PREPARATION OF IN-VITRO DERIVED BOVINE EMBRYOS FOR TRANSFER INTO RECIPIENTS

Selection of Recipients

Recipient cows should be on day 7 of the estrous cycle (d 0 = estrus). Cows can be selected based on estrus detection (with or without synchronization) or after synchronization of ovulation without estrus detection. There are many ways to achieve estrous synchronization. See Estrous Synchronization - A Reproductive Management Tool from Select Sires, Estrous Synchronization of Cattle from *Brangus World* and Synchronization Programs from ABS among other sources.

One way to synchronize recipients is by giving one injection of GnRH (100 μg, i.m.) followed seven days later by an injection of $PGF_{2\alpha}$ (25 mg/ml) followed by estrus detection. Cows seen in estrus (most within 48-96 h after PGF) are scheduled to receive an embryo on day 7 of the estrous cycle. Transfer without estrous detection can also be performed by using the OvSynch program typically used for timed artificial insemination. This timed embryo transfer (TET) procedure, which is still under development, may be useful when estrus detection is difficult such as during heat stress (see Ambrose et al., 1999 and Al-Katanani et al., 2002). Cows used for TET receive an intramuscular injections of 100 μg GnRH (Cystorelin) on day 0 followed by 25 mg $PGF_{2\alpha}$ on day 7 and 100 μg GnRH on day 9. Embryos are transferred 8 days after last GnRH injection (note: the optimal time for transfer has not been determined experimentally).

Harvesting and Transport of Embryos

1. Embryos produced by in vitro techniques (see Procedures for In Vitro Production of Bovine Embryos by Rivera et al) are harvested on day 7 or 8 after fertilization (day 0=day of insemination). For this purpose, excellent and good quality blastocysts (as described in the Manual of the International Embryo Transfer Society) are identified using a dissecting microscope and transferred into a sterile tube containing embryo transfer medium (we use HEPES-TALP using HEPES-TL from Biowhittaker but other media probably work well also). *Note: Many types of tubes will work for transport – we frequently use a microcentrifuge tube with 1 ml of medium and as many embryos as available.*
2. Tubes are sealed by placing a strip of Parafilm around the cap and loaded in a device to keep embryos warm during transport. *Note: Use a portable incubator from Minitub (catalog # 19180/0000) (inexpensive but less reliable) or Cryologics (INC-RB1) (more expensive but excellent) that can maintain temperature at 39.0°C. Both operate on batteries. The Minitub incubator an operate from a car cigarette lighter.*

Preparation of Materials for Transfer

The following is prepared on the day of transfer and transported to the embryo transfer site:

a. Slide warmer set at 39°C (if not available use a Styrofoam rack or other piece of Styrofoam as a platform for Petri dishes containing embryos to keep embryos from cold surfaces)
b. Bench paper (not necessary but helpful at creating a clean area to work on)
c. 70% Ethanol (wipe all areas with ethanol before starting any procedure)
d. Petri-dish to place embryos prior to pick up into transfer straw (Intergrid plate works very well because of the demarcations on the plastic)

e. Round Petri dish to transfer embryos from the tube were the embryos were transported (60 x 15 Petri dish works very well due to its small size)
f. 1 ml tuberculin syringes
g. Instrument to handle embryos – many are available as described in the Guide on Use of Instruments for Picking Up Oocytes and Embryos
h. Water bath
i. 200 μl pipet tips (yellow)
j. 0.25 ml French straw
k. Pipettor set at 70 μl
l. Embryo transfer rods (we use IMV 21" deep chamber rods from Agtech cat# F17)
m. Sheaths for embryo transfer rod (these have sideways openings to reduce contamination of the uterus – from Agtech; catalog number F18A)
n. Oversleeve Sanitary Chemise – a plastic sleeve used to cover the sheath and transfer rod manufactured by IMV (available from Agtech; catalog #F27A)
o. Additional sterile wrap (Dualpeel tubing from VWR works well for this step) for embryo transfer rods
p. Dissecting microscope
q. Plastic Pasteur pipets
r. Transfer medium (HEPES-TALP - ~ 15-30 ml)

Setup of Work Area at Transfer Site

a) Prepare a clean area at the farm were the preparation of straws will take place. A temperature controlled room works best but any draft-free area can be sufficient.
b) Wipe all surfaces with 70% ethanol.
c) Place bench paper (if available) on area where the embryo manipulations will take place.
d) Set slide warmer at 39°C (or set up Styrofoam rplatform)
e) Place round and Intergrid Petri-dishes on slide warmer
f) Set water-bath set at 38.5°C - *Alternatively, one could use the portable incubator to the tube containing transfer medium warm. If a portable incubator or a water-bath is not available, another alternative is to place the drops of medium into an Intergrid plate that is on top of the slide warmer. Place the lid on the plate to prevent evaporation. In about 5-10 minutes, the 70 μl drops of medium should be warmed up and ready for use.*
g) Transfer embryos from the tube in which they were transported into a round Petri dish with a plastic Pasteur pipet.

Loading Embryos into Straws (see Figure 1)

a) Locate, count and evaluate embryos for quality.
b) Insert 200 μl yellow tip onto the 1 ml syringe as shown (see Figure 1).
c) On a Petri-dish (i.e. Intergrid plate) place 3 microdrops of 70 μl holding medium side by side (**A**).
d) Place one blastocyst into the middle drop (**B**).
e) Insert the cotton plug side of a 0.25 ml French straw into the wide end of the pipet tip (**C-D**).
f) Aspirate one empty drop (**E**).
g) Aspirate air to create a ~0.5 cm column (**E**)
h) Aspirate the microdrop containing embryo (make sure that the embryo is aspirated by observing under the microscope; **F**)
i) Aspirate air to create a ~0.5 cm column (**F**)
j) Aspirate the remaining empty drop until the cotton plug is wet (**G**).
k) Remove transfer straw from syringe and place on slide warmer until use (**H**).

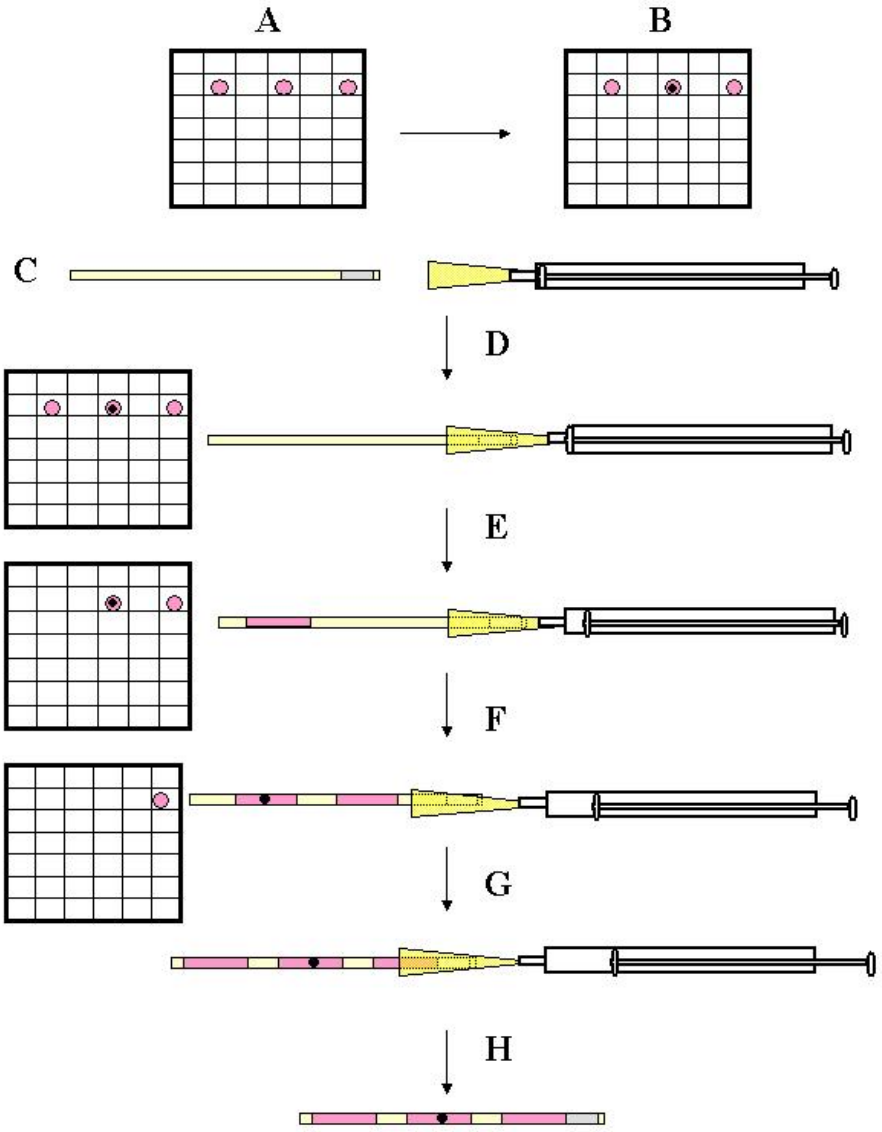

Figure 1. Loading embryos into straws.

Loading Straws into Transfer Pipettes (see Figure 2)

a) Open bag containing sterile transfer rod (**A**) *(use rod designed for 0.25 cc straws)*
b) Pull the plunger from the back of the transfer rod (**B**)
c) Insert the embryo containing French straw into the transfer rod cotton side first (do this by holding the straw *from the side of the cotton plug to minimize contamination of the straw). If resistance is encountered stop immediately and check if the plunger if pulled back* (**C**).
d) Move blue ring towards the center of the gun. Place a disposable blue sheath over the transfer gun without touching the front end. Push the sheath all the way to

the back of the rod (you may need to apply some pressure). Move blue ring to the back of the rod to fasten the sheath in place (**D**).

e) Place a chemise over the gun without touching the front end (**E**).
f) Place loaded rod into a protective bag (**F**).
g) Transport to the site of transfer in a horizontal position.
h) Remove rod from protective bag and keep plastic bag clean to reuse for next transfer
i) After completion of transfer, bring transfer rod back to the preparation area and wipe with ethanol. Do this by performing a single movement from the tip of the rod (cleanest area) to the back of the rod (dirtiest area).
j) Wait until ethanol has evaporated completely.

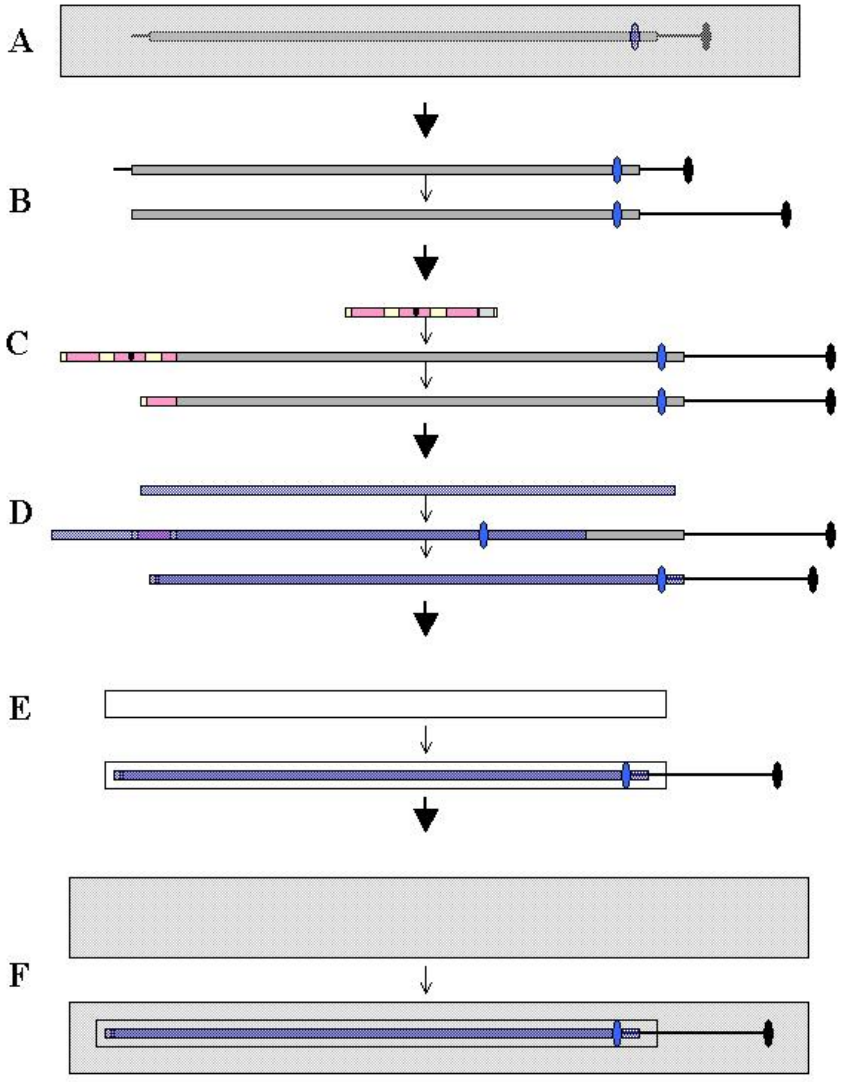

Figure 2. Loading straws into embryo transfer pipettes

Preparation of Cows for Transfer

a) Restrain the cow
b) Palpate recipient cows for the presence of the corpus luteum (CL) – if no CL present, do not use the recipient.
c) With chalk mark the side were the CL is present
d) Shave hair on the tailhead
e) Prepare tailhead for epidural by cleansing with betadine scrub followed by alcohol (70% ethanol)
f) Give an epidural by injecting 5 ml of lidocaine into the epidural space. *When the tail is relaxed, the recipient is ready for the embryo to be transferred.*

Embryo Transfer Procedure

In general, transfer is performed as for AI (see Artificial Insemination Technique *by Michael O'Connor. Special modifications and concerns are listed below*

a) Care must be taken to avoid contamination with feces. Clean the vulva thoroughly before inserting the transfer pipette. In addition, the vulvar lips should be opened before insertion of the pipette. This can be accomplished by the technician (by pushing the arm in the rectum downwards and back slightly) or by an assistant (by grabbing the vulvar lips and pulling backward).

b) The tip of the transfer pipette is placed approximately one-third of the way up the uterine horn ipsilateral to the corpus luteum. The embryo is then gently expelled from the pipette.