ABOUT EMBRYO TRANSFER

To achieve the best possible outcome in an ET program, animal management, animal synchronisation and the physical retrieving and transferring of the embryo must be well managed. Donor nutrition and health should be closely monitored and be on a rising plain during program preparation to ensure the best possible environment for superovulation to occur. Treatment and Artificial Insemination (AI) procedures must be accurate and performed to schedule to ensure best possible scenario in recovery of fertilised embryos. Recipients should also be on a rising plain of health and nutrition and should be selected on the basis of fertility, calving ability and mothering ability.

Embryo collection and transfer, due the scientific nature of the stages, can be difficult for many to comprehend without seeing it in practice. To begin the program, donors are superovulated using a hormone treatment programme, conducted by the client or breeding centre manager. Programmes are tailor made for individual donors and the dose and type of hormone used will depend on the age, breed and previous flushing history of the donor.

After completion of the hormone injection regime donors are inseminated twice, 12 and 24 hours from the onset of standing heat. Seven days later, embryos are 'flushed'. This is where the embryo technician visits the farm or breeding centre, with a goal of recovering the ova when they are most robust at 7 days of age.

Flushing

For embryo collection the animal is loaded into a cattle crush. The rectum is evacuated of faeces and then the vulva and perineal region are cleaned carefully.

The animal is flushed from both uterine horns using a sterile latex catheter. Flushing is performed by passing the catheter through the cervix and into one uterine horn. A cuff or balloon is inflated to keep the catheter in place and to isolate the tip of the uterine horn. Sterile flushing media is then gravity fed into the uterus until slight distension of the uterus is felt. The fluid is then drained from the uterus into a sterile embryo filter. The draining is

aided by a siphoning effect of the column of fluid in the exit junction of the catheter. This filling and emptying process is repeated several times. The fluid is then drained from the catheter, the cuff is deflated and the catheter repositioned into the opposite uterine horn. The process is then repeated. When complete the catheter is removed and the animal is released from the crush. The filter is removed and labelled. If several donors are to be flushed on one day, they are flushed in the order that they came into oestrous.

Once the flush has been completed the filter is transferred to the laboratory. A new filter is used for each flush.

In the Lab

The embryo filter is opened in the laboratory so the screen can be rinsed. The bottom part becomes the petri dish. Individual filters are then searched systematically for ova. Ova sink to the bottom of the dish and are retrieved by the assistant embryologist with a sterile micro-pipette with the aid of a microscope. Ova are placed into one well of a four well petri dish. Once all ova have been recovered they are separated into viable and non-viable ova. All embryos must be examined to ensure they have an intact 'zona' and are free of adherent mucus or other debris. Viable embryos are moved to a well containing fresh holding media. They are examined here for suitability for processing for freezing or transfer. Only embryos from an individual donor are handled at any one time.

Freezing Embryos

Embryos are frozen in either 1.5M Ethylene Glycol or 10% Glycerol. If they are frozen in Ethylene Glycol [EG] they are always frozen as one embryo per straw and straws are always transparent yellow. These embryos can be thawed at the crush side directly from the liquid nitrogen into 25 degree water ready to be loaded into the transfer gun, known as 'direct transfer'. Contrastingly embryos frozen in Glycerol are loaded into clear straws, and can contain more than one embryo per straw as embryos must be thawed under the microscope before being implanted. This method is not as common as the direct transfer method.

The embryos are frozen as soon as possible after processing and are loaded into the sterile straws which are sealed using a heat sealer. An individual label with embryo details is applied to the appropriate straw. The sealed straws containing the embryos are placed into the embryo freezer and held at -6°C until all embryos have been loaded. Each straw is seeded with a cotton bud four minutes after being placed into the embryo freezer to begin the formation of ice crystals

from the top of the straw. Once all embryos are loaded into the freezer the machine is set to run. Embryos are cooled at 0.5˚C per minute until they reach -32˚C. They are then plunged into liquid nitrogen at -196˚C.

At the completion of freezing, embryos are stored in the sterile liquid nitrogen container filled with clean nitrogen.

Transfer to Recipient Cows

To ensure recipients are at their most appropriate time for holding a transferred embryo, recipients should cycle within +/- 24 hours of the donor. To ensure greatest pregnancy rate this must be monitored to ensure recipients that cycle outside of this window are not transferred into. The transferring must be performed by practiced and competent technician with the least amount of internal manipulation to prevent local trauma in the uterus affecting the ability of the embryo to survive.

For transfer, the 0.25 ml straw containing the embryo is thawed and placed into a transfer gun, and a sterile sheathe is locked onto the end of the straw. The covered gun is then covered with a special plastic sleeve.

During transfer the sheath covered gun is passed via the vagina to the cervix, where the gun is forced to pierce the chemise and then guided through the cervix. This allows the gun to be passed into the uterine horn with minimal contamination. the embryo is then placed as far up the uterine horn on the side of the corpus luteum (CL) with as little manipulation as possible. Any local trauma during transfer will affect the ability of the embryo to survive.