Inducing timed ovulation in the mare

Susan Salter BSc Hons BVM&S MRCVS and Jonathon Pycock BVetMed PhD DESM MRCVS **compare and contrast various ovulating agents used to induce ovulation in mares at breeding, highlighting the advantages and disadvantages, efficacy and welfare implications associated with each**

Weatherbys documented 14,747 active thoroughbred broodmares in Ireland in 2019, almost twice as many as the UK which recorded 8,571. Ireland is the third biggest producer of thoroughbreds in the world after Australia and the USA.[1]

In addition, it is estimated that there are around 15,000 active breeders in the sport horse sector.[2] In order to maximise the efficiency of breeding, it is essential that timing of ovulation can be manipulated effectively. It is also imperative that attempts to manipulate timing of ovulation are not associated with subsequent delays in return to oestrus. The current Covid-19 crisis presents additional challenges and pressures of balancing the economic imperative to continue equine breeding with the public health requirement to minimise human contact and observe social distancing guidelines. Any measures that improve the efficiency of the breeding process have the dual benefit of improving economic efficiency and safeguarding public health. Presently in Ireland, there are two agents that are registered for the induction of ovulation: human chorionic gonadotrophin (hCG) and the deslorelin acetate implant.

HUMAN CHORIONIC GONADOTROPHIN

HCG is registered as Chorulon and is administered by intravenous or intramuscular injection. Each vial contains 1,500IU of a white crystalline powder which is reconstituted for injection and administered as a dose of 1,500-3,000IU (one to two vials). It is relatively inexpensive but has been largely superseded by deslorelin.

HCG will induce ovulation approximately 36 hours after administration when given to cycling mares in oestrus in the presence of at least a 35mm follicle, however ovulation can take up to 48 hours. This variability in timing of ovulation makes it harder to estimate the optimal time for artificial insemination, particularly with frozen semen, and often results in more frequent examinations. Non-response to hCG is common and warrants repeat induction of ovulation and re-breeding. This hinders optimum management of busy thoroughbred stallions.

With repeated use of hCG, there is a risk of anti-hCG antibody production and an associated reduction in efficacy of hCG in inducing ovulation. McCue et al (2004) investigated and showed that an initial ovulation rate of 68.3% within 48 hours reduced to 51.9% and 48.8% when hCG was used on subsequent cycles. They also showed that younger mares were more likely to ovulate within 48 hours than older mares when given hCG. Repeated use of hCG is, therefore, associated with decreased reliability in inducing timed ovulation and efficacy declines significantly with increased mare age making it unreliable for use in older mares.[3] In Ireland, hCG is still used since the deslorelin implant has labour, cost, welfare and safety implications.

DESLORELIN ACETATE – THE IMPLANT AND THE INJECTABLE

Deslorelin is a gonadotrophin releasing hormone (GnRH) receptor agonist. In either carrier form, it is a more reliable ovulating agent than hCG with ovulation occurring at approximately 40 hours post-administration. Comparisons between deslorelin and hCG have repeatedly indicated higher ovulation rates with deslorelin; for example, Gomes et al reported ovulation rates of 78.6% vs 50% in transitional mares and 68.8% vs 60% in mares which were cycling.[4] Samper et al. showed that 83.3% of mares had ovulated with hCG while 100% ovulated with deslorelin by 48 hours.[5]

Deslorelin acetate is registered in Ireland as Ovuplant, an implant that contains 2.1mg of active drug within a 3.6mm carrier pellet designed to facilitate sustained release. It is registered for subcutaneous implantation via an 11-gauge needle into the lateral neck region midway between the head and the shoulder. The carrier will be absorbed and was not designed to be removed; however, the presence of the implant has been demonstrated to be associated with a delayed return to oestrus and a subsequent extended inter-ovulatory period. [6,7,8] Johnson et al (2002) demonstrated a reduction in plasma LH and FSH concentrations following deslorelin implant administration for up to seven days, which is associated with an increased inter-ovulatory interval.[9]

This typically undesirable consequence of the implant is sometimes used deliberately to suppress ovarian activity for several months in competition mares by administering two implants.[10] Henderson et al (2012) showed that 60.8% of mares that received an implant (and remained barren on that cycle) had an inter-ovulatory interval of more than 22 days, compared to 30.4% in those mares who had the implant removed or received hCG instead of the implant.[7] Data collected by the authors indicated that when the implant was left in situ, mares had an inter-ovulatory interval that was at

least five days longer. This was in comparison to other cycles in the same mares during the same season both when the implant was removed post-ovulation or hCG was utilised as the ovulation induction agent (Salter, unpublished data).

To mitigate the negative effects of deslorelin implants on inter-ovulatory intervals, placement in the vulva and removal are recommended[6], even though this constitutes off-label use. Implants are placed, via the 11-gauge needle that is provided, into the sensitive vulval mucosa to enable removal. Removal of an implant necessitates a small but invasive surgical procedure (which some vets perform with, and others without, local anaesthetic). In the authors' experience, both administration and removal of the implant can be painful, especially in maidens who have healthy, sensitive vulval mucosa. Repeated administration and removal of the implant can also disrupt the barrier offered by an undisturbed functional vulval seal. Repeated vulval implantation, therefore, raises ethical questions and can also be unsafe for the attending veterinary surgeon if mares are not appropriately restrained within stocks. When placed into the lateral neck region, sub-cutaneous reactions occurred in 65% of cases in an Australian study.[11] Swelling, fibrosis and an increase in sensitivity to touch at the site of implantation are recognised complications of using the implant (Ovuplant; product guide). Due to the welfare implications and the availability of efficacious alternatives, the deslorelin implant has been withdrawn from the market in the US and is no longer permitted in some European countries.[8]

In Australia and the US, injectable deslorelin products are registered and available. In the UK, there is no registered product, however, an extemporaneous preparation is manufactured under license. Injectable deslorelin is an aqueous liquid which is easily administered via a small 21-gauge needle intramuscularly, therefore overcoming welfare issues associated with the implant. Normal inter-ovulatory intervals are reported following the administration of injectable deslorelin[12], eliminating the concerns associated with the use of the implant subcutaneously or necessitating its removal from the vulval mucosa. No injection site reactions were reported in association with the use of the deslorelin injection in a UK study.[12]

COVID-19

In light of the Covid-19 pandemic, hCG and the deslorelin implants are not advantageous. Each modality often requires more veterinary intervention including more ultrasonography. This is especially true on artificial breeding programmes when hCG is used and the use of the implant necessitates placement into the vulval mucosa and removal by a veterinary surgeon. This results in less social distancing, more human interaction and potential for Covid-19 transmission. These factors should be considered, especially in the current climate.

CONCLUSION

HCG is easy to administer and is safe, having no significant adverse effects on welfare. However, it is a less reliable option than other ovulating agents, may adversely impact the efficiency of subsequent breeding management and has reduced efficacy in older mares and mares who have had multiple hCG doses. Deslorelin, either as an implant or as an injection is more reliable and more effective. To avoid the risk of increased inter-ovulatory intervals and oestrous suppression, the implant must be used in an unauthorised manner which can be associated with pain and a compromise to the animal's welfare especially in light of reasonable alternatives. Injectable deslorelin overcomes the aforementioned complications while offering excellent efficacy but is not yet available in Ireland. Whether this product can be made available via special request remains unknown. However, altered practice conditions (due to the Covid-19 pandemic) and welfare issues may be additional reasons to justify a successful request.

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Is it septic? How to confidently diagnose a septic joint in the horse

David M Bolt, senior lecturer in Equine Surgery at the Royal Veterinary College Equine Referral Hospital outlines the clinical signs, diagnostic mechanisms, and management of septic arthritis in horses

Septic arthritis (SA) is commonly encountered in equine practice and represents a potentially life-threatening or career-ending emergency that requires immediate veterinary attention. Successful management of SA in adult horses includes several critical goals:

- Immediate and accurate recognition;
- Complete examination;
- Thorough timely elimination of infection;
- Reduction of inflammation and pain; and
- Return to normal function.

Joint infection in adult horses can occur after intrasynovial contamination after penetrating wounds, iatrogenic introduction after intraarticular injection or surgery and, very occasionally, hematogenous spread. Less commonly, SA can result from local extension of periarticular infection. Potentiating factors for establishing a bacterial infection include devitalised tissue, foreign material, nature and number of microorganisms involved, as well as local or systemic immunological compromise of the patient. Iatrogenic infection after intraarticular injection or surgery and penetrating wounds are the most common causes for SA in adult horses. With wounds, a variety of bacteria (gram-positive and gram-negative organisms) can be expected, whereas *Staphylococcus aureus* and other Staphylococci represent the most common isolates in iatrogenic septic arthritis. The size of the inoculum required to overcome synovial defense mechanisms depends mainly on the microorganism's virulence and pathogenicity and on predisposing factors in the articular environment, such as presence of certain intraarticular medications. Several medications used in equine practice including corticosteroids and polysulphated glycosaminoglycans have been associated with a higher risk to develop SA, due to their ability to inhibit normal synovial defense mechanisms. Under normal circumstances, the synovial environment is capable of controlling large inoculations with microorganisms and of preventing their proliferation and colonisation.

CLINICAL SIGNS AND DIAGNOSTIC METHODS

The main clinical signs of SA include joint effusion and variable lameness. If contamination has occurred from the outside, a wound or puncture may be visible. The degree of lameness can vary depending on size, age and type of the horse, duration of infection, as well as on pathogenicity and virulence of the infecting organism. Secondary periarticular oedema, cellulitis, or both can make identification of joint effusion difficult. Horses with open joint lacerations that are draining joint fluid are commonly not as lame as horses with closed joint infections. Mild hyperfibrinogemia and a 'high normal' white blood cell count (WBC) are common findings in peripheral blood analysis. However, in adult horses, the complete blood count (CBC) count and the biochemical profile are usually unremarkable.

SYNOVIAL FLUID ANALYSIS

The definitive diagnosis of SA is confirmed by cytological and microbiological analysis of synovial fluid that is obtained via an aseptically performed arthrocentesis. Knowledge of the regional anatomy and different accesses to a joint cavity are important to avoid introduction of microorganisms from an overlying cellulitis. Fluid should be collected into sterile plain and ethylenediaminetetraacetic acid (EDTA) tubes. Blood culture bottles should be used for aerobic and anaerobic cultures. Ideally, a minimum of 5ml of undiluted synovial fluid aspirate should be cultured with blood culture medium to maximise the chance of isolating the infecting organism(s). In the case of an unsuccessful aspirate or in joints with a small volume (eg. the distal tarsal joints), it may be necessary to first infuse the joint with sterile isotonic saline prior to obtaining an aspirate. Urea concentration in synovial fluid of joints closely mirrors the urea concentration in serum. This can, therefore, be used to accurately calculate the dilutation that occurs with this technique.

A cloudy or turbid appearance of synovial fluid is strongly suggestive of infection. The viscosity of synovial fluid is directly related to the hyaluronan content which decreases with SA. Mucinous precipitate quality (MPQ) or mucin clotting is a semi-quantitative measure of hyaluronan concentration and is poor synovial fluid of joints with SA. Practically, viscosity can be estimated by watching the synovial fluid drop from a syringe or by separating a drop between the examiner's thumb and index finger.

Normal synovial fluid protein is usually less than or equal to 2g/dL. Total protein concentration increases with SA and often rises above 4g/dL, although this varies and may often be lower earlier in the disease process. Normal synovial fluid has fewer than 200 cells per µl (less than 10% of neutrophils). Synovial fluid from infected joints can have a total WBC count (WBC) of more than 50,000cells/µl (predominantly neutrophils). Sequestration of inflammatory cells in intraarticular fibrinocellular conglomerates (pannus) can occur. Microorganisms can be directly identified on cytological smears in approximately 25% of cases.

SYNOVIAL BIOPSY

Biopsy of the synovial membrane can be performed during arthroscopic exploration and lavage of a joint. Although histology does not always allow for a clear distinction between infectious and non-infectious inflammation, co-culturing synovial biopsies and synovial fluid has been reported to increase the chance of obtaining a positive culture result in horses with SA and help with antibiotic choice. This technique is rarely used in equine practice.

DIAGNOSTIC IMAGING

Radiography

Diagnostic imaging is an essential part of the work-up of horses with suspected SA. Plain radiographs should first be obtained in order to evaluate for presence and extent of bone pathology. Bony involvement can include osteitis, osteomyelitis, physitis, osteoarthritis or fractures that communicate with or are related to the affected joint. Contrast radiography such as fistulograms or positive contrast arthrograms (see Figure 1) can be used to confirm communication of a wound with a neighboring joint or can help identifying cartilage damage that is not detected on routine radiographs.

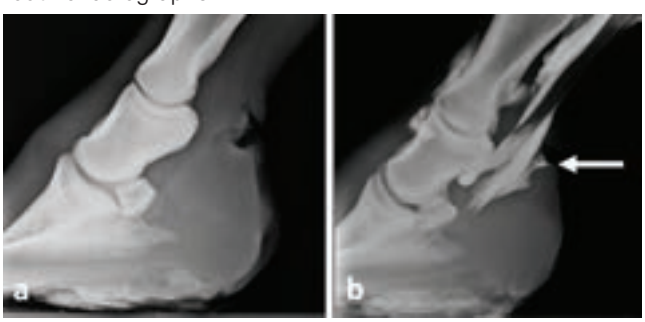

Figure 1: Radiographic study of a horse presented with a fresh laceration in the pastern area: A) plain radiograph; B) positive contrast tenogram. The digital flexor tendon sheath has been distended with iohexol and physiologic saline. The radiographic contrast material can be seen leaking from the synovial structure through the wound (arrow).

Ultrasonography

Ultrasonography is very useful in the diagnosis of SA. The technique can be used for identifying communication between wounds and adjacent joints, to determine the degree of effusion in the affected joint, to assess the nature of the synovial fluid, to subjectively identify synovial inflammation, to evaluate integrity of parts of the articular cartilage and to identify foreign bodies that are not readily identified on plain or contrast radiographs. In addition, ultrasonography can be used for guiding arthrocentesis during collection of synovial fluid for analysis. Ultrasonography can be difficult or impossible with fresh wounds where there is a large amount of subcutaneous air accumulation.

Advanced imaging modalities

Radionucleotide imaging is used extensively in human medicine to differentiate between septic and aseptic osteoarticular disease. Although radionucleotide imaging is not routinely used in equine practice, ^{99m}Tc-labelled leukocytes have been reported to help in the diagnosis of occult infections. Computed tomography (CT) and magnetic resonance imaging (MRI) are becoming increasingly available and affordable in the diagnosis of musculoskeletal disease in horses. Although plain and contrast CT and MRI studies can provide superior detail of bone and soft tissues over radiography, cost and the necessity to anaesthetise the animal for the majority examination of the appendicular skeleton in most referral centers preclude their routine use for the diagnosis of SA. On the other hand, standing-low field MR examination of the distal limb can provide very valuable additional information, particularly with solar penetrations.

CONCLUSION

Synovial fluid analysis remains the gold standard to confirm infection of joints, tendon sheaths and bursa. A rapid diagnosis is important in order to timely initiate the correct, targeted treatment, such as endoscopic lavage. This is particularly important with wounds in the vicinity of synovial structures. Unfortunately, it is not always possible to aseptically obtain a synovial fluid sample for laboratory analysis. Additional techniques, such as infusion and aspiration of sterile physiologic saline, as well as diagnostic imaging modalities, such as positive contrast radiography, ultrasonography and occasionally advanced imaging modalities can be helpful in these situations.

RECOMMENDED READING

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