

## **In vitro embryo production from terminal cows**

Cows which are terminally ill, down, injured and are about to die or be put down, do not necessarily mean a loss of valuable genetic assets. Evergen Biotechnologies Inc., a leading biotech company, with a lab located in Northeastern Pennsylvania, can help salvage the genetics of such cows. Using in vitro fertilization technology, the ovaries of such cows can be used for producing embryos and continuing genetic lines.

### **A. Follicle Stimulating Hormone (FSH)**

1. If the procedure can be planned in advance, then the cow can be given FSH shots to increase the number of follicles and recovered oocytes from the ovaries. Consult your Vet for the administration of FSH. A standard regime is as follows (using “Folltropin (Bioniche Animal Health, Pullman, WA, USA), which is the most widely used brand of FSH):

- a. When mixed there is 20cc of FSH in the vial. You will need all 20cc to complete the injection series.
- b. Refrigerate the vial between injections
- c. Use a separate needle for each injection.
- d. Give injections every 12 hours exactly
- e. All injections are given in the muscle of the hind leg.
- f. Example of injection series:

Day 1:	Morning: 4ml	Evening: 4ml
Day2:	Morning: 3ml	Evening: 3ml
Day 3:	Morning: 2ml	Evening: 2ml
Day 4:	Morning: 1ml	Evening: 1ml

### **B. Ovary removal and transport to the lab**

1. When the cow is about to die or be put down, consult your Vet for the best humane way to do it.

2. Inform Evergen as soon as possible so the lab can schedule the ovary processing and further procedures. Also, if any solutions need to be sent from the lab, they can be arranged.

3. Once the cow is deceased, have her abdomen cleaned and disinfected with 70 % alcohol. After having the abdomen opened, the ovaries need to be located, identified and cut out aseptically, wearing surgical gloves.

4. The ovaries are to be immediately put in warm (35 °C or 95 °F) saline, containing antibiotics such as penicillin and streptomycin or gentamycin). This solution can be provided by Evergen, or be arranged locally.

5. The warm saline containing ovaries is carried in a Styrofoam mailer box or thermos flask, to keep the ovaries at the required temperature during transit.

6. Bring the ovaries to Evergen lab within 4 to 8 hrs after removal.

7. The ovaries should be accompanied by identification (ear tag #, photo, registration number, etc) and other information such as date and time of ovary removal, cause of death, production record and reproductive history.

8. If registration is required, a blood sample from the jugular vein should be taken. Obtain 10ml of blood into a clean vial and maintain in refrigeration (4 °C or 40 °F).

### **C. Semen for IVF**

1. Evergen can provide sexed or non sexed semen for IVF.
2. Contact Evergen staff for Bull ID, availability and pricing.
3. When providing own semen, it should be accompanied by Bull name and NAAB Bull number.
4. At least three straws must be provided for IVF purpose.

### **D. Embryo transfer**

1. Embryos can either be transferred fresh or vitrified.
2. If fresh transfer is desired, recipient cows should be synchronized to come in heat on the day of IVF.
3. Vitrified embryos must be recorded and stored at Evergen's lab or the client's place in a liquid nitrogen tank (Liquid nitrogen and tank required).

### **E. Contact Information:**

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