



EMBRYO TRANSFER TECHNOLOGY (ETT)



Prepared by-

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EMBRYO TRANSFER TECHNOLOGY (ETT)

“Embryo transfer is a technique where embryos are collected from the donor females and transferred into uterus of recipients which serves as a foster mother for its development throughout the remainder period of pregnancy.”

Learning Objectives:

- ✓ Define the Embryo transfer (ET)
- ✓ Explain the steps of embryo transfer
- ✓ List the advantages of embryo transfer
- ✓ List the disadvantages of embryo transfer

History of Embryo Transfer

- The first successful embryo transfer was carried out in rabbit (1890) by **Walter Heap**.
- First lamb by ETT – 1949 by Berry.
- First calf by ETT – 1951 by Willet et al.
- In Swine -1951 by Kvansnickii
- In Asian Buffalo - 1983 by Drost et al.

Steps Involved In Embryo Transfer

- 1) Selection of donor
- 2) Selection of recipients
- 3) Estrus synchronization of donor and recipients
- 4) Superovulation (SOV) of donor (release of multiple eggs at single estrus)
- 5) Artificial insemination of donor
- 6) Embryo collection
- 7) Evaluation of embryos
- 8) Transfer of embryos/cryopreservation of embryos /micromanipulation

Applications of Embryo Transfer

- 1) Faster genetic improvement
- 2) Genetic screening
- 3) Disease control
- 4) Import and export
- 5) Circumvention of infertility
- 6) Twining in cattle
- 7) Conservation of endangered species
- 8) Research/production of clones/and genetic engineering

1. Selection Criteria Of Donor

- Superior individual performance
- Good productive performance of offspring
- Regular Cyclicity
- Ovaries must be free (No adhesions)
- Intact tubular genitalia (free from any abnormalities)
- Younger (4-8 years of age)
- Healthy and have good body weight

- Must have calved at least 60 days back (best 90-100 days postpartum)
- Normal postpartum history
- A history of no more than two breeding per conception
- Previous calves having been born at approximately 365 day interval

2. Selection Criteria of Recipient

- Healthy, free from infection and have good body weight.
- Regular cyclicity.
- Intact genitalia (free from any sort of abnormalities)
- Must have good cyclic CL of desired stage at the time of embryo transfer
- Exhibit calving ease, and that have good milking and good mothering ability.

3. Estrus Synchronization of Donor

- The donor cow should be synchronized to bring into estrus OR should have palpable CL on the ovary superovulatory process
- For this, any of the synchronization protocols can be used.
- Ov-synch, Co-synch, select synch hybrid synch, heat synch etc.

4. Superovulation of Donor Cow

- Procedure for increased ovulatory response by administration of hormones (gonadotropins) to produce several ova instead of one which is normally produced at each estrus.
- This large number ova is later on fertilized and embryo produced can be transferred to the recipients

- The basic principle of superovulation is to stimulate extensive follicular development through the use of a hormone preparation, which is given IM or SC with FSH activity.
- In Ewe, Doe and Cow an average of 12 ovulations can be expected. In Sow number of ovulations could be >20.
- SOV has not yet achieved in Mares due to ovulations occurring in at one site of ovary i.e. ovulation fossa.

Time of Superovulation

- For optimum response gonadotropin treatment is initiated during mid luteal phase of estrus cycle i.e. on days 9-14 of estrous cycle (Day 0 is estrus)
- Donor cows can be superovulated repeatedly at approximately 6-8 weeks interval.

5. Insemination of Donor (A.I.)

- Donor should be inseminated artificially 2-3 times at 12 hours interval after the onset of estrus. This is required because ovulation can occur over an extended time period.
- Fresh semen is preferred.
- If frozen semen then use double insemination dose at each insemination.

6. Embryo Recovery

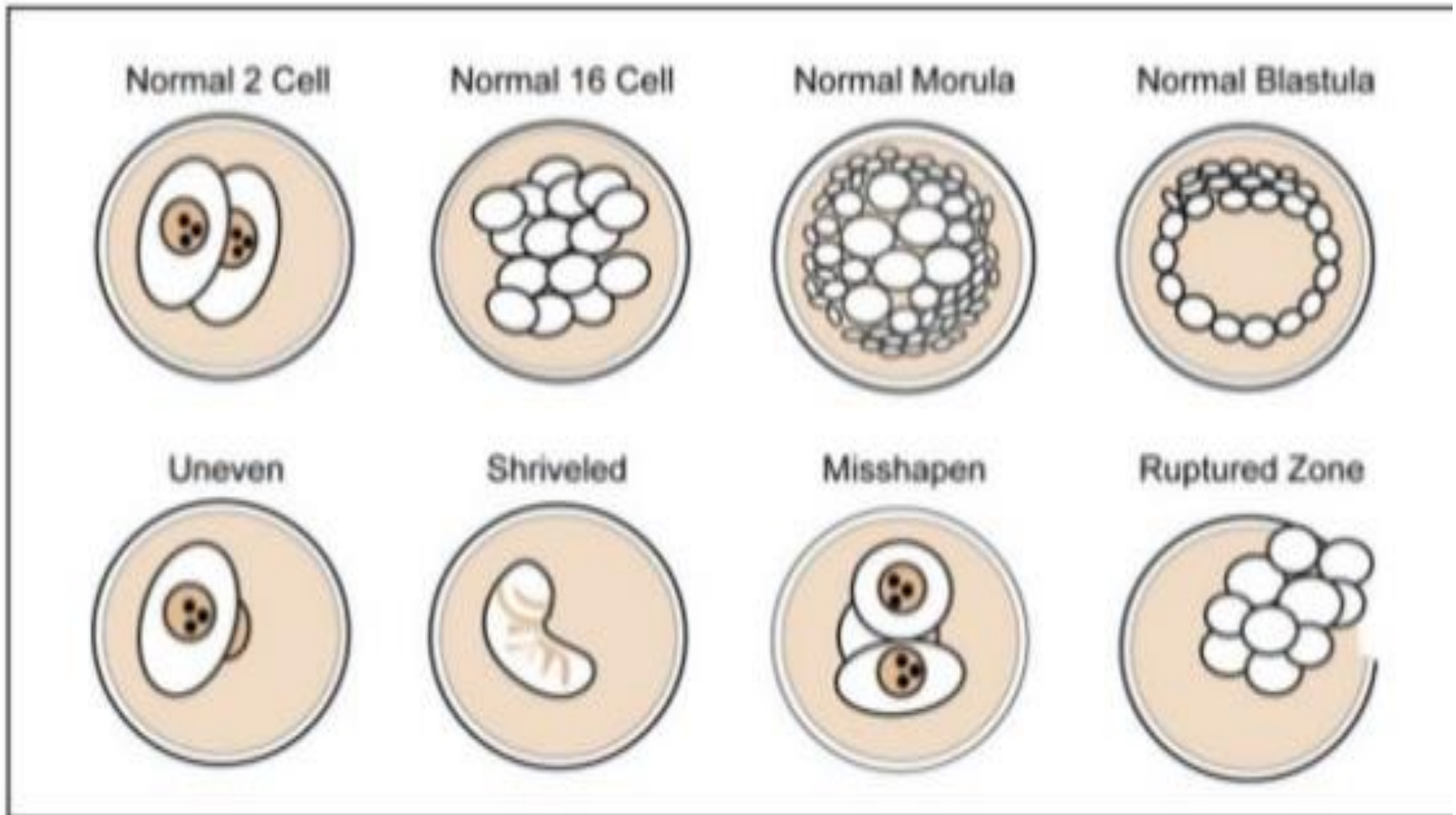
- Embryos can be collected by following methods:
 - A. Surgical method
 - B. Non-Surgical method
 - C. Laparoscopic method

A. Surgical Method

Most often used in Sheep, Goat and Swine through mid ventral incision under general anesthesia.

The method can be performed on day 3-4 after estrus in sheep and goat (8 cell embryo or less) and on 2-3 days after estrus in swine (4 cell stage).

Normal embryos will have between 2 and 64 cells.



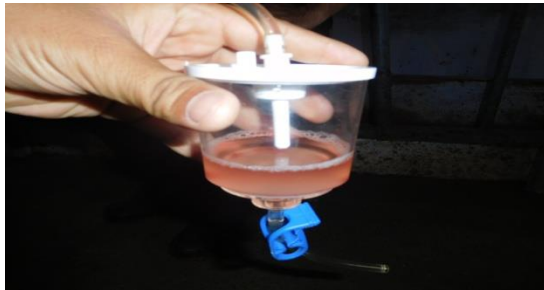
B. Non-Surgical Collection (Trans Cervical Method)

- Commonly performed in cattle, buffalo and mare.
- It involves 2 ways or 3 ways Foley or Woerllein catheter which allows flushing fluids to pass into the uterus and at the same time allows fluids to be returned from the uterus to a collecting receptacle.
- A small balloon near the end of catheter can be inflated just inside the uterine horn to prevent the flushing fluid from escaping through the cervix.

Trans Cervical Embryo Collection (Flushing of Donor)

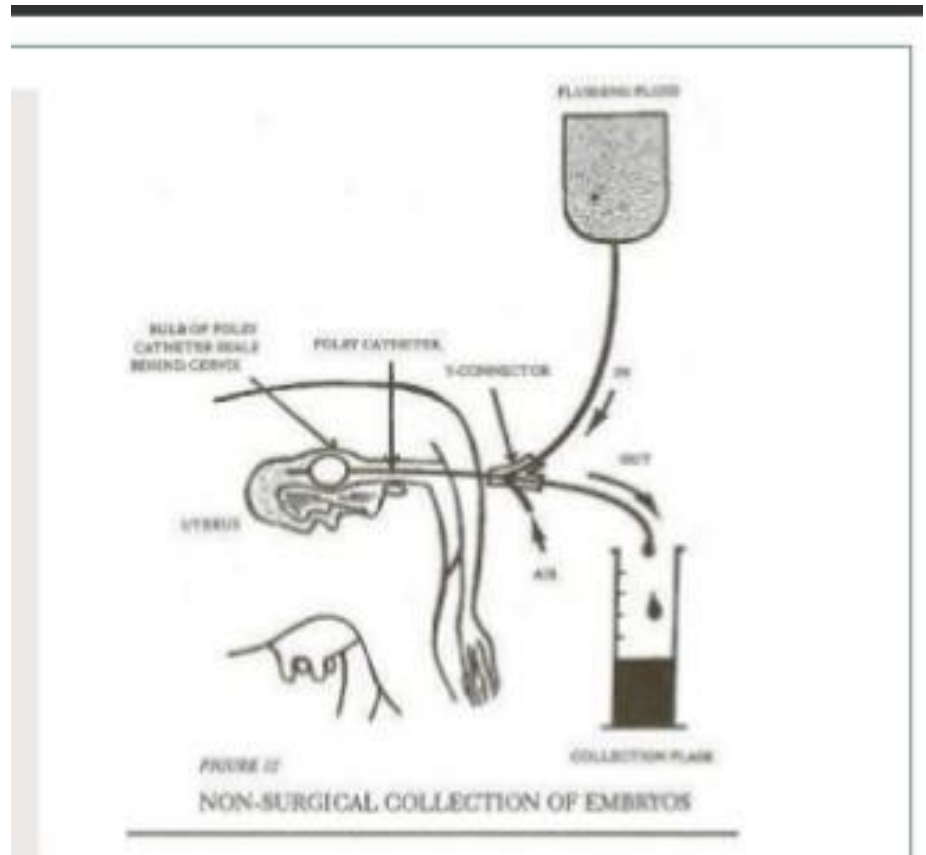


**Searching of Embryos in
Stereozoom microscope**





Foley's Catheter for Embryo Recovery from Donor



- Collection of bovine embryos should be made at 6-8 days post-breeding at compact morula or blastocyst stage.
- 6-7 days post ovulation at blastocyst stage in mare.
- The best flushing medium for embryo collection in most of the species is modified Dubecco's phosphate buffer saline. NS can be used in its absence.

- During final collection, oxytocin is administered @ 50 i.u. intravenously.
- Give large doses of intrauterine antibiotics to prevent infection.
- Injection of PGF2 α is also recommended to speed recoveries of ovaries and to prevent pregnancy, if viable embryos are not dislodged by the flushing.

C. Laparoscopic Embryo Collection

- Surgical collection is choice of method in sheep and goat due to inability to palpate reproductive tract.
- This has lead to the use of surgical techniques predominantly leading to adhesion formation.
- Laparoscopic is considered to results in fewer adhesions than traditional surgery.

7. Evaluation of Embryos

- After collection and before transfer to the recipients, the embryos are evaluated under stereozoom microscope at 50 to 100x magnification.
- Day 7 bovine embryos (compact morulla or blastocyst) are about 150-190 μ m in diameter and are still within the zona pellucida.
- Embryos are graded based on following characteristics
 - ✓ Compactness of the cells
 - ✓ Regularity of cells
 - ✓ Variation in cell size
 - ✓ Colour and texture of cytoplasm
 - ✓ Presence of vesicles, extruded cells, cellular debris

Using these criteria, the embryos are graded as:

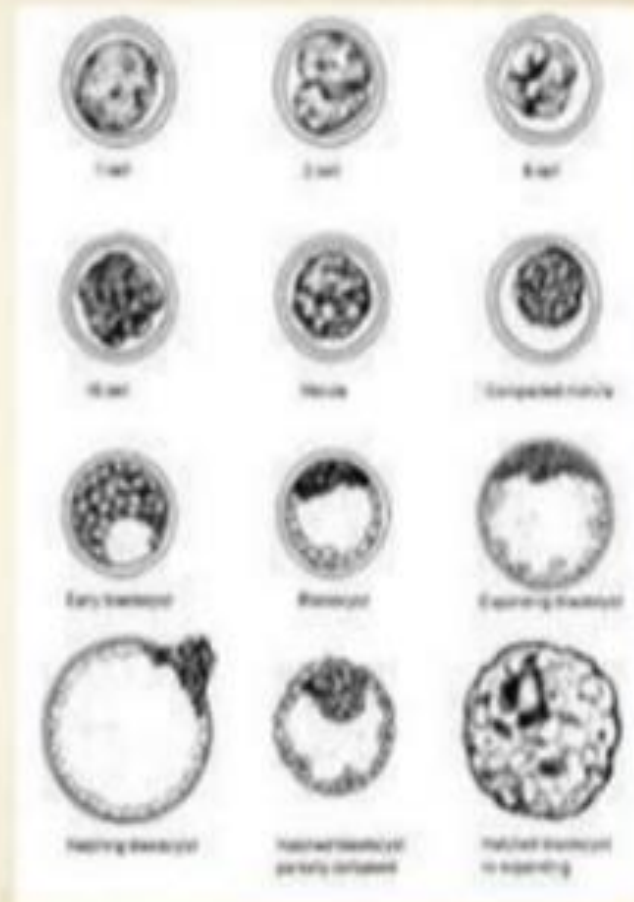
Grades	Types	Characteristics
I	Excellent	Symmetrical, compact, distinct outline, no blastomere extrusion, even granulation, neither very light nor very dark
II	Good	Somewhat asymmetric, even granulated with distinct outline, some blastomere extrusion
III	Fair	Hazy outline, extruded cell, asymmetric
IV	Poor	Uneven granulation, hazy outline, abnormal shaped
V	Degenerated	Developmental stage difficult to determine

Evaluation of the Embryos

Stages: Embryos are also evaluated for their state of development:

- Stage 1 (Unfertilized egg)
- Stage 2 (2 to 12 cell)
- Stage 3 (Early morula)
- Stage 4 (Morula)
- Stage 5 (Early Blastocyst)
- Stage 6 (Blastocyst)
- Stage 7 (Expanded Blastocyst)
- Stage 8 (Hatched Blastocyst)
- Stage 9 (Expanded Hatched Blastocyst)

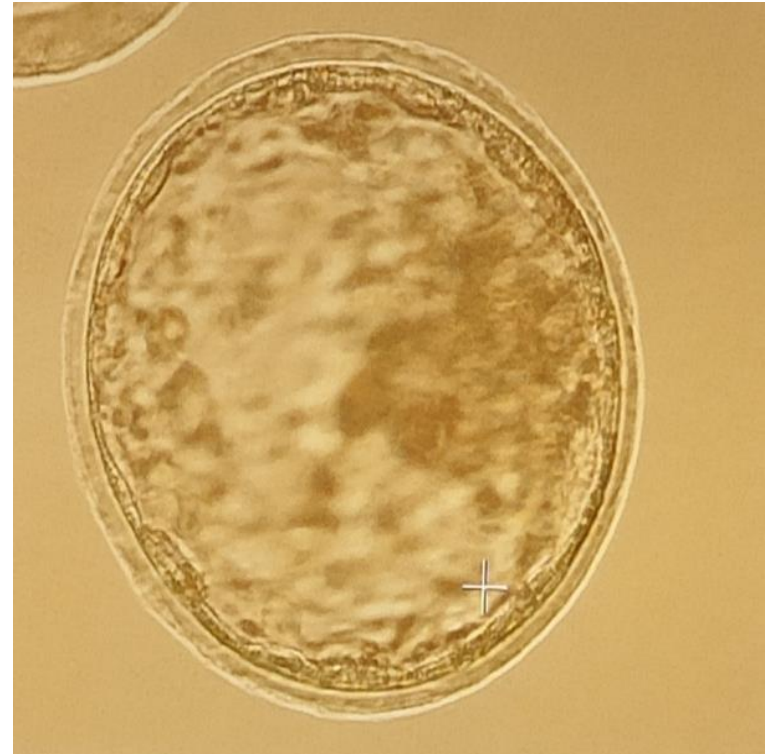
Stage 4, 5, and 6 embryos are best with the freezing and thawing procedures.



RINCKER



Morulla 16 Cell and 32 Cell



Expanded Blastocyst

Transfer of Embryo (Introduction to Recipients)

- Recipients should be in estrus within 12 hrs. of the donor so that it should possess good CL at the time of transfer.
- To maximize success rate of transfer, the recipient's estrus should be in sync with that of the donor.
- **Process of transferring embryos:** The recipient is palpated to determine the presence and location of the CL (Rt. Ovary vs. Lt. Ovary).
- Recipient is administered an epidural to relax the muscles in the pelvic area.

1. Surgical Method:

- It involves laparotomy incision, preferred in sheep, goat and pig. The uterine horn ipsilateral to ovary with CL is exposed. A small syringe fitted with 21 gauge needle is used for transfer.
- When the embryo is placed in the uterus, the needle is carefully inserted through the wall of the uterine horn whereas, when embryo is placed in oviduct then the needle is inserted through the infundibulum into the ampulla where the embryo is deposited.

2. Non-Surgical Method:

- Mostly used for cattle and mare.
- Flushed embryos after inspection are loaded into ET straw.
- If the embryo is frozen it is thawed in warm water bath (92°F) for <30s and placed in ET gun and covered with sterile sheath.
- The ET gun is passed through the vagina, cervix and into the uterine horn on the side as the CL. The embryo is deposited 1/3 the way up to the uterine horn.
- Pregnancy rate high when day of estrus of recipients and donor are within 24 hours.
- The embryos are typically transferred on day 7 of the estrous cycle.

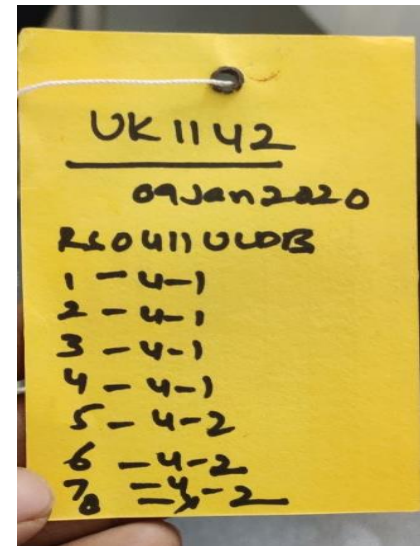
Storage and Cryopreservation of Embryos

- Embryos can be maintained at near body temperature in the media used for flushing during the period between recovery and transfer.
- If embryos are to be held for longer than 2 hrs. Up to 10 hrs.; a media containing 20% heat treated serum should be used as holding media.
- If embryos are cooled at 5°C (i.e. refrigerated temp.) , they can be maintained for 2-4 days.
- Cryopreservation of embryo is performed for longer period of time.

Embryo Freezing Unit



Marking of Straws →



Advantages of cryopreservation

- Long term storage of embryos
- Eliminates estrus synchronization in recipients
- Easy export and import
- Worldwide distribution

- Cryoprotectants like glycerol, ethylene glycol and DMSO are always needed for preservation of embryos
- **Thawing of Straws:** Straws are thawed before transfer of embryos to recipients. If 0.25 ml straws → 15 sec in air and 20 sec. in water bath at 37°C
- If 0.5 ml straws → 20 sec in air and 20 sec. in water bath at 37°C
- Exposure to air reduces damage to zona pellucida

Advantages of ETT

- Increase the number of offspring sired from superior females.
- Results in faster genetic progress.
- Obtain offspring from old or injured animals incapable of breeding or calving naturally.
- Increase farm income from sale of embryos.
- Export/import of embryos is easier than with live animals

Disadvantages of ETT Programme

- Costly and success rate are less than AI
- Cost and maintenance of recipient females
- Requires a technician with the skills to flush embryos from the reproductive tract.
- Possible spread of diseases through recipients.



THANK YOU