International Canine Semen Bank,
P.O. Box 651 • Sandy, Oregon 97055
Telephone: (503) 663-7031
Email: ik9sb@aol.com, Website: www.ik9sb.com

CONFIDENTIAL

ICSB-BUFFER HS
FOR TREATING BLOOD IN A CANINE SEMEN COLLECTION

In cases where blood is seen in the canine ejaculate, the ICSB buffer "HS" can protect the sperm cells from the effects of blood components in most cases. What happens in ejaculates containing heavy rbc contaminants is that clotting will occur, which binds the sperm cells in the clot, rendering the sperm cells unable to reach the egg to fertilize.

To avoid this clotting, ICSB has developed a procedure which keeps the sperm cells motile and free swimming so that the ejaculate can still be used for artificial insemination, shipping fresh chilled for delayed artificial insemination, or for semen freezing. The key to keeping the semen in a viable state depends on how quickly the following procedures can be performed.

1. As soon as the ejaculate is collected and a dark red color is noted, dilute the specimen quickly with an equal volume of the ICSB "HS" buffer. If this is not available, use fresh normal saline (0.9% NaCl). The "HS" buffer does a better job of protecting the sperm cells than the NaCl, but if the semen is to be used quickly for insemination, it will keep the sperm cells swimming freely for several minutes. If the ICSB "HS" buffer is used, it can protect the sperm cells for several hours.

2. Once the buffer is added, mix gently by sealing the open end of the test tube with Parafilm, inverting 3 times gently, then let the specimen sit for 5 minutes. After this, the semen can be centrifuged for 3 minutes at 1,200 to 1,400 x g. Remove the supernate and resuspend the sperm plug in either the ICSB "HS" buffer, or the normal saline at room temperature, or if proceeding to process for freezing, adding the appropriate amount of the ICSB canine semen freezing medium. Gently resuspend the sperm plug using a Pasteur pipette and rubber bulb to force the buffer against the plug, causing the plug to resuspend in the buffer. Be sure the plug is completely resuspended. At this point, the specimen should be ready for use. This procedure will usually result in a specimen that is free of clotting. If you have any questions about this procedure or use of the buffer, please call or e-mail us.