



## SOP 6.08 Embryo collection in horses

**Version and Date of Issue:** Ver 2, April 2024  
**Written by:** Jennifer Clulow  
**Review due date:** March 2027

### Purpose

- (1) The objective of this standard operating procedure is to provide guidance to the Charles Sturt University staff on:
  - a. The procedure for the flushing of an embryo from a donor mare.

### Scope

- (2) This procedure applies to any person who is involved in AEC approved projects involving embryo collection from the mare.
- (3) 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

- (4) Closed-in boots and protective clothing must be worn when carrying out this procedure. Prior to starting, the donor mare is restrained in a crush to reduce movement and to minimise the chance of injury to the mare or the inseminator/collector. The donor mare may require sedation or smooth muscle relaxants to prevent straining. These drugs are to be administered at the attending veterinarian's discretion.
- (5) Wrap the mare's tail and tie it out of the way with a quick release knot
- (6) Clean her anus, perineum and surrounding areas thoroughly using the clean hand, dirty hand technique
- (7) Thoroughly dry the area when cleaning is finished
- (8) The flushing catheter (Foley catheter) comes sterile and wrapped. It consists of 2 main parts, the catheter that enters the mare and the Y-tubing that attaches to the bag containing the flush fluid.
- (9) Before connection, the flow clamps are to be closed to ensure no fluid is lost prior to the flushing.
- (10) Both parts of the catheter can then be attached, and the Y-tubing can be attached to the flushing fluid bag (specific embryo flushing solution commercially sourced). Flushing fluid is allowed to run into the tubing to prevent the influx of air into the uterus.
- (11) The operator puts on a fresh, clean examination glove. A sterile surgical glove may be placed over the top depending on the operator's preference.
- (12) The tip of the sterile Foley catheter with 80 mL balloon is cupped in the operator's sterile hand. At this stage the balloon is deflated.
- (13) Clean, non-spermicidal lubricant is added to the back of the operator's hand and forearm.
- (14) With the non-sterile hand, the operator parts the lips of the vulva (assistance from another person may be required) and gently enters the sterile hand into the vestibule, rotating back and forth to spread lubricant around the circumference of the vestibule and the vagina.



## **SOP 6.08 Embryo collection in horses**

- (15) Once in the anterior vagina, the operator's index finger is introduced through the cervix and is used as a guide to pass the tip of the Foley catheter into the uterus. Care is taken to ensure the balloon of the catheter is anterior to the internal ostium of the cervix.
- (16) The index finger is removed from the cervix and the operator gently holds the external ostium of the cervix around the catheter.
- (17) An assistant fills the balloon with air using a syringe until the operator indicates a secure fit. Up to 80 mL of air or fluid may be introduced into the balloon during this step.
- (18) Once the operator confirms that the outlet clamp is closed, the inlet clamp is then opened resulting in 1 to 2 L of flush fluid passing into the uterus with gravity.
- (19) Once all of the fluid is within the uterus, the inlet tube is clamped, and the outlet tube clamp is opened to allow the fluid to flow out through the embryo filter (commercial embryo filters are available). It is important that approximately 1 to 2 cm of fluid remains within the filter to avoid desiccation of the embryo.
- (20) The efflux fluid may be re-collected into the original fluid-bag for use in subsequent flushes.
- (21) The uterus is flushed several times. Approximately 5 minutes prior to the final flush, 10-20 units of oxytocin is injected intravenously or intramuscularly to assist with uterine contractions (ecbolic effect). The operator's hand may also be withdrawn from the vagina and inserted rectally to assist with manual massaging the uterus during the flushing procedure.
- (22) At completion of the final flush an assistant withdraws all air or fluid from the balloon of the catheter via a syringe. The catheter is withdrawn from the joiner and held upwards so that all fluid flows out of the catheter and tubing and through the embryo collection filter.
- (23) The donor mare is injected with a luteolytic dose of prostaglandin F2 alpha, the tail wrap is removed, and the mare lead from the crush and returned to her accommodation.
- (24) The embryo filter and associated fluid is taken to the laboratory to allow searching for the embryo.

## **Drugs, chemicals, or biological agents**

- (25) Acepromazine, Xylazine, Propantheline bromide, Hyoscine ('Buscopan'), Oxytocin and Prostaglandin F2 $\alpha$ .

## **Impact of procedure on wellbeing of animals.**

- (26) This procedure causes minimal, or nil impact on animal well-being.

## **Animal care**

- (27) Mares should be observed for signs of discomfort for up to 30 minutes following the last procedure. Mare can be returned to routine management.

## **Pain relief**

- (28) Not required.



## SOP 6.08 Embryo collection in horses

### Reuse and repeated use

- (29) In a teaching/research context, the procedure may be repeated up to 2 times at approximately 15- minute intervals.

### Qualifications, experience or training necessary to perform this procedure

- (30) Demonstrator: Veterinarian experienced in uterine lavage of the mare. Thorough knowledge of the physiology, endocrinology and anatomy involved.
- (31) Students: Veterinary Science. Prior experience with handling horses and background knowledge of anatomy, physiology and endocrinology is desirable. **Students must work under supervision at all times.**

### Record requirements

- (32) Completion of AEC approved daily monitoring forms.
- (33) **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)

- (34) Nil

### Glossary

- (35) Nil

### References and relevant links

- (36) Nil