

## POST MORTEM EMBRYO PRODUCTION

### HOW TO REMOVE AND PACK OVARIES AFTER EUTHANIZATION

The ovaries are removed aseptically quickly after the mare has been euthanized. Barbiturates are toxic for the oocytes, so try to be as well prepared as possible to reduce the contact time. Alternatively, the mare could be euthanized with a nail gun or put in general anesthesia (as an example a sedation followed by a knock-out with Ketamine or Zoletil), to remove the ovaries and afterwards euthanize the mare. If Barbiturates are the only possible way, be as fast as possible. The ovaries need to be removed in a few minutes.

#### Preparation:

- Prepare a styrofoam box (semen box etc.) that is able to keep the temperature stable with a ballast-fluid (as an example a rectal glove with water).
- The temperature of the ballast-fluid depends on the transport time to VetEmbryo:
  - If the transport time is less than 2 hours, the temperature of the ballast fluid should be between 33-37°C
  - If the transport time is more than 2 hours, the temperature of the ballast fluid should be around 22°C
  - It is extremely important that the temperature does *not* get over 37°C or less than 20°C
  - In a very warm weather, it might be necessary to take a freezer pack in the bottom of the box under the 22°C ballast fluid. Remember to measure the temperature in the box to make sure that the oocytes do not get a heat- or cold shock
  - The ovaries can also be transported in an Equitainer
- Before the euthanization 1 Liter of physiological, sterile fluid (as an example 0,9% NaCl, Ringers Lactate or Embryo flush) is warmed-up to 33-37°C
- For the extraction of the ovaries you need:
  - A scalpel
  - Rectal gloves
  - A bag for the ovaries: A clean rectal glove or a bag approved for food added a small amount of the pre-warmed physiological, sterile fluid to keep the ovaries moist.
  - An extra rectal glove/bag for a double layer and closure to avoid leakage.
- Immediately after the mare has been euthanized and the reflexes are gone, the ovaries are removed.
- Make an incision half way between the last rib and the hip. The ovaries are localized and loosened from the ligaments and oviducts. Both ovaries can be removed from the same side or the mare can be turned around and a second flank incision is made.
- After the ovaries have been removed, they need to be thoroughly cleaned with pre-warmed physiological, sterile water. *All* the blood and dirt need to be removed.
- The ovaries are packed in a rectal glove with a small amount of pre-warmed physiological, sterile water to keep them moist. Another rectal glove is used for a double layer and closure.
- The rectal gloves with the ovaries are put into the styrofoam box with the ballast-fluid.
- *Remember! A sample of hair for DNA, if the offspring has to be registered in a certain breeding association.*

Picture-guide on the next page

Ballast-fluid in a rectal glove with the right temperature



Transport time < 2 hours: 33-37°C



Transport time > 2 hours: 22°C



The fluid used for cleaning the ovaries/keeping the ovaries moist during the transport: Sterile, physiological 33-37°C (0,9% NaCl, Ringers Lactate or embryoflush).



A 10 cm flank incision half way between the last rib and the hip



The ovary is localized and loosened from the ligament and oviduct.



Both ovaries can be removed from the same side, or the mare can be turned around and another flank incision is made.



Things to remember!

Oocytes are extremely sensitive to toxic substances so do not use any other type of fluid, plastic bags etc. than mentioned here.

Always measure the temperature before sending the ovaries! The right temperature is very important for the survival of the oocytes.

Clean the ovaries thoroughly with pre-warmed sterile, physiological fluid 33-37°C



Put the clean ovaries in a rectal glove with a small amount of sterile, physiological fluid 33-37°C.



Close the rectal glove and put it into another glove to reduce the chances of leaking. In cold weather, more than one ballast-fluid can be used to keep the temperature stable



Things to remember!

Remember to take some hair from your mare for DNA, if the DNA of your mare is not known. This is important for registering in a breeding association.

If you need any help or further information during the procedure, do not hesitate to contact us!

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