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DOI: 10.1080/17415349.2015.1028770

Equine reproduction Part 1: Artificial insemination

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ABSTRACT: Equine artificial insemination is widely performed in equine and mixed practice using chilled and frozen semen. To facilitate a safe and cost effective procedure, veterinary nurses should have an appropriate level of knowledge of the endocrinology and reproductive anatomy in the mare, as well as the insemination procedure, in order to assist the veterinary surgeon.

Introduction

Equine artificial insemination is becoming more popular amongst clients due to the reduced cost and stress of transporting the mare (and perhaps foal) to stud. It also increases the genetic pool, as there is an increased availability of stallions within or between countries and is safer for the mare as she will not risk injury being covered by the stallion. In addition there is a reduction in the risk of sexually transmitted diseases between mare and stallion.

Reproductive anatomy of the mare

The *vulva* forms the external opening of the reproductive tract and this is the first effective barrier to protect the uterus from ascending infections. The two vulval lips should be full and firm and meet evenly at the midline, as abnormal vulval shape can cause the mare to be prone to sucking air and foreign material into the vagina and uterus, consequently leading to infection and reduced conception rate.

The *clitoris* is contained within the clitoral fossa at the lower end of the vulva. *Taylorella equigenitalis* (CEM), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* can become established here, therefore, if the mare is going to be naturally, covered this area is swabbed and cultured before she goes to stud to prevent the transmission of disease. When the mare is in oestrus the clitoris is repeatedly exposed; this is termed 'winking'.

The *vestibule* extends from the vulva to the *vestibulo-vaginal constriction* just in front of the external urethral opening. The vestibulo-vaginal constriction is an important seal which prevents entry of foreign material into the vagina and this is the secondary line of defence.

The *vagina* is a hollow tubular structure which extends from the vestibulo-vaginal constriction to the external opening of the *cervix*. The *hymen* is a fold of skin at the junction between the vagina and the vestibule, overlying the external urethral opening, continuing on either side of the vagina. This structure is complete in maiden mares and will be broken during insemination.

The *cervix* is a thick-walled tubular structure that acts as the tertiary line of defence. The cervix alters in size, consistency and shape according to hormonal changes during the oestrus cycle. During oestrus the cervix is flaccid and this allows insemination of semen and also drainage of uterine fluid. During pregnancy and dioestrus it is firm and closed due to elevated levels of progesterone.

The *uterus* is a T-shaped, muscular organ made up of a cranial body with left and right uterine horns. It is suspended by the *broad ligament* within the pelvic cavity and abdomen. The uterus is lined by secretory epithelium and possesses a thick muscular layer, this muscular layer is responsible for the changes in uterine tone during oestrus, dioestrus and early pregnancy.

The *uterine tubes*, also known as *Fallopian tubes* or *oviducts*, lie at the end of the uterine horn and extends to the ovaries. They are the site of fertilisation and the early embryo resides here for 5.5 days before entering the uterus.

The *ovaries* are broad-bean-shaped, and mature oocytes are shed from the follicle within the ovary. During spring and summer the ovary contains several follicles of different sizes prior to ovulation.

Oestrus endocrinology

Increasing daylight is transmitted from the retina of the eye to the pineal gland, inhibiting melatonin secretion. This reduction in melatonin stimulates the hypothalamus to produce increased levels of gonadotropin-releasing hormone (GRH), which stimulates the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are released by the anterior pituitary gland. FSH stimulates growth and development of follicles in the ovary and LH is involved in their final maturation. Both FSH and LH stimulate the ovaries to produce oestrogens which cause the mare to become receptive to the stallion.

Signs exhibited by the mare during oestrus include raised tail, urinating frequently and winking. The vulva is long and relaxed, and the cervix becomes pink and moist.

Hormones used to manipulate the oestrus cycle

A number of hormone-based drugs can be used in horses to manipulate their oestrus cycle, in order to induce ovulation.

Synthetic progestogen

Synthetic progestogen (e.g. Regumate Equine, MSD Animal Health) is an oral preparation given in feed to assist in the stimulation of the onset of cyclical ovarian activity. It is given for 10–15 days then stopped; the mare will come into oestrus and ovulate 10 days later.

Human chorionic gonadotropin (HCG)

Human chorionic gonadotropin (e.g. Chorulon, MSD Animal Health) is a complex glycoprotein, which can be administered intravenously or intramuscularly once the mare's follicle is greater than 35 mm in diameter (ascertained by ultrasound) and the mare is showing signs of oestrus. The mare will ovulate within 48 hours of administration.

Gonadotropin-releasing hormone (GRH)

A gonadotropin-releasing hormone such as Ovuplant (Dechra Ltd), an implantation tablet containing 2.1 mg deslorelin, can be administered subcutaneously in the lateral neck region midway between the head and shoulder. It induces ovulation within 48 hours by increasing the concentration of endogenous luteinising hormone (LH) during oestrus in normally cycling mares with a follicle greater than 30 mm in diameter.

Prostaglandins

Prostaglandins can be used to shorten the normal luteal phase, inducing oestrus 3–6 days following administration, however this will not work in the first 4–5 days post ovulation.

Preparation of the mare prior to insemination

The mare's passport and microchip should be checked to ensure that they match those in the paperwork supplied with the semen. The mare should be put in a well-lit environment inside stocks and restrained adequately to reduce the chances of injury. The mare's tail is bandaged and tied out of the perineal region. The veterinary surgeon (VS) should empty the mare's rectum of faeces to prevent contamination, her vulva and perineal area should then be cleaned using warm water and cotton wool and then dry paper towel as water is spermicidal.

Procedure using fresh or chilled semen (Box 1)

Insemination using chilled semen can be performed by the VS or AI technicians. i.e. individuals that have attended a recognised course of training in the EU and have been certified as proficient by the course provider. They are allowed to inseminate mares by the simple trans-cervical route under the Veterinary Surgery (Artificial Insemination of Mares) Order 2004 (as amended 2010).

Box 1. Equipment required for artificial insemination

- Stocks
- Ultrasound scanner
- AI insemination catheters
- Non-spermicidal lubricant
- Disposable shoulder-length gloves
- Tail bandage
- Clean bucket with warm water
- Cotton wool
- Disposable (paper) towelling
- Scissors
- Haemostats
- Water bath
- Stopwatch
- Microscope
- Warm stage (37°C)
- Slides
- Cover slips

The mare should be inseminated 0–24 hours prior to ovulation. To be sure of the timing, the VS will perform daily palpations and ultrasound examinations per rectum, looking for a large, soft follicle around 45 mm in diameter **(Figure 1)** and a marked endometrial oedema pattern. Chilled semen is sent to the establishment via courier on the morning prior to ovulation following the veterinary surgeon's request, usually around 36–48 hours prior to ovulation.

The inseminator should use a sterile obstetric glove and a small amount of sterile, non-spermicidal lubricant (such as KY Jelly) should be placed on the top of the hand around the knuckles to facilitate the procedure. The semen is infused from a two-part syringe **(Figure 2)** with plastic plunger, as rubber-headed plungers may be spermicidal, into a sterile catheter introduced via the cervix to a point halfway along the uterine body.

Once the semen has been placed in the uterus, the sperm cells travel up one of the horns until they reach the oviductal

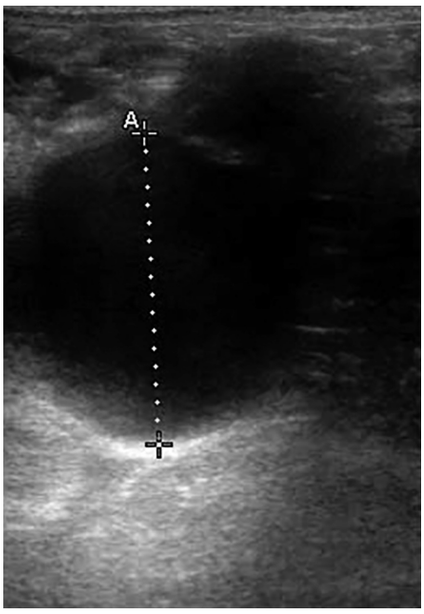

Figure 1. An equine follicle as seen on ultrasound; the diameter is indicated by the dotted line

Figure 2. The two-part insemination syringe

papilla, or utero-tubal junction. This is the entrance to the oviduct or uterine tube and is located at the tip of the uterine horn.

Once sperm cells have passed through this junction and are in the oviduct, they can fertilise the oocyte. Following insemination, a small amount of semen should be warmed to 37°C and examined for progressive motility and gross abnormalities.

Fresh semen

Fresh semen insemination uses the same technique as above, however the semen is collected directly from the stallion, and inseminated straight into the mare without the use of extenders, (liquid diluent that is added to the semen as a preservative). The seminal fluid that contains the semen keeps the sperm alive throughout the procedure. The major drawback to the use of fresh semen is that the stallion and mare must be on the same stud at the same time.

Frozen semen insemination technique

Equine semen is frozen in 0.5ml French plastic straws like those used for cattle, and between 1 and 8 straws are required per insemination or one dose may arrive in a 5ml straw. Each equine dose contains a minimum of 800 million sperm cells.

Semen usually arrives at the hospital or yard of destination in a vapour shipper **(Figure 3).** The typical shipper maintains a static holding temperature of approximately -196°C for 5–7 days. If all of the semen in the tank is not used before the end of the holding period, it must be transferred to a long-term storage tank **(Figure 4).** This is done using long haemostats, or similar forceps, which are pre-cooled by lowering them for a few seconds below the frost line visible at the neck of the storage tank; this prevents warm forceps from touching frozen straws **(Figure 5)**. The straws of semen should be transferred from the shipper into the storage tank in under 5–8 seconds to prevent crystal formation, which could potentially cause damage to the frozen semen.

Figure 3. A typical frozen-semen vapour shipper. Picture courtesy of Equine Reproductive Supplies Ltd

Figure 4. A typical long-term, frozen-semen storage tank

The paperwork received with the vapour shipper should be checked and recorded in the semen log book, recording the location (goblet colour), number of straws/dose, colour of straws, size of straws, stallion's name, mare's name and thawing instructions. If the semen is imported, it should be accompanied by a valid original health certificate and import licence.

The insemination dose then needs to be transferred from the storage flask using long haemostats into a heat bath for thawing. Macrotubes (5.0 ml) should be thawed at 50°C for 40 seconds and 0.5 ml straws should be thawed at 37°C for 30 seconds. The straws are then removed from the water bath and dried thoroughly, as it is necessary to limit semen exposure to water, which may be spermicidal.

Whilst the straws are being thawed, the vet should be placing the deep intrauterine catheter ready for insemination. The crimped or powder end of the straws should then be cut and placed into the insemination catheter with the cut end of the straw facing into the catheter. Using the deep intrauterine metal stylet, the straws are pushed down inside the catheter and the semen is ejected from the tube into the uterus. The empty straws are removed and disposed of and the procedure is

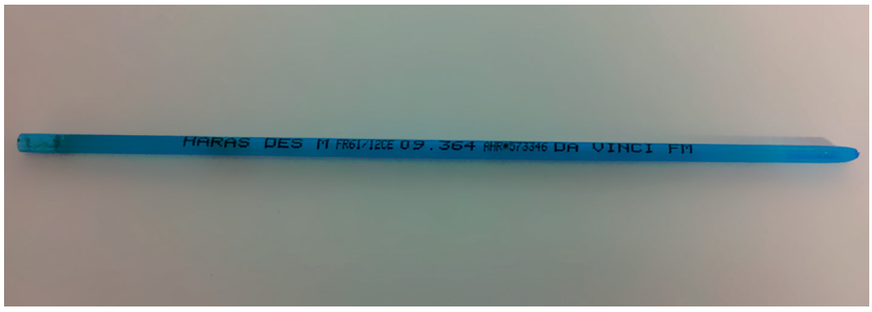

Figure 5. A frozen semen straw

repeated until all the straws are used. A small amount of semen is kept for analysis of its progressive motility. The empty straws should be stored under the mare's, owner's and stallion's name and insemination date in case the foal's DNA needs testing against the semen used to inseminate the mare.

The use of frozen semen has many advantages in preference to chilled semen. These include the increased availability of competition stallion's semen both nationally and internationally. A considerable stock of semen can be kept indefinitely, which allows access to semen from deceased stallions, however it should be noted that frozen semen is not suitable for all mares, especially those with known susceptibility to post-insemination endometritis.

Acknowledgments

Equine Reproductive Supplies Ltd for the use of their equipment images.

Further reading

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