

FROZEN SEMEN PROTOCOL 5ML MACRO AND 0.5 ML STRAW

This protocol is appropriate for semen, frozen by Eurequine, in 5ml straws, from Eurequine Stallions and others. One straw is one insemination dose and contains 600 - 800 million spermatozoa extended with non pathogenic egg yolk extender, Cryomax LE, Botucrio, SBS Cryosystem or INRA Freeze. This semen is EVA negative and free of any other detectable equine disease. Post thaw motility has typically been between 35% and 70%. Even though several pregnancies, and embryo transfers have been accomplished with the use of one straw it is not unusual to use more than 1 dose per cycle, one insemination hours before and one right after ovulation.

5ML MACRO

To prepare the semen for insemination with **5ml** macro straw:

- Prepare extender: 10cc Universal dual sugar basic formula(Exodus) or INRA96 without additional antibiotics, warm to 37 degrees Celsius in sterile container, then put in incubator. If above mentioned extenders are not available no extender should be used.
- Take one straw from storage tank, immediately put in water that is 50 degrees Celsius. Submerge for 45 seconds, remove from 50 degree water, wipe of excess water as it could be spermicidal.
- If no extender is used remove thawed straw from water and keep in incubator until mare is prepared.
- With air bubble on top cut sealing ball off of the straw. Turn cut side in container and cut plug off other side allowing semen to run in container, mix with extender and store in incubator until mare is prepared for a.i.
- Draw up the semen in to insemination syringe and pipette for a.i. just as would be done with fresh semen.

0.5 ML STRAW

For **<u>0.5ml</u> straws** (6-10 per dose):

• Thaw the straws in a 46 degrees Celsius water bath for 20 seconds. Remove immediately, dry and follow routine insemination protocol for using frozen semen.

The timed protocol below is less labor intensive than continually checking the mare - and also very successful. Examine mare during estrus

- Day 0 The day you detect a 35-40mm follicle. Administer HCG or Deslorelin at approximately 4 PM.
- Day 1 Inseminate 1 dose of frozen semen at 4 PM (24 hours post injection).
- Day 2 Inseminate 1 dose of frozen semen at 8 AM (40 hours post injection).
- Day 3 confirm ovulation or inseminate mare at 8 AM with a 3rd dose if she has not yet ovulated

If breeding certificate is desired email us a vet certificate with the insemination dates and the information of the mare(s) inseminated. The AHS and possibly other registries allow the mare owner themselves to do all paperwork needed for registry of foals with frozen semen.

Call or text us at (916) 203-2247 if there are any questions concerning this frozen semen. Sincerely,