturales previamente diagnosticados; el paciente fue intervenido en estación utilizando neurolepto analgesia. Se realizó la técnica *Whitehouse* modificado en cada bolsa gutural para extraer la totalidad de los condroides. Se realizó tratamiento antibiótico y antiinflamatorio, pre y post quirúrgico, con Trimetoprim-Sulfadiazina y Flunixin Meglumine vía sistémica; además Penicilina G sódica local mediante video endoscopia. Posterior a la intervención el paciente fue dado de alta sin complicaciones y finalmente se evidenció la resolución completa de la condición respiratoria. El reporte de este caso permitirá conocer con detalle la técnica *Whitehouse* modificada, las ventajas que presenta al ser una técnica en estación, sus retos quirúrgicos y principales consideraciones; ya que en Colombia a la fecha no hay portales al respecto en un CCC.

Palabras clave: empiema, linfadenopatía, equino, cirugía.

Keywords: empyema, lymphadenopathy, equine, surgery.

Surgical correction of mandibular fracture in a mare by interdental cerclage: a case report

Corrección Quirúrgica de Fractura Mandibular en una Yegua por medio del Cerclaje Interdentario: un reporte de caso

Darío A. Cedeño Quevedo ¹, DMV Esp MSc PhD; Lina M. Lucero Yandún ²; Elmer C. Portilla Domínguez ³

¹ Líder, Grupo de Investigación en Equinos, Universidad de Nariño, Departamento de Salud Animal, Ciudadela Universitaria Torobajo, Pasto, Colombia. dcedeno@udenar.edu.co; ² Investigador, Grupo de Investigación en Equinos, Universidad de Nariño, Departamento de Salud Animal, Ciudadela Universitaria Torobajo, Pasto, Colombia. lininis27@gmail.com; ³ Investigador, Grupo de Investigación en Equinos, Universidad de Nariño, Departamento de Salud Animal, Ciudadela Universitaria Torobajo, Pasto, Colombia. k96portilla@gmail.com

Resumen

Una yegua de raza Caballo Criollo Colombiano con 64 meses de edad presenta mala oclusión con desgarramiento de la mucosa bucal. Al realizar la proyección radiográfica oral se detecta una fractura abierta transversal en el cuerpo mandibular a nivel de la región de la placa alveolar derecha. El protocolo de anestesia se inicia con maleato de acepromazina a dosis de 0.04 mg/kg vía intramuscular e inducción con xilacina a dosis de 1.1 mg/kg y ketamina a dosis de 2.2 mg/kg por vía intravenosa. Se utilizó una anestesia total intravenosa con el protocolo de triple goteo para mantenimiento (2 g de ketamina + 500 mg de xilacina en 1 litro de guaifenesina al 5%) en infusión constante a una tasa de 2–3 ml/kg/h. Después de limpiar y desbridar la herida se realiza una incisión a través de la mejilla directamente sobre los premolares 406 y 407. Con una broca calibre 1,4 mm se perfora un orificio entre los espacios interalveolares de los premolares. Se pasa el alambre a través del agujero perforado, el cual es dirigido rostralmente y atado a través de los agujeros previamente hechos entre los incisivos 402 y 403. Los cables, se tuercen para aumentar la compresión en la línea de la fractura. Después de apretar, los extremos de los cables son doblados. El manejo postoperatorio se basó en la administración de ketoprofeno durante 5 días, Penicilina G a dosis de 20.000 UI/kg intramuscular y desinfección diaria de la herida con Clorhexidina 0,12%. El alambre se dejó 4 semanas, observando una rápida cicatrización y consolidación de la fractura. El restablecimiento de las funciones de aprehensión y masticación se obtuvieron rápidamente. Se puede afirmar que esta técnica proporciona una estabilidad segura, es fácil de realizar y de bajo costo.

Palabras clave: *fractura mandibular, cerclaje interdentario, cirugía.*

Keywords: *mandibular fracture, Interdental cerclage, surgery.*

Medicina

Amikacin sulfate regional intravenous perfusion in the treatment of a penetrating injury to the equine frog: a case report

Perfusión intravenosa regional de sulfato de amikacina en el tratamiento de una herida perforante en la ranilla de un equino: un reporte de caso

Dario A. Cedeño Quevedo ¹, DMV, Esp, MSc, PhD; Juliana C. Peñafiel Maldonado²; Karen V. Lasso Rosero ³

¹ Líder, Grupo de Investigación en Equinos, Universidad de Nariño, Departamento de Salud Animal, Ciudadela Universitaria Torobajo, Pasto, Colombia. dcedeno@udenar.edu.co ² Investigador, Grupo de Investigación de Equinos, Universidad de Nariño, Departamento de Salud Animal, Ciudadela Universitaria Torobajo, Pasto, Colombia. jcarito.95@hotmail.com; ³ Investigador, Grupo de Investigación de Equinos, Universidad de Nariño, Departamento de Salud Animal, Ciudadela Universitaria Torobajo, Pasto, Colombia. karenlasso1@gmail.com

Resumen

El paciente es un caballo criollo colombiano de 42 meses de edad, mantenido en una pesebrera en el municipio de San Juan de Pasto-Nariño. Manifiesta una claudicación 5/5 del miembro posterior izquierdo. El caballo sufrió una herida penetrante profunda, causada por la perforación de un tornillo en el surco paracuneal de la ranilla del casco causando infección en la parte posterior del casco. Al examen clínico durante la palpación de la extremidad se encontró pulso digital positivo y surco coronario con aumento de la temperatura, además, de dolor en la ranilla al realizar el examen del casco con pinzas de exploración. Para llevar a cabo el procedimiento de drenaje en los bulbos y realizar la perfusión digital intravenosa se anestesia siguiendo el protocolo anestésico para campo: inicialmente se tranquiliza al animal con acepromacina, después de 30 minutos se realiza una anestesia total intravenosa (TIVA) con el triple goteo KXG (ketamina 1 g + xylazina 250 mg en 0,5 litro de guaifenesina al 5%). Para realizar la perfusión regional digital sobre la vena digital medial se coloca un torniquete (12 cm de ancho) en la parte proximal del metatarso, se fija

un catéter calibre 22. Previa a la perfusión se realiza una exanguinación. La infusión se realiza lentamente con 2 gramos de amikacina diluida en 10 ml de solución salina al 0.9%. El torniquete se deja en su lugar durante 10 minutos después de administrar el antibiótico. La perfusión fue hecha cada 24 horas durante tres días. El caballo logró la recuperación completa y volvió al entrenamiento 1 mes después de la lesión.

Palabras clave: *claudicación, bulbos, drenaje, vena digital.*

Atresia coli in colombian creole foals: a report of three cases

Atresia coli en potros de raza criollo colombiano: reporte de tres casos

Susana Franco A ¹, DMV, MSc; Juanita Rico A ², DMV, MSc.

¹Directora Centro de perinatología equina Foal Care, Cajicá Cundinamarca, miembro del grupo de investigación de enfermedades de grandes animales Universidad Nacional de Colombia sede Bogotá; ² Práctica privada en sabana de Bogotá.

Resumen

Se presentan tres casos de potros raza criollo colombiano, prematuros (<320 días de gestación) con motivos de consulta: depresión, prematurez y cesárea. Muestran signos de inmadurez como: bajo peso al nacimiento, orejas caídas, frente abombada, laxitud de ligamentos peri articulares, poco o ninguna evacuación de meconio también dolor abdominal y poca motilidad intestinal. Se les realizó hematología sin alteraciones, medición de IgG (<400mg/dl de IgG) a las 24 horas de vida y ultrasonido abdominal con distensión intestino delgado y baja motilidad. Se les administro calostro o plasma según el caso, antibioterapia profiláctica, fluido terapia y soporte cardiovascular (solución de Ringer Lactato más dextrosa al 3-5%). Se les realizaron múltiples enemas, infusiones de lidocaína y metoclopramida como estimulantes de la motilidad intestinal sin respuesta. Entre las 36 y 72 horas de vida mostraron dolor abdominal con distensión y amotilidad intestinal, sin respuesta a la terapia médica y mueren por arresto respiratorio. Al examen post mortem muestran atresia coli grado II. Los problemas congénitos son poco frecuentes en potros recién nacidos, la atresia coli ha sido reportada en potros de varias razas, pero no hay reportes en caballo criollo colombiano (Young *et al.*, 1992, Equine vet J 24(1) 60-62). La atresia coli es una causa de dolor abdominal en potros después del segundo día de vida, (Schneide and Leipold 1978, Equine Pract. 27, 285-290) es de manejo quirúrgico y tiene un mal pronóstico, por lo cual es motivo de eutanasia; tanto por razones económicas como congénitas (Estes and Lyall 1979, J equine med. Surg. 3, 495-498). Este es el primer reporte de atresia coli en caballo criollo. Es un diagnóstico diferencial importante a tener en cuenta en potros con dolor abdominal agudo en los primeros días de vida, principalmente en potros prematuros.

Palabras clave: *neonato, prematuro, atresia, intestinal.*

Keywords: *foal, Premature, intestinal atresia.*

Immune-mediated keratopathy in equine: a case report

Queratopatía inmunomediada en equino: un reporte de caso

Natalia Pérez Restrepo ^{1*}, MVZ, MSc; Nicolás Gaviria Loaiza ², MVZ

¹ Grupo de Investigación en Ciencias Agropecuarias - GlSCA, Facultad de Medicina Veterinaria y Zootecnia, Fundación Universitaria Autónoma de las Américas, Circular 73 No. 35-04, Medellín, Colombia. Clínica Veterinaria - ULULA Oftalmología Veterinaria, Calle 13 No. 43E-27, Medellín, Colombia. ² Bienestar Equino, Calle 27 No. 35-04, Medellín, Colombia. *Correo electrónico: nataliaperezrestrepo@gmail.com

Resumen

Se llevó a consulta un equino, hembra, raza appaloosa x cuarto de milla, de 10 años de edad, debido a que hace 8 meses muestra una lesión corneal en el Ojo Derecho (OD). Al examen clínico general no se evidenciaron otras anormalidades, pero a la exploración oftalmológica se halló en OD marcado blefaroespasmio y epífora, hacia el ecuador presentó una opacidad corneal que comprometía el epitelio y estroma, con neovascularización superficial ramificada y sin presencia de ulceración; en el resto de estructuras oculares y pruebas realizadas a ambos ojos no se encontraron alteraciones significativas. Se procedió a tomar muestras de la lesión para su posterior estudio citológico y bacteriológico, en el cual se identificó infiltrado celular inflamatorio denso sin presencia de microorganismos patógenos. Por lo anterior se estableció el diagnóstico de queratitis estromal superficial inmunomediada. Se efectuó una queratectomía superficial para retirar el defecto, posterior a la cicatrización corneal, se formuló tratamiento tópico con soluciones oftálmicas de Ciclosporina 1% fórmula magistral, y un triconjugado con Polimixina B sulfato + Bacitracina de zinc + Dexametasona. Pasado 1 mes se observó excelente evolución, logrando la transparencia de la córnea. Las queratitis no ulcerativas son un grupo de enfermedades, siendo las de origen inmunomediado en gran parte únicas de los equinos, cuya etiopatogenia implica la inmunorespuesta corneal que da lugar a las lesiones reportadas como principal motivo de consulta oftalmológica (Matthews A. *et al.* Vet Ophthalmol. 2009; 1:10-6). Los caballos afectados generalmente responden a tratamiento con inmunomoduladores, sin embargo, la causa subyacente de la enfermedad sigue siendo enigmática, debido a que es una enfermedad crónica que no tiene predisposición racial y afecta a ambos géneros (Braus B.K. *et al.* Vet Immunol Immunopathol. 2017; 187:48-54). El diagnóstico y tratamiento oportuno de las queratitis inmunomediadas en equinos permite establecer un correcto pronóstico, y garantizar en el tiempo una adecuada capacidad visual en los pacientes.

Palabras clave: córnea, oftalmología, queratectomía, queratitis.

Keywords: cornea, ophthalmology, keratectomy, keratitis.

Equine Fibrous histiocytic sarcoma and nasal adenocarcinoma: a case report

Sarcoma fibroso histiocítico y adenocarcinoma nasal en un equino: un reporte de caso

Leonardo A Parra Moyano ¹, MV, MSc; Viviana E Castillo Vanegas ², MV, eMSc; Katherinie Tiuso Barbosa ³, MV, eMSc, Lorena Gutiérrez Medina ⁴, MV; Manuel A Mahecha ⁴, MV.

¹ Profesor tiempo completo, Universidad CES, Facultad de Medicina Veterinaria y Zootecnia, Medellín, Colombia;

² Profesor Catedra Universidad CES, Facultad de Medicina Veterinaria y Zootecnia, Medellín, Colombia — Laboratorio de diagnóstico Vitalab, Rionegro, Colombia; ³ Estudiante de Posgrado, Universidad CES, Medellín, Colombia;

⁴ Médico Veterinario. Práctica privada, Medellín, Colombia.

Resumen

El sarcoma fibroso histiocítico y el adenocarcinoma son neoplasias malignas, localmente invasivas, que pueden generar metástasis a través del drenaje de nódulos linfáticos. Su ocurrencia en equinos es baja y, los signos clínicos no son comunes, dependiendo del lugar de presentación. Se reporta el caso de un sarcoma fibroso histiocítico y un adenocarcinoma en un equino macho de nueve años de edad, raza Criollo Colombiano con anamnesis de asimetría unilateral de la cara de un mes de evolución, hace ocho meses presenta secreción nasal bilateral mucopurulenta con sangrado nasal después de ser ejercitado. Al examen clínico se encontró exoftalmia, con fistula ventral al ojo derecho con secreción purulenta, aumento de tamaño sobre el hueso maxilar. Se realizó estudio citológico donde se evidenció severa reacción inflamatoria y atipia celular (pleomorfismo severo, poiquiloanisocariosis, nucléolos, basofilia citoplasmática severa) correspondiente a neoplasia de estirpe mesenquimal. El estudio radiográfico de cráneo reporta pérdida del contorno normal del hueso en zona de la órbita con neoformación ósea. Debido a las estructuras comprometidas, y al mal pronóstico, se decide realizar eutanasia. Se observó una masa multilobulada

que involucra espacio retro-ocular, seno frontal, maxilar craneal y caudal con pérdida de la arquitectura. Se diagnosticaron dos neoplasias en el mismo sitio anatómico. La primera de células fusiformes con crecimiento invasivo, organizado en grupos o células individuales, algunas rodeadas y separadas por intersticio eosinofílico acelular, irregular y amorfo con inmunoreacción positiva para vimentina, CD11c y CD1a. La segunda neoplasia de origen epitelial, organizada en acinos recubiertos por una o varias capas. Células cuboides, cilíndricas y algunas irregulares, algunas vacuoladas, pérdida de la polaridad. La primera neoplasia con inmunoreacción positiva citoqueratina AE1/AE3. El diagnóstico final corresponde a un Adenocarcinoma tubular y neoplasia maligna de origen mesenquimal anaplásica, siendo el primer reporte del caso en equinos.

Palabras clave: *caballo, neoplasia, histiocito, células fusiformes.*

Keywords: *horse, neoplasia, Histiocyte, spindle cells.*

Chronic sinusitis by *Aspergillus* spp. in a Netherland saddle horse KWPN: a case report

Sinusitis crónica por *Aspergillus* spp en caballo silla holandés KWPN: un reporte de caso

Cesar Andrey Galindo ¹, DMV, MSc, PhD; Juan Manuel Restrepo Méndez ², DMV.

¹Profesor Universidad Federal de Sergipe, Aracaju, Brasil. cesardmv@yahoo.com

²Director Horse Center Colombia, Bogotá, Colombia. horsecentercolombia@gmail.com

Resumen

Caballo Silla Holandés de 15 años de edad es remitido por presentar hace año y medio signos clínicos respiratorios, epistaxis y descarga nasal mucopurulenta del ollar izquierdo. Al examen clínico manifiesta descarga nasal mucopurulenta y serosanguinolenta de olor fétido del ollar y conducto nasolagrimonal izquierdo, asimetría y aumento de tamaño del seno maxilar izquierdo y sonido mate a la percusión. Se realizó videoendoscopia de vías aéreas altas, radiografías L-L y ecografía de la región maxilar izquierda, evidenciando placas de moco y pus en la base de los cornetes dorsal y medial, degeneración de la mucosa, congestión e hiperemia en nasofaringe y orofaringe del lado izquierdo, al igual que la bolsa gutural izquierda, hueso estilogiloideo y músculo digástrico, la radiografía muestra corrientes de secreción en la base del seno maxilar indicando contenido, la ecografía del músculo masetero evidencia pérdida de fibras musculares y fibrosis del músculo. Se realiza sinusostomía del seno maxilar izquierdo bajo neuroleptoanalgesia donde se retira contenido mucopurulento y se lava con solución salina e itraconazol. Se realiza videoendoscopia de los senos frontal, etmoidal y esfenopalatino encontrándose normales, se remueven mediante videoendoscopia placas en cornetes, se envían para histopatología y cultivo, dando como resultado *Aspergillus* spp. Se continúa tratamiento con Itraconazol a dosis de 3 mg/kg BID por 20 días, antibiótico, Meloxicam y lavados diarios del seno maxilar mediante dren. El paciente continuó con secreción serosanguinolenta por el conducto nasolagrimonal, en la videoendoscopia se evidencia pérdida del 80% de la mucosa; se realizan 3 puntos de drenaje en el músculo masetero obteniendo salida de secreción serosanguinolenta con detritos de la cápsula fibrotica, a la biopsia se evidencia miositis necroticosupurativa hemorrágica severa, compatible con Aspergilosis. Se concluye que usando métodos diagnósticos como la radiografía, ecografía, pruebas de laboratorio y videoendoscopia de vías aéreas altas y conducto nasolagrimonal se llegó al diagnóstico final de sinusitis por Aspergilosis.

Palabras clave: *aspergilosis, caballo, videoendoscopia.*

Reproducción

Effect of seminal plasma components on the stallion semen quality

Efecto de los componentes del plasma seminal en la calidad del semen equino

Alexandra Úsuga Suarez ¹, Benjamín A. Rojano ², Giovanni Restrepo Betancur ³

¹ Investigadora Grupo de Investigación en Ciencias Animales (INCA-CES), Universidad CES, Cl 10a #22 - 04, Bloque C, Piso 4, Sala 7, Medellín, Colombia. ausuga@ces.edu.co; ² Investigador Grupo de Investigación en Ciencia de los Alimentos y los Productos Naturales, Universidad Nacional de Colombia Sede Medellín, Calle 59 A No. 63-20 Bloque 16, Oficina 413, Medellín, Colombia. brojano@unal.edu.co; ³ Investigador Grupo de Investigación en Biotecnología Animal (GIBA), Universidad Nacional de Colombia Sede Medellín, Calle 59 A No. 63-20 Bloque 50, Oficina 306, Medellín, Colombia. grestre0@unal.edu.co

Abstract

The importance of seminal plasma (SP) components for stallion semen quality is little known. Among the components of stallion SP, some enzymatic and non-enzymatic antioxidants which protect sperm from the injurious effects of reactive oxygen species (ROS) have been identified. This study aimed to evaluate the relationship between SP components and stallion semen quality. Semen of 30 stallions was collected, and then, SP was recovered and lyophilized. In SP total protein (TP) was assessed by Bradford method; vitamin C (CVIT), vitamin E (EVIT) and vitamin A (AVIT) were evaluated by high-performance liquid chromatography (HPLC) method; iron (Fe), copper (Cu), magnesium (Mg), and zinc (Zn) were assessed by flame atomic absorption spectroscopy. Semen motility (MOT), abnormal morphology (AM), sperm vitality (SV), and plasma membrane integrity (PMI) were evaluated. Levels of component of SP were established by a distribution analysis. Generalized linear models were fitted. Comparisons of means were done with Tukey's test. A high level of EVIT, Cu, Fe, and Zn in SP resulted in higher PMI while high level of Cu and Fe resulted in better SV. A high level of CVIT, had a deleterious effect on SV and the normal morphology of semen. Likewise, a low level of AVIT showed better results for SV and PMI. For TP, the results were less consistent because while a medium level of TP showed higher percentages for MOT, a high level of it had better results for the SV and PMI of semen. In conclusion, the level in which ions, vitamins and proteins are present in the seminal plasma influences the quality of stallion semen.

Keywords: seminal plasma, components, stallion, sperm quality.

Palabras clave: plasma seminal, componentes, equino, calidad seminal.

Cervicitis as a cause of subfertility in the Colombian Creole mare

Cervicitis como causa de subfertilidad en la yegua Criolla Colombiana

Jair Perez Osorio ^{1*}, MVZ, MSc, PHD; Astrid Paredes Cañón ², MV.

¹ Docente Titular, Grupo REMEAT, Universidad de La Salle, Cra. 7 No. 179 - 03, Tel. (571) 677 26 99 Bogotá, Colombia, jairperez@unisalle.edu.co; ² Médica Veterinaria Autónoma, Gerente BioAnimal SAS, Calle 152 n 9-80 apto 304 Torre 2, tel 6277335. Bogotá - Colombia. bioanimalsas@gmail.com

Resumen

Una de las principales barreras anatómicas del tracto reproductivo de la yegua es el cérvix o cuello uterino; este actúa como un importante mecanismo de defensa el cual debe preservar su morfoestructura y función durante la vida reproductiva en la hembra equina. En el presente estudio, se realizó la evaluación clínica

reproductiva completa de 58 yeguas, para buscar resultados que puedan explicar los cuadros de subfertilidad e infertilidad. Las yeguas evidenciaban cuadros de repetición de servicios, pérdida embrionaria temprana, secreción vulvar, intervalos ovulatorios cortos, y endometritis crónica, las cuales fueron sometidas a un exhaustivo examen clínico reproductivo, en donde se realizaron pruebas de palpación transrectal, ecografía, vaginoscopia, cultivo endometrial, citología, y biopsia endometrial. En las yeguas evaluadas fue posible observar a través de la vaginoscopia problemas de cervicitis, oclusión cervical total, oclusión cervical parcial, fibrosis cervical, invaginación, adherencias, y cérvix tortuoso; las bacterias comumente aisladas en los cultivos fueron, *E. Coli*, *Shigella Spp*, *Klebsiella Spp*. Y *Streptococcus Spp*. En la citología, fue posible evidenciar polimorfonucleares en la clasificación de I a III. En lo que respecta a la biopsia endometrial se encontraron endometritis clasificada en Grado III, Grado IIB, Grado IIA y Grado I, observándose cambios leves, moderados y severos en la morfoestructura del endometrio. Se determina que 26/58 yeguas presentan bacterias, 10 presentaron *Klebsiella spp*; 9 presentaron *Shigella spp*; 4 presentaron *Streptococcus*; 3 presentaron *E. Coli*. 32/58 yeguas presentan endometritis, 18 con clasificación Grado I, 9 con clasificación Grado IIB y 5 con clasificación Grado III. La cervicitis es uno de los casos clínicos más relevantes que causa sub e infertilidad en la yegua Criolla Colombiana, ya que su pronóstico es de reservado a malo, su tratamiento únicamente puede lograrse a través del empleo de *stems cells*; esta entidad patológica genera así mismo pérdidas económicas significativas en yeguas adultas, seniles y hembras donantes de embriones.

Palabras clave: cérvix, palpación transrectal, vaginoscopia, biopsia.

Keywords: *cervix, transrectal palpation, vaginoscopy, biopsy.*

Testicular degeneration in Colombian Creole Horse Treated with Bioregulatory Medicine

Degeneración Testicular en Caballo Criollo Colombiano tratado con medicina Biorreguladora

Jair Perez Osorio ¹, MVZ, MSc, PhD.

¹ Docente Titular, Grupo REMEAT, Universidad de La Salle, Cra. 7 No. 179 – 03, Tel. (571) 677 26 99 Bogotá, Colombia, jairperez@unisalle.edu.co.

Resumen

La degeneración testicular es la causa primaria de los cuadros de infertilidad y subfertilidad en los sementales. El objetivo de este estudio es presentar un cuadro de degeneración testicular ligado a una etiología de carácter nutricional en un semental de alto valor genético. Se realiza medición testicular del caballo, ultrasonografía, espermiograma, evaluación computarizada a través del CASA, viabilidad a través de eosina nigrosina y morfología espermática utilizando Diff Quick. El semental presento un elevado score de condición corporal, 8 en la escala de 0 a 9; al realizar la medición testicular se obtuvo: ancho testicular 9,6 cm, consistencia flácida, ultrasonografía con áreas hiperecogénicas, vena y arteria testicular normal, el DSO = 3,5 billones encontrado a través del volumen testicular; parámetros espermáticos: (44% defectos de cabezas, 18% defectos de pieza intermediaria, 35% defectos de cola, y 16 % células normales); en el análisis computacional CASA: progresividad 3%; rápidos 1,28%; velocidad curvilínea 51,9 µm/s; velocidad lineal 15,7 µm/s; velocidad media 27,6 µm/s; linealidad 30,41%; Índice de rectitud 57,14%; índice de oscilación 53,22%; Amplitud lateral de la cabeza 2,73%; la tasa de fertilidad del caballo fue muy baja, asociado a la mala calidad espermática, cuadro de obesidad y termorregulación deficiente, estableciendo un cuadro de degeneración testicular ligado al factor iatrogénico y nutricional. Se instauró tratamiento con Vigan-tol, Ascorvex y Aceite de oliva extra virgen; se inició una detoxificación con Lymphomyosot, nux vomica homaccord y berberis homaccord por 2 meses consecutivos, 3 veces por semana, administrados por vía intravenosa; al segundo mes se realizó tratamiento con hepeel, hepar compositum, selenium, coenzyme compositum, hormeel, testis compositum, hypophysis suis, durante dos meses consecutivos tres veces por semana. Este es el primer relato de caso clínico previamente diagnosticado y tratado con medicina

biorreguladora, presentando mejora significativa en su calidad seminal, posterior efectividad en preñeces y aumento en su libido, con disminución del escore de condición corporal y mejora del rendimiento atlético.

Palabras clave: *degeneración testicular, semen, reproductor, fertilidad.*

Keywords: *testicular degeneration, sperm, stallion, fertility.*

Effect of centrifugation with EquiPure® on seminal traits of Colombian Creole Horses after refrigeration using fluorescent dyes

Efecto de la centrifugación con coloide (EquiPure®) en las características espermáticas pos refrigeración, empleando sondas fluorescentes

Angélica M Vélez Rodríguez ¹, MV; Alexandra Úsuga Suárez ², MVZ, PhD; Astrid Paredes Cañón ³, MV; Giovanni Restrepo Betancur ⁴, MVZ, MSc, PhD; Luis Bernardo Murcia ⁵, MV; Cesar Camacho ⁶, MVZ, Esp, MSc, PhD(c); Jair Pérez Osorio ¹, MVZ, MSc, PhD.

¹ School of Agricultural Sciences, La Salle University, Bogotá – Colombia; ² Faculty of Veterinary Medicine and Animal Science, CES University, Medellín, Colombia;

³ Bioanimal, Bogotá – Colombia; ⁴ Faculty of Agricultural Sciences, National University of Colombia, Medellín-Colombia; ⁵ Horse Breeders Mancillas National Police of Colombia;

⁶ Reprolab- Faculty of Veterinary, Departament of Animal Medicine: Equine - UFRGS.

E-mail: jairperez@unisalle.edu.co

Abstract

The use of colloids for the centrifugation of equine semen allows for the selection of the most viable sperm before undergoing cryopreservation and artificial insemination. The Objective was to determine the effect of centrifugation with EquiPure® on the mobility of refrigerated equine semen in the presence of two diluents. The ejaculates (spermatic fraction) of five Colombian Creole horses were collected. Only samples with a minimum of 80% total motility (MT) were used. The samples were divided into four aliquots, which were then subjected to the following treatments: Control-Kenney® (T1), Control-BotuSemen®Special (T2), Equipure-Kenney® (T3), Equipure-BotuSemen®Special (T4). For T3 and T4, centrifugation was performed with 5 ml of EquiPure® plus 5 ml of semen using (falcon tubes 50 ml), for 20 min at 300 g before dilution. Each sample was then subjected to refrigeration at 4 °C for 48 h. At 0 and 48 h of refrigeration seminal traits was evaluated using a SCA® system, the MT, Curvilinear Velocity (VCL) and Linearity (LIN) of the spermatozoa were evaluated. We used a randomized block design analysis of variance and comparison of means using a Turkey test. MT values were found at 0 h of 55.2^a, 67.3^{ab}, 69.9^{ab} and 79.2^b, and at 48 h of 39.2^a, 59.2^{ab}, 50.2^{bc} and 71.5^c, for T1, T2, T3 and T4, respectively (p < 0.05). Non-statistical differences were found between the treatments for VCL and LIN (p > 0.05). The samples were subjected to the use of fluorescent dyes (acridine orange, Carboxyfluorescein Diacetate and propidium Iodeto) were found statistically significant differences (p < 0.05), for the treatments with colloid centrifugation and re-suspension with Equipure-BotuSemen®Special (T4), 69.2 + 9.7; compared with the other Control-Kenney® (T1) treatments, 33.5.0 + 6.0; Control-BotuSemen®Special (T2), 63.5.0 + 6.0; Equipure-Kenney® (T3), 18.4 + 3.0. Sperm separation by centrifugation with Equipure® colloid and its subsequent dilution with BotuSemen®Special may improve the conservation of motility and; showing greater integrity of DNA and integrity of plasmatic membranes of refrigerated equine semen for up to 48 hours after seminal collection for use in artificial insemination programs.

Keywords: *colloid, cryopreservation, sperm quality, spermatozoa.*

Palabras clave: *coloide, criopreservación, calidad espermática, espermatozoide.*

Freezing of stallion semen with modified INRA-82 extender with two combinations of glycerol and dimethylformamide

Congelación de semen de sementales con extensor INRA-82 modificado con dos combinaciones de glicerol y dimetilformamida

José Carlos González ¹, MVZ; Giovanni Restrepo Betancur ², MVZ, MSc, PHD; Astrid Paredes Cañón ³; Jair Perez Osorio ^{4*}, MVZ, MSc, PHD.

¹ Faculty of Veterinary Medicine, University of Cordoba, Monteria – Colombia;

² Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Medellín, Colombia.

³ Bioanimal SAS, Bogotá, Colombia; ⁴ School of Agricultural Sciences, La Salle University, Bogotá, Colombia
E-mail: jairperez@unisalle.edu.co

Abstract

The individual or combined use of cryoprotectants in the diluent for the freezing of equine semen is a determining factor in the post-thawing viability of spermatozoa. The objective of this investigation was to evaluate the effect of the modified INRA-82 diluent, supplemented with two combinations of cryoprotectant glycerol and dimethylformamide, on the post-thaw quality of equine semen. The semen was obtained from stallions of the following breeds; Argentinean Sport horses, Belgian horses, American Quarter Horses, Percheron, American Paint Draft horses, Zangersheide and Selle Français breed located in the breeding facilities of the National Colombian Police (Facativá-Cundinamarca). Each semen sample was divided into two equal parts that were afterwards diluted in BotuSemen® at 37 °C. After two hours, each sample was centrifuged at 800g for 12 minutes and the pellet was resuspended in the modified INRA-82® diluent previously supplemented according to the T1 treatments: 2.5% dimethylformamide (DMF) and 2.5% glycerol (GLY) or T2: 4% DMF and 1% GLY, both at a concentration of 200×10^6 cells/mL. Samples were packed in 0.5 mL straws, which were then refrigerated at 5°C for 80 minutes followed by exposure to liquid nitrogen vapors for 15 min. The samples were stored in liquid nitrogen until thawed in water at 37°C. Post-thawing evaluation of sperm motility was performed through the Sperm Class Analyzer® computerized system; the vitality and morphology of spermatozoa were evaluated through Eosin-Nigrosin staining; the functional integrity of the membrane was measured by the HOST hyposmotic test and the structural integrity of the membrane was analyzed using fluorescent SYBR14 / IP probes. The statistical analysis was performed using generalized linear models and the comparison of means by the Tukey test. A higher proportion of morphologically normal sperm was observed for T1 (70.7%) in comparison to T2 (67.0%) ($p < 0.05$). Similarly, the structural integrity of the membrane was greater for spermatozoa cryopreserved with T1 (51.6%) as opposed to T2 (43.8%) ($p < 0.05$). Although no significant statistical difference was found ($p > 0.05$), the results of total motility (59.7% vs. 54.0%) and progressive motility (24.1% vs. 21.0%) were slightly higher for T1. In conclusion, the supplementation of the seminal extender INRA-82 modified with the combination of 2.5% DMF and 2.5% GLY has a greater protective capacity for equine sperm during the cryopreservation process, compared to the use of the combination of 4% DMF and 1% GLY in this same diluent.

Key words: cryopreservation, extender, semen, stallion, cryoprotectants.

Palabras clave: criopreservación, extensor, semen, semental, crioprotectores.

Fetal eye measurement as gestational age predictor in colombian criollo mares: preliminary data

Medición del globo ocular como predictor de edad gestacional en yeguas criollas colombianas: datos preliminares

María José Quintero ¹, Daniel Ángel ^{2*}, Isabel Catalina Vélez ².

¹ Bienestar Equino, Medellín, Colombia; ² Grupo de Investigación en Ciencias Animales (INCA-CES), Universidad CES, Medellín, Colombia. * danielangel30@gmail.com

Resumen

Determinar la edad gestacional en equinos es una herramienta clínica que permite hacer una evaluación crítica de los pacientes. Se han reportado medidas del globo ocular para determinar la edad gestacional (Turner *et al.*, AAEP PROCEEDINGS, 52, 2006), no obstante, se presentan variaciones entre razas. El objetivo de este estudio es determinar un modelo predictor de días de preñez (DP) en yeguas criollas colombianas (CC) con más de 120 días de gestación. Se tomaron mediciones del globo ocular del feto en 17 yeguas CC entre 4 y 20 años de edad utilizando ultrasonografía transcreactal. Se enfocó el cristalino y se tomaron tres medidas, largo (tocando tangencialmente el cristalino), ancho (perpendicular al largo), y volumen de 2 ejes, medida generada por el software del ultrasonido (SonoScape A6 Medical Corp, China). Se realizaron 3 repeticiones de cada medición y se obtuvo un promedio. Para determinar el modelo se utilizó un análisis de regresión logística. Tres modelos fueron analizados: A. Combinación de las tres variables ($DP = 164.46424 - 0.10754 * L - 4.30571 * A + 18.58648 * V$; $R^2 = 0.89$), B. Únicamente volumen ($DP = 100.14766 + 14.03691 * V$; $R^2 = 0.88$), C. Largo y ancho ($DP = -109.63473 + 4.21984 * L + 9.24481 * A$; $R^2 = 0.87$). No hubo diferencias en el promedio de gestación (PG) vs. el PD en ninguno de los modelos (204 días), La menor diferencia entre PG y PD para los tres modelos fue de 1 y el máximo de 59 días de gestación. Los análisis preliminares arrojaron un r similar a lo reportado por otros investigadores (Turner *et al.*, AAEP PROCEEDINGS, 52, 2006). Las variaciones entre PG y PD pueden ser dadas por diferencias en el tamaño de los fetos de la misma edad. Adicionalmente, la ultrasonografía es una técnica dinámica que exige exactitud de la imagen obtenida para garantizar repetición de la técnica. Se requiere aumentar el tamaño de la muestra para confirmar los hallazgos aquí reportados.

Palabras clave: gestación, parto, globo ocular, edad gestacional.

Keywords: gestation, parturition, eye, gestational age.

Clinical management of placentitis in mares with cloned fetuses: preliminary data

Manejo clínico de placentitis en yeguas con fetos clonados: datos preliminares

Juanita Rico Almanza ¹, MV, MSc; Susana Franco Ayala ², MV, MSc; Ernesto Rojas ³, MV; Estefanía Betancourt ⁴, MV; Laura Rubiano ⁵, MV.

¹ Práctica Privada en Sabana de Bogotá. ² Investigadora propietaria Centro de perinatología equina Foal Care, Cajicá Cundinamarca, miembro del grupo de investigación de enfermedades de grandes animales Universidad Nacional de Colombia sede Bogotá. ³ Director Biohorse SAS. ⁴ Foal Care. ⁵ Foal Care.

Resumen

Un grupo de yeguas con gestación de alto riesgo producto de clonación se sometió a un protocolo terapéutico, buscando prevenir pérdidas gestaciones avanzadas y favorecer la salud postnatal en sus potros. El objetivo del presente reporte es evaluar la respuesta al tratamiento e identificar si mejora la tasa de supervivencia.

de las crías. Se seleccionaron cinco animales que tenían parámetros ultrasonográficos anormales compatibles con placentitis y con alteraciones al cuadro hemático. Las yeguas fueron tratadas con penicilina/estreptomicina, gentamicina, pentoxifilina y flunixin meglumine. La respuesta placentaria al tratamiento fue monitoreada a partir de exámenes ultrasonográficos seriados hasta el parto. Después del parto se inspeccionó macroscópicamente la placenta y se correlacionó su peso con el del potro. Adicionalmente, se registró la tasa de supervivencia de los neonatos. Todas las yeguas tratadas se encontraban con gestaciones >320d, presentaban engrosamiento de la unión útero placentaria (≥ 15 mm) y pliegues placentarios, reducción de la cantidad de líquido amniótico e incremento de la actividad fetal (score 2 y 3). En el perfil hematológico había una tendencia a un mayor recuento total de leucocitos con linfocitosis (3/5 animales), polimorfonucleares neutrófilos activos (4/5 animales) y tendencia a un incremento de globulinas. El seguimiento ultrasonográfico evidenció: estabilización en las mediciones útero placentarias, reducción de mínimo un punto en el score de actividad fetal (4/5 animales) e incremento del líquido amniótico (3/5 animales). Macroscópicamente 3/5 placentas (75%) mostraron cambios compatibles con placentitis, pero solo en una, la correlación del peso neonatal con el placentario estaba alterada (>11% PV potro). La tasa de supervivencia en estos potros fue del 40%, superior al 10% calculado en animales no tratados. Estos resultados preliminares sugieren que la implementación de estrategias terapéuticas para casos de placentitis en gestaciones producto de clonación, favorecen la función fetoplacentaria conllevando a mejorar la tasa de supervivencia en potros clonados.

Palabras clave: *equino, gestación, unión útero placentaria.*

Keywords: *equine, gestation, uteroplacental unit.*