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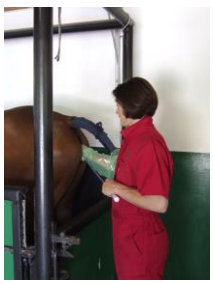
Equine Reproduction

Mini Series

Session One: Mare Reproduction

Madeleine Campbell BVetMed MA (Oxon) MA (Keele) Phd DipECAR DipECAWBM MRCVS
European and RCVS Recognised Specialist in Equine Reproduction



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CPD Solutions: Mare Reproduction.

Dr Madeleine Campbell
BVetMed (Hons) MA(Oxon) MA(Keele) PhD DipECAR Dip ECAWBM (AWSEL) MRCVS

Hobgoblins Stud and Equine Reproduction Centre

LEARNING OBJECTIVES

- How to undertake and interpret a breeding soundness examination
- Timing of breeding using natural cover, and fresh, chilled and frozen semen
- Insemination of fresh, chilled and frozen semen using transcervical, deep intrauterine and hysteroscopic methods
- Post-breeding assessment of mares
- Treatments for breeding – induced endometritis
- Timing and methods of pregnancy diagnosis in the mare

BREEDING SOUNDNESS EXAMINATION

Ideally, a breeding soundness examination would be carried out on each mare at the start of the breeding season to ascertain the state of the mare's reproductive health, in order provide any necessary treatments before expenditure on stud fees and veterinary services commences. In reality, breeding soundness examinations are often confined to times when the mare is changing hands (though note that a Five Stage Vetting does not routinely include assessment of the reproductive system); for mares which remain non-pregnant at the conclusion of the breeding season ('barren mare examinations'), or if a problem becomes evident during the breeding season. Mares which are being assessed for a particular use e.g. as embryo transfer recipients will also often undergo a breeding soundness examination.

The list of diagnostic tools which may be used during a breeding soundness examination is long, including

- General clinical examination.
- Rectal and *per vaginal* palpation.
- Ultrasound.
- Speculum examination.
- Clitoral swabbing.
- Endometrial bacterial/mycology.
- Endometrial cytology.
- Endometrial biopsy.
- Endoscopy.
- Oviductal patency tests.
- Hormonal /chromosomal tests.

Which of those tests are employed is at the clinical discretion of the veterinary surgeon, and dependent upon the reason for examination. Typically, a basic breeding soundness examination would include clinical examination, testing for venereal disease, palpation and ultrasound imaging of the mare's reproductive tract and uterine bacteriology, cytology and possibly biopsy. Other tests may be added according to need.

(i) General physical exam.

? ability to carry foal to term e.g. ventral musculature rupture; previous colic surgery with mid-line incision; pelvic fracture. ? concurrent disease which would affect ability to carry foal to term e.g. laminitis; COPD.

(ii) Testing for venereal disease

Testing should be carried out according to the annually published HBLB Codes of Practice https://codes.hblb.org.uk/

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Bacterial venereal diseases of the mare are the same as those of the stallion i.e. Contagious Equine Metritis Organism, *Klebsiella pneumoniae* capsule types 1,2 and 5 and *Pseudomonas aeroginosa.* For full details of each of these diseases see the current HBLB Codes of Practice.
Where testing for these diseases is required, mares are screened for these diseases according to protocols established in these codes each year. In brief, mares are categorised as either high or low risk mares, and are subsequently tested by a specified regime of clitoral and endometrial swabbing. The sites for clitoral swabbing are the lateral clitoral sinuses; the central clitoral sinus and the clitoral fossa. A paediatric or ENT swab must be used as conventional veterinary swabs are too small to penetrate the clitoral sinuses. Clitoral swabs may be taken at any stage of the cycle after January 1st each year.
Endometrial swabs must be taken in oestrus. Endometrial swabs may be obtained either via a speculum (unguarded technique) or by the use of a 3-part guarded swab, which is not protruded fully from its casings until it is inside the uterus. Use of a guarded swab technique minimises contamination with bacteria from the vulva, vagina and external cervix.
All swabs must be stored in Amies transport medium sent off to an approved laboratory (listed in The Veterinary Record each year).

The main viral disease which is transmitted during coitus and in all types of semen is Equine Viral Arteritis virus. All mares should have a blood test taken after January 1st and within 28 days of them entering a stud/AI Centre to prove that they are not currently infected with EVA and thus do not pose a threat to other mares/stallions. EVA testing should also form part of any breeding-soundness examination. Testing for Equine Infectious Anaemia (EIA) (which can also be venereally transmitted) is also prudent if a mare has been in an area in which EIA occurs, or covered using a stallion or the semen of a stallion from such an area.

(iii) Examination of external genitalia.
Vulva (angle, length); vagina (adhesions; discharge); cervix (deficits; adhesions; cervicitis; normal for stage of cycle?).

(iv) Rectal and vaginal palpation (stage of cycle).
Can you identify one cervix; one uterine body; two uterine horns; and 2 ovaries. Is the size, location and consistency of each structure normal? E.g. are the ovaries of the size which you would expect them to be for the time of year? In winter would expect 2 small ovaries; during vernal transition would expect 2 large ovaries with many follicles on each; during summer cyclicity would expect 2 similar sized ovaries although one may have a dominant follicle on it. Are the ovaries a similar size to each other (if not, why not? ?Granulosa cell tumour). Are the ovaries, uterus and cervix doing what you would expect them to be doing for the stage of the cycle which you believe the mare to be in? E.g. in a mare which is teasing well in July and therefore probably in oestrus would expect a flaccid uterus, an open cervix and two medium-large ovaries. A tight cervix in the presence of all the other indicators would be inconsistent and would suggest cervical “fibrosis”.

(v) Speculum examination of the vagina and cervix.
Can you see any discharges on the floor of the vagina (endometritis; urovagina)? Are there any adhesions of the vagina (persistent hymen; foaling trauma) or cervix (foaling trauma)? Is the colour, position and patency of the cervix as you would expect it to be for the stage of the cycle which you believe the mare to be in? (e.g. oestrus – patent, pink, flaccid and dropping towards the floor; dioestrus - white, closed, sticking straight out).

(vi) Ultrasonography of the uterus and ovaries.
Uterus: assess for stage of cycle. In anoestrus, expect to be small in diameter, no uterine oedema; no free fluid. In oestrus and in the vernal transition, expect to be large in diameter; good uterine oedema (cartwheel pattern); no or minimal free fluid. In dioestrus, expect to be intermediate in diameter; no uterine oedema; no free fluid.

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Ovaries: (i) Assess for stage of cycle. In anoestrus expect 2 small ovaries; no corpora lutea; no follicles > 20 mm (usually smaller). In oestrus, expect 2 medium - large ovaries; at least one follicle of >25mm; no corpora lutea. In vernal transition expect 2 medium – large ovaries; no corpora lutea; many medium-large sized follicles. In dioestrus should have at least one visible CH / CL.
(ii) Assess for abnormalities e.g. anovulatory haemorrhagic follicles (AHF); ovarian tumours; *paraovarian* cysts (NB mares do not have true ovarian cysts).

(vii) Endometrial bacteriology

The purpose of testing for non-venereal uterine pathogens of the uterus is to detect those pathogens which may affect the mare's own fertility (although they pose no risk to the stallion).
Samples for bacteriology and cytology can be obtained using either a uterine swab (see above) or small volume uterine lavage.
The uterus of a normal mare should be free of bacteria during dioestrus and should be free of bacteria or have a very scanty growth of commensual bacteria during oestrus. It is normal to take an endometrial swab during oestrus as the cervix is open, the immune system of the uterus active, and the risk of causing a persistent infection by swabbing therefore reduced. Some authors, however, argue that a dioestrus swab is more meaningful. If an endometrial swab is taken in dioestrus it is advisable to administer a prostaglandin injection following swabbing in order to bring the mare into oestrus and enable her to eliminate any contamination of the uterus which was caused by the swabbing. Endometrial swabs for non-venereal diseases are routinely run for aerobic bacteriology, although for a complete breeding soundness examination anaerobic and fungal culture are also advisable. Bacteria most commonly grown from equine uterus:*Strep zooepidemicus; E coli (haemolytic);Staph aureus; Bacteriodes fragilis; Klebsiella spp; pseudomonas spp.*
Fungi most commonly grown: *Candida spp; Aspergillus spp; Mucor spp*
NB NEVER take an endometrial swab from an mare or administer a prostaglandin injection without first ensuring for yourself that the mare is not pregnant.

(viii) Endometrial cytology.

Because of the potential to pick up contaminants from other parts of the reproductive tract in the course of taking an endometrial swab, endometrial bacteriology should ALWAYS be interpreted in conjunction with endometrial cytology. A cytological specimen is most commonly obtained by the same techniques used for bacteriology, although a uterine 'cytobrush' is also sometimes used. A slide is made from the endometrial swab or centrifuged low volume lavage sediment, and subsequently stained with e.g. Diff-Quik (modified Wright-Giemsa). Polymorphonuclear cells (PMNs) are not present in samples from normal mares. The presence of PMNs is indicative of active inflammation. In conjunction with a positive bacteriology result, this indicates a genuine acute infectious endometritis. A positive bacteriology with a negative cytology suggests that the bacteriology result is attributable to contaminants. Other cells which may be seen on endometrial cytology include lymphocytes (indicative of chronic antigenic stimulus); eosinophils (indicative or pneumovagina or fungal infection) plasma cells (indicative of immunogenic stimulation) and haemosidirin-laden macrophages (indicative of the foal heat).

TIMING OF INSEMINATION

Principles of timing:

The equine oocyte lasts c 6 hours once ovulation has occurred.
Freshly ejaculated semen from a normally-fertile stallion lasts a minimum of 24 hours (and 48-72 hours for many stallions).
Capacitation takes c 6 hours.
Therefore for natural cover and insemination with fresh or chilled semen the ideal is to inseminate the mare within 6 – 24 hours before ovulation so that capacitated semen are waiting in the oviduct to fertilise the oocyte the moment ovulation occurs.

Natural cover in the wild: stallion would probably cover the mare at least once daily so that her reproductive tract had a constant supply of semen throughout the oestrus period and the peri-ovulatory period.

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Management for natural cover/insemination with fresh semen (i.e. collected on-site).
Most studs aim to minimise number of times each mare covered.
If veterinary involvement is minimal and for stallions with few mares/year, studs may simply rely on teasing to detect oestrus and cover the mare every other day until she will no longer stand to be covered. This essentially mimics the situation in the wild, and should ensure that the oviducts have a supply of semen throughout the peri-ovulatory period.
The disadvantages of this system are (i) that it is time consuming and increases the stallion's workload (ii) it relies heavily on teasing, which is not always accurate and (iii) it results in mares being covered many times, which is a problem for mares which are susceptible to endometritis.
The alternative management system is to have a greater degree of veterinary involvement and to use the same system as that described below for chilled semen. Because the semen being inseminated is fresh, it will last longer within the reproductive tract of the and therefore covering / inseminating within 48 hours pre-ovulation is sufficient to achieve good pregnancy rates using most stallions.

Management for chilled semen insemination
Chilled semen: normally only have chance to order semen once per cycle (constraints of delivery; stallions heavily booked).
Greater veterinary involvement advisable.
Aim to inseminating within 6 – 24 hours before ovulation (semen is normally already 24 hours old by the time insemination occurs, if being sent overnight).This is achieved by:

(a) Accurate detection of oestrus and impending ovulation
(Teasing); palpation of uterus, ovaries and cervix; ultrasonography.
Main criteria: mare must be in oestrus with open(ing) cervix; flaccid uterus, good uterine oedema and at least one follicle of at least 35mm diameter.
NB the presence of a large follicle alone is not sufficient to determine that the mare is ready to inseminate/close to ovulation, since mares can have large follicles and indeed can ovulate when they are in dioestrus.

(b) Use of ovulation –inducing agents
Once (a) is achieved, administer an ovulation-inducing agent e.g hCG (Chorulon) or GNRH analogue (Ovuplant; deslorelin injectible; Buserelin).
Inseminate the mare 24 hours after administration of the ovulation-inducing agent and expect her to ovulate within 12 (24) hours after that.

Management for frozen semen inseminations
Frozen semen undergoes capacitation-type changes as a result of the freeze-thawing process, and consequently only lasts 6-12 hours once thawed (stallion variation).
Therefore need to inseminate closer to ovulation.
Increased veterinary involvement and expense necessary.
If 2 or more doses of semen, follow protocol for fresh/chilled semen, scan at 24 hours post-ovulation induction agent. If mare has ovulated, inseminate. If not, re-scan and inseminate at 30 hours after ovulation-induction agent and if has not ovulated at 30 hours post induction scan every 12 hours after that until ovulation occurs. Inseminate with the second insemination dose when ovulation is detected. (NB this protocol assumes that ovulation is likely to occur by c40 hours post induction)
If only one dose of semen, scan at 24 hours post-ovulation agent and then every 6 hours until ovulation is detected, inseminate immediately.

Possible problems with multiple inseminations (endometritis).
Huge variation in longevity of frozen-thawed semen.

INSEMINATION
There are three routinely performed methods of artificial insemination (AI) in the mare. In the UK, the first (only) may be performed by qualified technicians, as well as veterinarians, under an exemption to the Veterinary Surgeons Act. Otherwise in the UK, insemination of mares is considered an act of veterinary surgery and as such restricted to MRCVSs.

For all three methods, the mares must be adequately restrained (ideally in stocks, if not sedated), the tail bandaged, and the perineum thoroughly cleaned prior to insemination. Operators should wear clean rectal gloves and use sterile, non-spermicidal AI gel. Risk of rectal tear.

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(1) Uterine body insemination
Insemination is performed by passing a (usually rigid) artificial insemination catheter into the uterine body via the cervix. The semen is preloaded into a non-spermicidal syringe, and attached to the catheter. The semen is gently expelled into the uterine body by depressing the syringe. It is not usually necessary to use air to flush semen through the catheter as the volumes of (typically fresh or chilled) semen being used with this technique are usually large. The advantage of this system is simplicity, and the fact that it is not necessary to palpate the mare *per rectum.* The disadvantage is that semen is deposited in the uterine body, not the uterine horn, which may reduce pregnancy rates where insemination volumes are very small and do not stimulate physiological uterine contractions (which normally help to move the sperm towards the utero-tubal junction).

(2) Deep uterine horn insemination
A flexible artificial insemination catheter is used. The mare's rectum is emptied before cleaning of the perineum. The catheter is introduced into the uterus via the cervix, and then fed into and to the tip of the uterine horn ipsilateral to the dominant ovarian follicle, using *per rectum* palpation. One of three methods may be used to pass the semen (usually frozen:thawed) down the catheter. Either (i) a couple of millimetres of air is drawn up into a syringe. The syringe is then attached to the flexible catheter. Semen is drawn up into the distal end of the catheter, so that it sits within the catheter but does not usually (depending on volume) enter the syringe. Semen is expelled by gently depressing the syringe, so that all of the semen is deposited at the tip of the uterine horn, with the small volume of air which was in the syringe being used to flush the last of the semen out of the catheter. Or (ii) the same technique is used, but the semen is drawn up into an narrow gauge inner tube (supplied with some flexible catheters) in order to avoid wasting semen in dead space. Or (iii) the straws in which the semen was frozen are thawed, the distal end is cut off, and the straws are fed one by one down the catheter, and the semen is then depressed from each straw, one at a time, using a metal stylet which is supplied with the catheter. One straw is withdrawn from the catheter before the next is used. The advantage of this technique is that it facilitates deposition of the semen close to the utero-tubal junction. The disadvantages of this technique are increased time, increased technical ability, and the fact that it may require assistance from a second person to pass semen straws etc..

(3) Hysteroscopic insemination
This technique was originally developed for use with frozen semen of poor quality, or where only low doses were available. Hysteroscopy is performed using a flexible endoscope, after insufflation of the uterus with air. The utero-tubal junction ipsilateral to the dominant ovarian follicle is visualised. The semen is drawn up into a flexible narrow gauge catheter which is passed down the endoscopic channel. The semen is deposited onto the utero-tubal junction. The advantage of this system is that it allows for accurate deposition of the semen on the utero-tubal junction. The disadvantages are cost, time, and the fact that introducing an endoscope into the uterus tends to cause endometritis in mares which are prone to uterine inflammation.

ASSESSMENT OF THE MARE AFTER BREEDING

Post-breeding endometritis is the most common cause of subfertility in mares. All mares experience a transient endometritis following cover or insemination, which is a normal physiological reaction to the presence of sperm cells and of debris and bacteria from the stallion's penis in the uterus. Normal mares resolve this endometritis through an open cervix via immune defences and uterine contractions within 24 hours post-covering / AI. Mares which fail to resolve the physiological endometritis associated with coitus are known as "susceptible" to post-coital inflammation and infection. In these mares, the uterus is not clear of infection and inflammation by the time the embryo descends into the uterus from the oviduct at day 5-6 post-ovulation, and so the embryo is killed. Typically, these mares are simply not pregnant at the first pregnancy diagnosis scan.

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Pre-disposing factors for non-venereal post-breeding endometritis

(a) Poor vulval conformation resulting in “windsucking” of air and faeces into the vagina.
(b) Poor vestibular seal.
(c) Cervical deficit (poor cervical seal).
(d) Poor uterine contractility.
(e) Abnormal position of the uterus within the pelvis/abdomen (typically sunken into the abdomen in older multi-parous mares).
(f) Failure of the cervix to relax in oestrus (so-called “fibrotic cervix”).
(g) Postulated inadequate immune response to the presence of semen and bacteria within the uterus.

Diagnosis of non-venereal post-breeding endometritis.

For swabbing, culturing and cytological sampling and assessment see above.
The use of ultrasonography to detect free fluid within the uterus is also very common, and is often the mainstay of diagnosis. Many practitioners will treat non-venereal post-breeding endometritis presumptively based on demonstrating the presence of fluid within the uterus and a knowledge of the pathogens which are most commonly responsible, and will use swabs either to confirm their presumptive diagnosis or only if the patient fails to respond to routine treatment.

Treatment of non-venereal post-breeding endometritis.

Treatment aims at

(a) ***Physically clearing the uterus*** of debris and inflammatory exudates (large volume uterine lavage using isotonic solutions (e.g. 1-2 litres lactated Ringer’s solution); ecbolic agents e.g. oxytocin; prostaglandin (NB do not use prostaglandin after ovulation as it may cause luteolysis)). Encourage mares to move around / exercise, particularly immediately after lavage and ecbolic treatment.

(b) ***Resolving infection*** (intra-uterine antibiotics e.g. ceftiofur; penicillin and gentamycin; anti-fungal treatments e.g. enilconazole). **BUT NB** that many cases of post-breeding endometritis are sterile, and use of anti-microbial therapies is not warranted. Routine post-breeding intrauterine antibiotic infusion used to be common but can no longer be justified, in light of concerns about antimicrobial resistance. The risk of using intrauterine treatments is that one introduces infection when doing so, particularly after ovulation, when the cervix is closing and uterus is under the dominance of progesterone. Recent work has shown that intramuscular ceftiofur does reach therapeutic minimum inhibitory concentrations in the uterus for up to 24 hour after treatment. Earlier work in pregnant mares showed that oral trimethoprim potentiated sulphonamides also penetrated the uterus. There may therefore be rationale in using systemic rather than intrauterine antibiotics, particularly if treating after ovulation.

Systemic antibiotics may also be more appropriate than intrauterine antibiotics in mares with uterine biofilms. Recent work suggests that in some mares persistent bacterial endometritis is attributable to the development of a ‘biofilm’ i.e. an extracellular matrix produced by a community of bacteria. The biofilm increases resistance to antimicrobial treatment by physically reducing penetration of the drug, and by reducing the metabolism of bacteria. The presence of a biofilm ultimately allows a subpopulation of bacteria which are resistant to antimicrobial drugs to persist in the uterus.

Treatment with dilute hydrogen peroxide and with chelating agents are reported as being unsuccessful as methods of disrupting biofilms in the horse.
N- Acetylcysteine (NAC) (the use of 30ml of 20% NAC solution added to 100ml of sterile saline (a 3.3% solution)) has been recommended as a method of dissolving mucus / biofilms in the equine endometrium. This solution is infused into the mare’s uterus, and left for 24 hours. After 24 hours, the uterus is lavaged (using sterile 0.9% saline). The NAC and lavage treatments are repeated until the lavage comes back clear of debris.

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(c) ***Immunomodulation of the uterine environment***.
Modulation of the inflammatory response associated with mating using the following agents has been reported:

Dexamethasone injected intravenously at the time of breeding.
Prednisolone PO BID for 4 days, beginning 48 h before breeding.

(NB Take great care using steroids in mares which have concurrent bacterial endometritis as immunosuppression can exacerbate infection. Take great care in using steroids in mares with a history of or likely predisposition to laminitis Warn owners!)

Intrauterine infusion of platelet rich plasma (to downregulate the exacerbated inflammatory response in mares susceptible to post-breeding endometritis).

Immunostimulants such as Cell wall extract of *Mycobacterium phlei* (Settle, Bioniche Animal Health) or a suspension of *Propionibacterium acnes* (Eqstim, Neogen Corp).which help to restore homeostatic local inflammatory mechanisms (mares susceptible to post-breeding endometritis have been reported to express higher levels of pro-inflammatory cytokines and lower levels of interleukin-10 (which inhibits production of pro-inflammatory cytokines) in their uteri compared to non-susceptible mares:

(d) ***Correcting any underlying anatomical abnormality*** which is predisposing to non-venereal endometritis (eg Caslick's vulvoplasty to correct a sloping vulva and consequent "windsucking"; Pouret's operation in more serious cases; correction of urovagina).

PREGNANCY DIAGNOSIS IN THE MARE

Diagnosis of pregnancy can be made by rectal palpation , or by hormonal assay, but is most commonly made using uterine ultrasonography with a 5 or 7.5 mHz linear array probe.

Rectal palpation is impossible to perform reliably before about day 30 of pregnancy; it impossible to detect early twins, and it is impossible to detect unilateral twins even late on.

Hormonal methods of pregnancy diagnosis

Serum progesterone
Day 18-20 days onwards. In pregnant mares should remain above 6.3 nmol/L until around day 200 of gestation. False positives and false negatives.
.
Equine chorionic gonadotrophin (eCG)
eCG is secreted by the endometrial cups. It first appears in maternal blood 37-40 days after ovulation, peaks at day 55-70, then declines steadily and disappears completely from maternal serum between days 100 -140.
False negatives: may occur from samples collected before day 35 and after day 90.
False positives: If embryonic death occurs after day 35. Does not correlate with viability of the foal.
.
Oestrone sulphate
Produced by the equine foetoplacental unit and are released into the maternal circulation.
Oestrone sulphate is only produced by a combination of viable foetus and placenta, therefore confirms that a foetus is alive.
Detected in maternal circulation from day 60, peaks at around day 150, then slowly declines

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Ultrasonographical pregnancy diagnosis

Mares should be appropriately restrained (in stocks, or sedated), to minimise the risk of a rectal tear and to personnel.

The entire uterus is imaged transrectally in a systematic fashion, using a 5 or 7.5 mHz linear array probe.

Embryo first detected as a hypoechoic sphere with a hyperechoic line at the top and botoom ('specular echoes') after 10 days of pregnancy at least (***day 12*** with most ultrasound scanners).

After ***day 16*** the embryo stops moving and becomes fixed at the base of one uterine horn. It is about 1.5-2cm diameter.

The embryo proper can be visualized towards the ventral surface of the vesicle at around ***day 20-21.***

The ***heart-beat*** can be seen from day 21/22, though it is more reliably visualised day 25 onwards.

Between ***days 21 and 40***, the allantoic membrane can be seen ('signet ring appearance')

At around ***35 days*** of pregnancy the embryo proper is approximately half-way up the the vesicle.

Twin detection is easiest at the 15-16 day stage (this is necessary because although an embryo can be detected from day 12 using most ultrasound machines, asynchronous ovulations can occur up to 4 days apart) In most cases the vet may suspect twins if a multiple ovulation was detected.

A minimum of two pregnancy diagnosis scans (14-16 days and 25-30 days) are recommended to reduce the chances of 'missing' a twin conception. Many stud vets routinely perform a third pregnancy diagnosis time around day 42 (when organogenesis is completed), and some stud fee terms require a scan to prove that the mare is pregnant on October 1st.

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