



SOP 6.03 Collecting semen from the stallion using artificial vagina (AV)

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Purpose

- (1) The objective of this standard operating procedure is to provide guidance to the Charles Sturt University staff on:
 - a. collection of semen from a stallion
- (2) Semen is most commonly collected from the stallion by using an artificial vagina, having the stallion either mount a mare in heat, or a dummy. However, semen has also been collected from individual stallions without them having to mount any object. In these situations, the stallion has been trained to gain and maintain an erection so that the artificial vagina can be placed over the penis and semen collected. Semen has also been collected with the aid of drugs such as Imipramine. If the stallion is anaesthetised, semen can be collected with an electro ejaculator similar to that used in a bull. This technique may be useful if a catastrophe has occurred, and semen needs to be collected from an injured animal prior to euthanasia or risky surgical procedures. Collecting semen from the stallion requires a minimum of three people if a mare is used. Two people are generally required if the stallion is trained to mount a dummy.

Scope

- (3) This procedure applies to any person who is involved in AEC approved projects involving collection of semen from a stallion using artificial vagina (AV).
- (4) All researchers and teaching staff using animals for scientific purposes must be competent. For definition of competency refer to Charles Sturt University's Policy on 'Animal Care Competency Training and Assessment'

Details of procedure

Equipment:

- (5) Equipment that should be available at the collection area includes, the loaded artificial vagina, palpation sleeves, tail wrap, tubing for filling the artificial vagina, KY gel, plastic collection bottle, and filter for semen collection.

Procedure

- (6) Ensure the AV has been correctly prepared. Obtain a mare that has teased in heat, apply a tail wrap to prevent tail hair from interfering with the collection and use suitable restraint to ensure the mare remains still throughout the procedure. Restraint may include one or a combination of sedation, nose twitching, sidelines or breeding hobbles. Ear twitching is NOT permitted under any circumstances during teaching or research activities.



SOP 6.03 Collecting semen from the stallion using artificial vagina (AV)

- (7) All personnel involved in the semen collection process (mare handler, stallion handler and the semen collector) must wear protective shoes, helmet and protective vest.
- (8) Have an assistant hold the mare firmly. Ensure the ground surface is not slippery or unstable. Have the stallion handler quietly approach with the stallion, halting approximately 5 meters from the mare. Allow the stallion to gain a full erection before proceeding further. If grossly contaminated, gently wipe the erect penis with warm water and cotton wool. *Use of detergents, disinfectants and soaps are contraindicated due to spermicidal qualities and the risk of destroying commensal organisms, possible allowing pathogens to multiply.* Remove the 'bean' which is a build-up of smegma commonly found in the urethral diverticulum. If a pre-ejaculatory swab is required, now is the time to insert a sterile swab approximately 5 cm into the urethra. Ensure the AV is lubricated and get in position of the near side of the mare at the level of the flank. Hold the AV in the left hand. Have the stallion handler approach from behind the mare, ensuring the stallion approaches quietly rather than rushing at the mare. Allow the stallion handler to walk past you, then as the stallion mounts, place the right hand on top of the penis and deflect it laterally into the AV. Drive the AV up along the shaft of the penis. Bring your right hand around underneath the artificial vagina and apply pressure by squeezing in the sides of the opening at the bottom between your little finger and base of the thumb. Place your index finger on the under surface of the penis to feel for pulsations as the stallion ejaculates. Flagging of the tail is an indication of ejaculation. When the stallion dismounts keep the artificial vagina in a vertical position to allow any semen to get out of the hot part of the AV and into the collection bottle as quickly as possible. This is facilitated by undoing the valve cap to release water and reduce the pressure within the AV. As soon as possible after the collection, remove the filtering device from the semen collection bottle so that the gel is quickly separated from the semen. *Note that exposure of semen to the gel portion for too long a duration can reduce sperm viability.* Once the gel portion has been removed:
 - a. place the bottle containing semen in an incubator maintained at 37 degrees Celsius for further analysis/processing.
 - b. For fresh semen insemination, quickly assess motility and extend the semen on a one: one basis.
 - c. If the semen is to be chilled extend the semen by at least one part semen to three parts extender

Drugs, chemicals, or biological agents

- (9) Usually none, pain relief is not required for this procedure.
- (10) Optional (under the direction of a veterinarian)
 - a. Mild sedation for jump/mount mare if required (Acepromazine, Xylazine)
 - b. Stallions with poor libido - hCG (Human Chorionic Gonadotrophin) or GnRH (Gonadotrophin Releasing Hormone) may be used 30 minutes before collection
 - c. Xylazine, Imipramine, Diazepam if required (for chemical ejaculation)

Impact of procedure on wellbeing of animals

- (11) This procedure causes minimal, or no impact on animal well-being.



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- (12) Nose twitches can lead to unwanted behaviour. Misuse of twitches can lead to physical damage, head shyness, and negative behavioural outcomes both in the short and long term.

Animal care

- (13) Both stallion and the jump/mount mare should be observed for signs of discomfort for up to 30 minutes after the procedure.

Reuse and repeated use

- (14) In a teaching/clinical/ research context, the semen collection may be repeated up to 2 times at approximately 1 hour interval.

Qualifications, experience or training necessary to perform this procedure

Demonstrator

- (15) Theriogenologist / Veterinarian OR it can be carried out by a Theriogenology technician / Equine Science graduate having experience with this procedure under the direction of a veterinarian. Thorough knowledge of the physiology, endocrinology and anatomy involved.

Students

- (16) Veterinary Science; Equine Science.
(17) Prior experience with handling horses and background knowledge of anatomy, physiology and endocrinology is desirable

Record requirements

- (18) Routine monitoring sheet recording. In addition, details of all equine use including procedures should be reported to the technical staff for documenting on Ardex software.

Associated documentation (including pictures if available)

- (19) None required

Glossary

- (20) None required

References and relevant links

- (21) None required