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EMBRYO TRANSFER

Introduction

Embryo transfer is a process of removing one or more embryos from the reproductive tract of a donor female and transferring them to one or more recipient females.

Embryos also can be produced in the laboratory via many techniques, such as *in-vitro* fertilization (IVF) or somatic cell cloning. But the actual transfer of an embryo is a step in a series of processes that includes some or all of the following:

- Estrous synchronization of both donor and recipient cows.
- Superovulation of donors.
- Insemination (fertilization).
- Collection of embryos.
- Isolation, evaluation, and short-term storage of embryos.
- Micromanipulation, and genetic testing of embryos.
- Freezing of embryos.
- Embryo transfer.

Steps for Embryo Transfer in Cattle

Virtually all commercial embryo transfers use nonsurgical recovery of the embryos rather than surgical techniques. The process involves several steps and considerable time as well as a variable expense.

A. Selection of the Donor Female

The first step is selecting a donor cow. It has been suggested that prospective donor cows in embryo transfer programs be selected on the following **criteria**:

1. The cow should have a normal reproductive tract and postpartum history, especially about estrous lengths of 18 to 24 days.
2. Both beef and dairy cows should be at least 60 days postpartum before the transfer procedure begins.
3. No parturition difficulties or reproductive irregularities
4. Both the very obese cow and the thin cow will have reduced fertility, so the donor must be in an appropriate body condition score at the time of embryo transfer.

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B. Selection and Preparation of Recipient Females

Proper recipient herd management is critical to embryo transfer success. Reproductively sound cows, that

1. show calving ease
2. have good milking and mothering ability are recipient prospects.
3. They must be on a proper plane of nutrition.
4. synchronization of the estrous cycles between the donor and the recipients, optimally within one day of each other. The critical point regarding recipient cow estrous synchronization is the timing must match the time of insemination of the donor cow so that the donor and the recipients have a similar uterine environment seven days later when the transfer takes place.

C. Superovulation of the Donor Cow

Superovulation of the donor cow is the next step in the embryo transfer process. Superovulation is the release of multiple eggs at a single estrus. Cows or heifers properly treated can release as many as ten or more viable eggs at one estrus. Approximately 85 percent of all normal fertile donors will respond to superovulation treatment with an average of five transferable embryos .

Some cows that are repeatedly super ovulated at 60-day intervals may produce a smaller number of oocytes over time. The basic principle of super- ovulation is to stimulate extensive follicular development with follicle-stimulating hormone (FSH).

D. Insemination

Many embryo transfer technicians will choose to inseminate the cow several times during and after estrus. One system is to inseminate the super ovulated cow at 12, 24, and 36 hours after the onset of standing estrus.

Using high-quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The correct site for semen placement is in the body of the uterus. This is a small target (1/2 to 1 inch) just in front of the cervix.

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E. Flushing the Embryos

Flushing is a process to collect the embryos nonsurgically, and it involves a small synthetic rubber catheter is inserted through the cervix of the donor cow, and a special medium being flushed into and out of the uterus to collect the embryos seven days after estrus.

This collection procedure is relatively simple and can be completed in 30 minutes or less without harm to the cow.

1. A pre-sterilized style is placed in the lumen of the catheter to offer rigidity for passage through the cervix into the body of the uterus.
2. When the tip of the catheter is in the body of the uterus, the cuff is slowly filled with approximately 2 mL of normal saline.
3. The catheter is then gently pulled so that the cuff is seated into the internal os of the cervix. Additional saline is then added to the cuff to completely seal the internal os of the cervix.
4. A connector with inflow and outflow tubes is attached to the catheter.
5. A pair of forceps is attached to each tube to regulate the flow of flushing fluid.
6. The fluid is sequentially added and removed by gravity. The fluid in the uterus is agitated rectally, especially in the upper one-third of the uterine horn.
7. One liter of fluid is used per donor. Many embryo transfer technicians use a smaller volume and flush one uterine horn at a time.
8. Each uterine horn is filled and emptied 5-10 times with 30 to 200 mL of fluid each time, according to the size of the uterus.
9. The embryos are flushed out with this fluid and collected in a filter with the fluid. The pores in the filter are smaller than the embryos, so excess fluid drains out of the filter without losing the embryos.
10. Embryos are separated from the flush media and examined under a microscope to determine their quality and stage of development.

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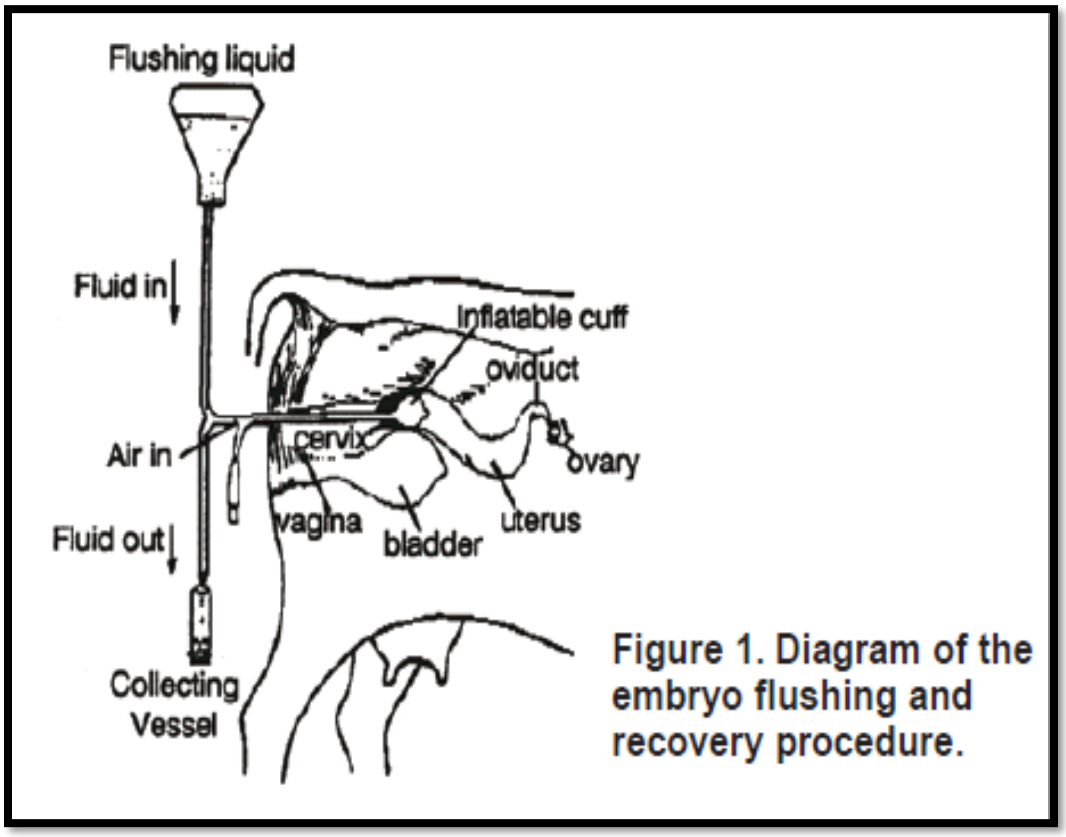

Figure 1 Embryo flashing

F. Evaluation of the Embryos

As the individual embryos are located using a microscope, they are evaluated for their quality and classified numerically as to the potential likelihood of success if transferred to a recipient female. The major criteria for evaluation include:

1. Regularity of shape of the embryo
2. The compactness of the blastomeres (the dividing cells within the boundaries of the embryo)
3. Color and texture of the cytoplasm (the fluid within the cell wall)
4. The overall diameter of the embryo
5. Presence of extruded cells
6. Regularity of the zona pellucida (the protective layer of protein and polysaccharides around the single-celled embryo)
7. Presence of vesicles (small bubble-like structures in the cytoplasm)

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Embryos are classified according to these subjective criteria as:

Grade 1: Excellent or good

Grade 2: Fair

Grade 3: Poor

Grade 4: Dead or degenerating

Embryos also are evaluated for their stage of development without regard to quality.

These stages are also numbered:

Stage 1: Unfertilized

Stage 2: 2 to 12 cells

Stage 3: Early morula

Stage 4: Morula

Stage 5: Early blastocyst

Stage 6: Blastocyst

Stage 7: Expanded blastocyst

Stage 8: Hatched blastocyst

Stage 9: Expanding hatched blastocyst

Embryo quality is also of utmost importance in the survival of the freezing and thawing stress. Grade 1 embryos generally are considered the only ones to freeze.

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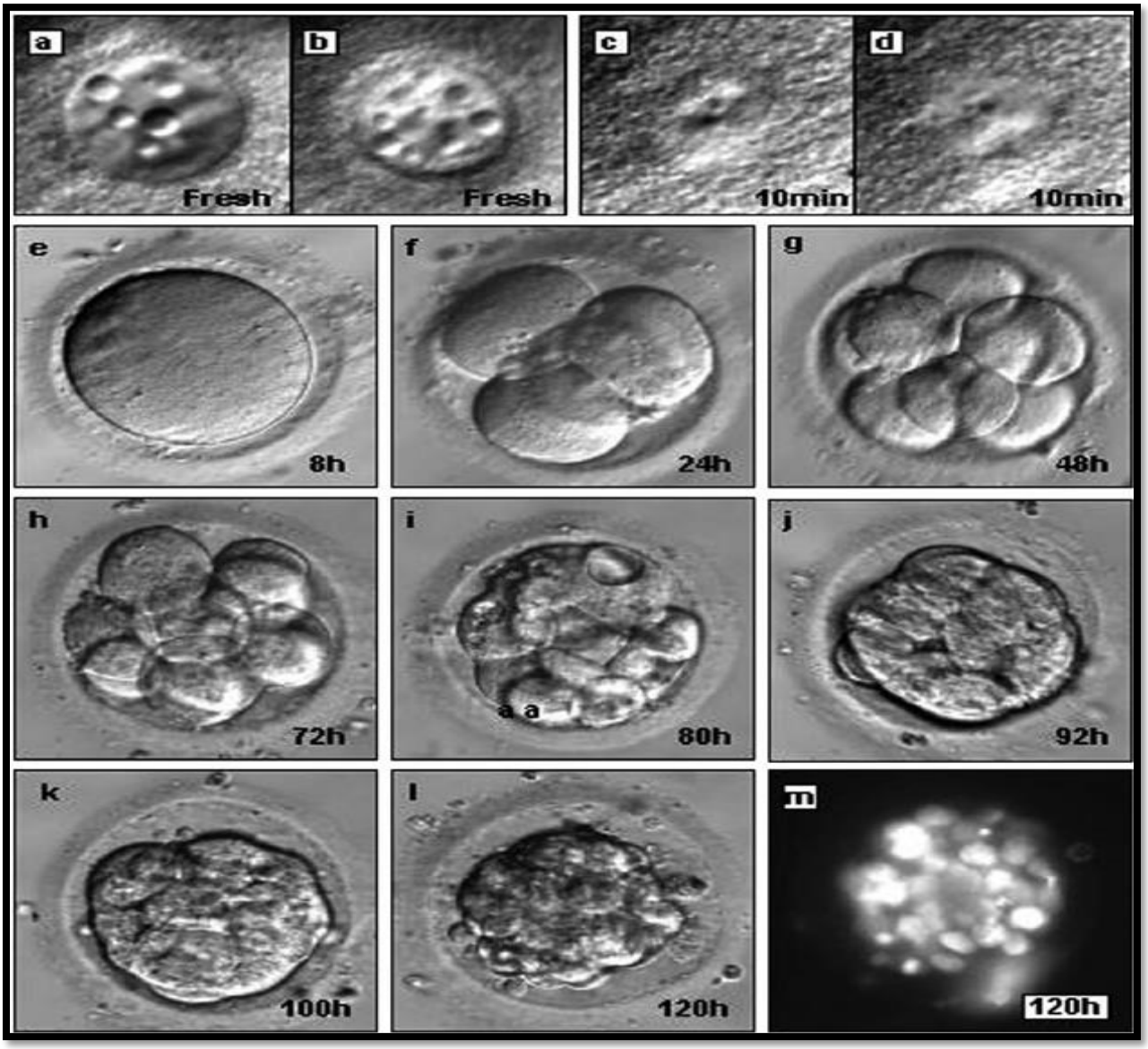

G. Transfer of the Embryos

1. The transfer of the embryo into the recipient cow first requires "loading" the embryo into a 1/4mL insemination straw. This is done under microscopic viewing with the aid of a 1mL syringe and requires considerable practice, patience, and dexterity.

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2. Just before embryo transfer, the ovaries of the recipient are palpated rectally to determine which ovary has ovulated.
3. Open the vulva of the recipient cow, the transfer gun or insemination rod is carefully passed through the cervix.
4. The tip of the rod is then allowed to slide into the horn on the same side of the ovary with an active corpus luteum.
5. The embryo is gently expelled in the forward tip of that uterine horn. Great care is taken to not cause damage to the lining of the uterus.
6. Embryo flushing and embryo transfer are both done after an epidural anesthetic has been given to block contractions of the digestive tract and aid in the ease of manipulation of the cervix and the uterine horns.
7. Embryos should be transferred as soon as possible after the flush (within 8 hours at least).