

## **Fronzen Semen Thawing instruction**

Frozen semen packaged on 0,5 ml straws

### **Please read carefully before opening container.**

The frozen semen will be shipped to you in a special cryogenic liquid nitrogen container. The straws containing the frozen semen are maintained at  $-198\text{ }^{\circ}\text{C}$ . To avoid damaging the spermatozoa and prevent possible injury to the handler, the following guidelines should be strictly adhered to:

1. Always wear gloves and protective eye-wear while handling the liquid nitrogen container:
2. Do not allow container to be tipped or layed on its side. They are not sealed completely. If this does occur, return the container to its upright position as quickly as possible and contact the sender.  
If any nitrogen has spilled, it will evaporate in a matter of seconds.
3. Do not open the container until ready to transfer straws to a storage container or until you are ready to inseminate the mare. If the straws are to be transferred to a liquid nitrogen storage container, this should only be performed by a technician experienced in the handling of frozen semen. Exposing of the straws to room temperature for more than 2 – 3 seconds will initiate thawing and cause irreverable damage to the spermatozoa.
4. Dilution after thawing should be avoided because the freezing extender can react with your extender and it is possible that these reaction can destroy the sperms.  
To be successfull, please use the special equipment from “Mini tüb”.

Necessary equipment:

1. Long handled foreceps for grasping the straws
2. One water bath – maintained at  $38^{\circ}\text{C}$ .
3. Insemination equipment: Fa. Minitüb GmbH, Hauptstr. 41, Tiefenbach,  
Tel.: +49 – (0) 8709 – 2077, 2078, 2079, Fax: +49 – (0)8709 – 3190

Preparing the mare:

Ideally, the mare is inseminated within 6 hours of an ovulation. It is very strongly recommended, that the mare be palpated and ultrasounded at least every twelve hours, when ovulation is close at hand. Inseminations should occur within 8 hours prior to ovulation and within no more than 8 hours after ovulation. Since precise prediction of ovulation is difficult, the above procedure is critical.

When palpation and ultrasound indicate the mare is ready to be bred:

1. Prepare the mare aseptically prior to opening the liquid nitrogen container.
2. Wep the mare`s tail and clean the vulva and perineal area thoroughly.
3. Be sure to dry the area well with clean paper towels.

Thawing the frozen semen:

For one insemination you need 3 to 4 straws.

1. Prepare the constant temperature water bath at 38°C.
2. Open the liquid nitrogen container and pull canister slightly upward until straws are visible. Do not under any circumstances allow the top of the canister to rise higher than the neck of the container!
3. Grasp straw with forceps and plunge directly into 38°C water bath for exactly 10 seconds.
4. Remove the straw from the water bath and dry off completely. Any water that would leak into the straw is spermicidal.

Confirm identification on the straw for the correct stallion that is bred to this mare.

Cut off the straw at the sealed end. Place the open end of the straw into the insemination pipett. For insemination we prefer the special instrument of MINITÜB Comp. Germany. You can change the straws while leaving the pipette in the uterus. Don't dilute the semen after thawing!!

It is imperative that the mare is rechecked in 12 hours.

If she has not ovulated repeat the insemination procedure. If she has ovulated you may choose whether or not to repeat the insemination procedure for the last time.

NOTE: Each insemination dose contains 600 – 1000 million total sperm , every straw has a volume of 0,5 ml volume. A minimum of 30% progressive post-thaw motility is expected. The number of straws used for a single insemination, may vary according to the freezing procedure employed .

**Please consult the Frozen Semen Transaction Report for how many straws you have to use for one insemination.**

It is important to deposit the entire insemination dose into the uterus for optimum results.

**Please do not hesitate to contact Gentrans if there are any questions concerning the procedure for using this freezing semen.**

**Tel :** + 49 5492 1394  
**Fax :** + 49 5492 2485  
**E- mail :** [info@pferdeklunik-muehlen.de](mailto:info@pferdeklunik-muehlen.de)