UNIT V - ANIMAL TECHNOLOGIES

Lesson 2: Embryo Transfer Technologies

Objective/Competency: Describe the process of embryo transfer.

Study Questions

1. **What is embryo transfer?**
2. **What are the advantages of embryo transfer?**
3. **What equipment is needed for embryo transfer?**
4. **How is the donor managed for superovulation?**
5. **What are the steps in the embryo flush process?**
6. **What are the steps in transferring the embryo to the recipient?**
7. **What is cloning, and how is it accomplished?**
8. **What are the benefits of cloning?**
9. **What is in vitro fertilization (IVF)?**

References

1. *Biotechnology: Applications in Agriculture (Student Reference)*. University of Missouri-Columbia: Instructional Materials Laboratory, 1998, Unit V.
2. Transparency Master
   a) TM 2.1: Catheter
3. Activity Sheets
   a) AS 2.1: Examining Embryos (Instructor)
   b) AS 2.1: Examining Embryos (Student)

UNIT V - ANIMAL TECHNOLOGIES

Lesson 2: Embryo Transfer Technologies

TEACHING PROCEDURES

A. Review

As discussed in Lesson 1, the use of artificial insemination in the livestock industry has enabled livestock producers to select highly desirable male animals for their breeding programs. However, producers must own many high quality female animals to improve a herd's genetic potential rapidly. Through the development of embryo transfer technologies, producers can obtain high quality embryos and improve the genetic potential of their herds more quickly.

B. Motivation

Congratulations! Your two-year-old heifer has been named Grand Champion at four major Angus shows across the country. You have put a great deal of hard work and money into this animal, and now you have the opportunity to make money by raising or selling calves. You could use embryo transfer. If semen from a prizewinning bull is used in the embryo transfer process and three or four live births are predicted, would you choose to use embryo transfer technology? What are the factors that might influence your decision?

C. Assignment

D. Supervised Study

E. Discussion

1. Remind students that an embryo is a fertilized egg. Ask them to explain what embryo transfer is.

What is embryo transfer?

a) Embryo transfer (ET) is the process of transplanting embryos from a donor female to a recipient female.
b) Embryo transfer has six basic steps.
    1) Synchronization of estrous in donor and recipient
    2) Superovulation of the donor
    3) Breeding the donor
    4) Recovering the embryos from the donor through flushing
    5) Isolating and examining the embryos
    6) Transferring embryos to the recipients or freezing the embryos

2. Ask students to identify when embryo transfer might be of the greatest benefit.

What are the advantages of embryo transfer?

a) Increases the reproductive potential of superior females
b) Increases the rate of genetic improvement in a herd
c) Allows female animals to be progeny tested more easily and accurately
d) Provides a way to use breeding stock from other countries

3. Point out to students that some of the equipment used in embryo transfer is similar to the equipment used in AI. Show students a semen straw and an embryo straw as examples. Discuss the equipment used in ET.

What equipment is needed for embryo transfer?

a) Equipment needed to flush a donor
   1) Catheter
   2) Stylet
   3) Collection cylinder
   4) Shoulder-length glove
   5) Lubricant
   6) Syringes
b) Equipment needed to examine the embryos
   1) Microscope
   2) Pipette embryo-handling tool
   3) Gridded search dish
c) Equipment needed to transfer the embryo to a recipient
   1) Embryo straw
   2) Embryo transfer gun

4. Ask students to recall the name of the hormone that causes the follicle (egg or ovum) to be released at ovulation. Tell students that if large amounts of this hormone (FSH) are given, multiple eggs will be released.

How is the donor managed for superovulation?

a) If prostaglandin is used to manipulate the heat cycle, preparation of the donor cow begins ten days after she is in standing heat with an injection of the hormone FSH.
b) FSH injections are given once in the morning and once in the evening until a total of seven injections have been given.
c) Prostaglandin is injected in the morning and evening on the third day of the procedure, which causes the donor to come into estrus in 48 hours.
d) When estrus begins, the donor is bred naturally or through AI.

5. Ask students where the egg cells (ova) are produced. Explain that each female animal has thousands of immature ova in their ovaries. Review the reproductive cycle of the female, focusing on ovulation. Have students complete AS 2.1.

What are the steps in the embryo flush process?

a) The embryo flush process is performed seven days after breeding.
b) An epidural anesthesia is injected into the space between two cervical vertebrae.
c) With the aid of the stylet, the catheter is inserted into the vagina, through the cervix, and into the right uterine horn.
d) The technician must palpate the donor carefully to guide the catheter into the right location.
e) The inflatable bulb near the end of the catheter is inflated to block off the uterine horn while it is being flushed.
f) The sterile flushing solution is allowed to flow into the uterine horn under the force of gravity until 500 milliliters of the solution is in the uterine horn, and the horn is then massaged to loosen the embryos.
g) When the fluid has filled the uterine horn, the outlet tube of the catheter is opened and the fluid and embryos are collected in a collection cylinder.
h) This process is repeated in the left uterine horn.
i) The embryos settle to the bottom of the collected fluid, and the fluid is siphoned off so the embryos can be counted and characterized, or examined for quality.

6. Describe the procedure for transplanting the collected embryo into the recipient.

What are the steps in transferring the embryo to the recipient?

a) Embryos that are to be transferred to recipients are loaded into plastic embryo straws.
b) They are then prepared to be either transferred into recipient cows or frozen in a container of liquid nitrogen at -320 degrees Fahrenheit.
c) The embryo transfer gun is loaded with a straw and inserted into the vagina.
d) It is guided through the cervix and into the uterus, where the embryo is expelled.

7. Ask students to give examples of animals that have been cloned. Explain that sheep, cattle, and goats have all been cloned.

What is cloning, and how is it accomplished?

a) Cloning - the asexual reproduction of an organism in which the resulting organisms are identical
b) Two methods of cloning
    1) Splitting an embryo
        (a) A researcher physically splits the embryo into two halves as it is dividing.
        (b) Each half is transferred to a recipient and develops normally.
    2) Nuclear transfer
        (a) A cell is extracted from a parent organism, and an electrical pulse is used to fuse it to an unfertilized ovum that has had its nucleus removed.
        (b) The new cell has a diploid number of chromosomes and will develop as if it were a natural embryo; however, it must be stimulated to act like a fertilized ovum and begin dividing.

8. Ask students to list some reasons why a group of genetically identical animals could be useful.

What are the benefits of cloning?

a) Cloning can increase the number of highly prized animals produced.
b) Fewer animals could be used in live animal tests because all of the animals--control animals and experimental animals--would be identical.
c) Animals genetically altered to produce pharmaceuticals could be cloned, which would reduce the cost of producing the animals.

9. Ask students to recall what in vitro means (“in glass”). Explain that IVF is one of the newest biotechnologies. A few dairy producers have begun to use it.

What is in vitro fertilization (IVF)?

a) IVF is a process in which immature follicles are collected from the ovaries of a female animal, stimulated to mature, and fertilized outside the female reproductive tract.
b) The fertilized embryos can be transferred to recipient animals.

F. Other Activities

1. Using a female reproductive tract from a slaughterhouse, simulate the embryo transfer process.

2. Show the video “Embryo Transfer” from Creative Educational Video (CEV), which is available from MVRC.

3. Contact an ET technician or veterinarian and ask if he or she would bring the ET equipment to class or if the class could borrow the equipment for a day so students could do an equipment identification activity.

4. Take students to a farm or veterinarian's office to observe an actual or simulated nonsurgical flush.

G. Conclusion

In the early 1980s, embryo transfer was an expensive and difficult process involving the surgical removal of embryos. Today, more and more producers, particularly those who produce breeding animals, are looking to embryo transfer to give them more progeny from their high performing animals.

H. Answers to Activity Sheet

AS 2.1

Answers will vary.

I. Answers to the Evaluation

1. d
2. b
3. d
4. c
5. b
6. d
7. 3
8. 2
9. 4
10. 1

11. Students may list any two of the following.
    - Cloning can increase the number of highly prized animals produced.
    - Fewer animals could be used in live animal tests because all of the animals--control animals and experimental animals--would be identical.
    - Animals genetically altered to produce pharmaceuticals could be cloned, which would reduce the cost of producing the animals.

12. Embryo transfer (ET) is the process of transplanting embryos from a donor female to a recipient female.

13. IVF is a process in which immature follicles are collected from the ovaries of a female animal, stimulated to mature, and fertilized outside the female reproductive tract. The fertilized embryos can be transferred to recipient animals.

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EVALUATION

Circle the letter that corresponds to the best answer.

1. Which of the following is not an advantage of embryo transfer technology?

   a. Embryo transfer allows for easier and more accurate progeny testing of female animals.
   b. Embryo transfer provides a way to use breeding stock from other countries.
   c. Embryo transfer increases the rate of genetic improvement in a herd.
   d. Embryo transfer reduces the management needed for breeding programs.

2. A stylet is used to:

   a. Insert the embryo into the recipient.
   b. Insert the catheter into the donor for the flushing procedure.
   c. Manipulate the embryos when counting and grading them.
   d. Collect the embryos after they have been flushed.

3. The hormone that causes a cow to superovulate is:

   a. Prostaglandin.
   b. Syncromate-B.
   c. Progesterone.
   d. Follicle stimulating hormone.

4. How many days after a donor cow has been superovulated and bred is the flush procedure done?

   a. 2
   b. 3
   c. 7
   d. 11

5. The process in which a cell is extracted from a parent organism and fused into an ovum that has had its nucleus removed is called:

   a. In vitro fertilization.
   b. Nuclear transfer.
   c. Embryo splitting.
   d. Embryo flushing.

6. Which of the following is not equipment required for embryo transfer?

   a. Catheter
   b. Straw
   c. Collection cylinder
   d. Surgical supplies

Superovulation involves several steps. Put the following steps in the correct order by placing "1" by the first step, "2" by the second step, etc.

7. Prostaglandin is injected into the donor cow.

8. The donor cow is injected with FSH.

9. The donor cow is bred.

10. Ten days pass after estrous is detected in the donor cow.

Complete the following short answer questions.

11. What are two reasons cloning may be useful?

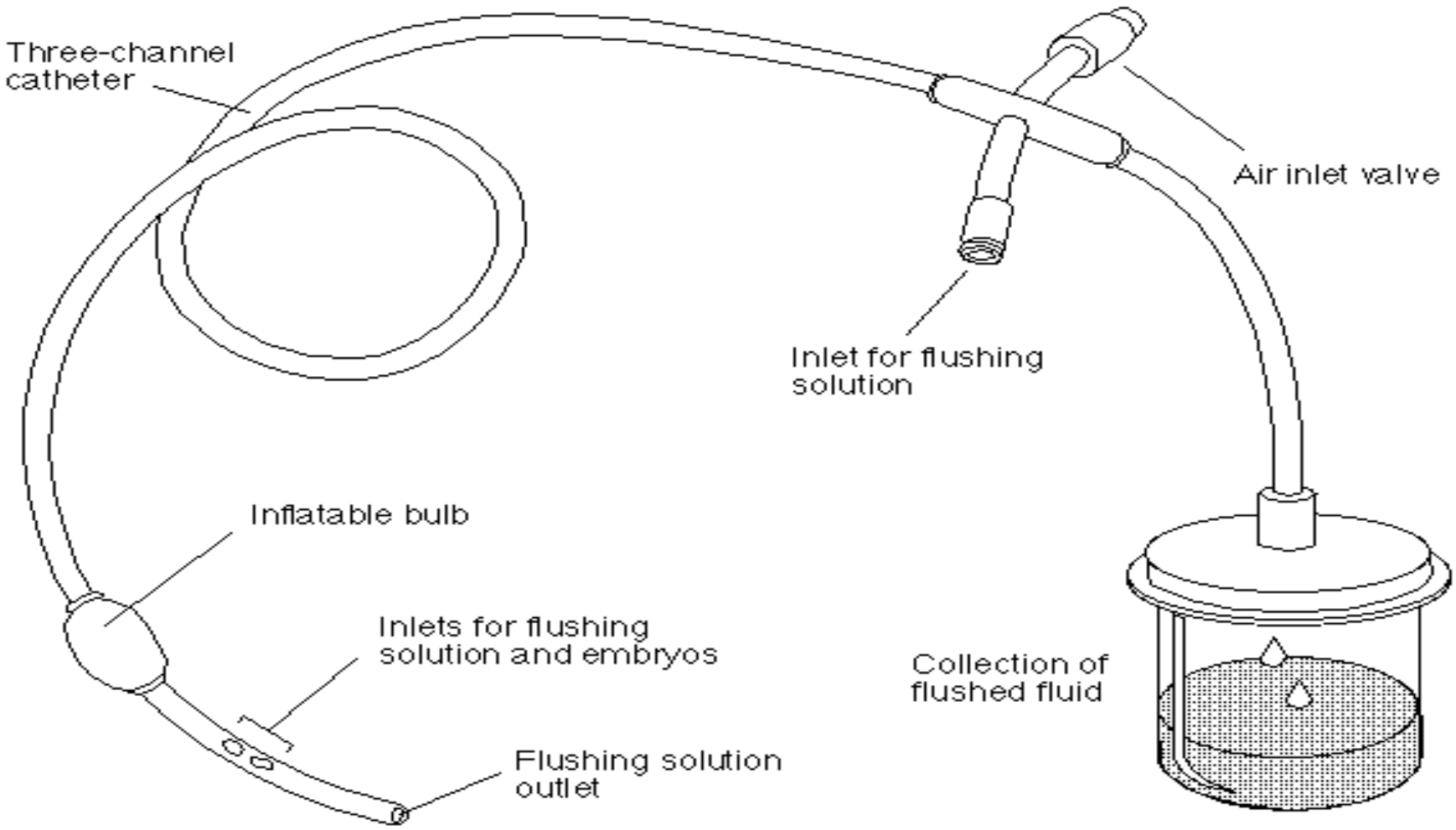
    a.

    b.

12. What is embryo transfer?

13. What is in vitro fertilization?

Catheter


Three-channel catheter
Air inlet valve
Inlet for flushing solution
Inflatable bulb
Inlets for flushing solution and embryos
Collection of flushed fluid
Flushing solution outlet

Lesson 2: Embryo Transfer Technologies

Examining Embryos

Objective: Examine and grade embryos.

Materials and Equipment:

1-5 microscopes (magnifying **less** than 100x, preferably 10x and 50x)
4-6 petri dishes (preferably gridded dishes)
500 ml of embryo-holding media or flushing fluid
Liquid nitrogen tank
Warm water bath

Procedure:

Before conducting this activity, frozen cattle embryos must be obtained from a local breeding service or veterinarian. Embryos can also be obtained from ABS (American Breeders Service) for a fee; the price charged for these embryos will vary.

Obtain a tank containing liquid nitrogen from a local veterinarian or livestock breeder to store the embryos correctly. Remember to thaw the embryos properly by first allowing them to warm in the air for 10 to 15 seconds and then placing them in a warm water bath. Place a known number of embryos in a gridded petri dish containing holding solution and label it “A.” Place a single Grade 1, Grade 2, or Grade 3 embryo in each of the other petri dishes and label them “B,” “C,” and “D”; these dishes should contain holding media and have a lid.

Note: It can sometimes be difficult to find Grade 3 embryos, since they are not often frozen.

Divide students into groups. The number of groups will depend on the number of microscope observation stations. Place one or more of the petri dishes containing embryos at each station. Demonstrate how to remove the cover and focus the microscope so that the embryos in the dish are visible. Have the students examine the embryos and complete the activity.

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500 ml of embryo-holding media or flushing fluid
Liquid nitrogen tank
Warm water bath

Procedure:

1. Use the microscope to observe each of the petri dishes prepared by your instructor.

2. Count the embryos in dish "A" and record the number in the space provided. _____

3. Grade the embryos in dishes "B," "C," and "D." The different classifications used when grading embryos are described below.

   Grade 1: The embryo is nearly perfect. More than 98 percent of the cells in the embryo are apparently active. It is rounded in appearance.

   Grade 2: The cell mass of the embryo is apparently 70 to 98 percent active. Some cells are extruded from the surface of the embryo, so it may not be rounded in appearance.

   Grade 3: Less than 70 percent of the cell mass of these embryos is apparently active. Several cells are extruded from the surface of the embryo, which may be severely malformed.

   Degenerate: None of the cell mass of the embryo appears active. It may be flat or concave in shape.

4. Answer the following questions.

   a. What is the grade of the embryo in dish "B"?

   b. What is the grade of the embryo in dish "C"?

   c. What is the grade of the embryo in dish "D"?