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Centrifugation of Equine Semen for Cooled Transport

A common question we at SBS receive concerns the proper processing of abnormal ejaculates for cooled semen shipments.

Stallions that consistently ejaculate semen with a low concentration of sperm (>100 x 10^6/ml) pose a particular problem when one is utilizing cooled semen. The detrimental effects of seminal plasma on spermatozoa from dilute ejaculates of many stallions are not overcome by a dilution of even 3:1. In these cases, it may be necessary to remove the seminal plasma and concentrate the spermatozoa by centrifugation or dialysis. Centrifugation of semen may only be an option on large farms or in clinical laboratories where experienced technical assistance can be employed, because centrifugation may be damaging to spermatozoa of some stallions, particularly if performed improperly.

Centrifugation of equine semen is routinely used when concentrating spermatozoa for cryopreservation: consequently, there are a number of protocols and diluting media used. The following is a simple centrifugation protocol developed by SBS that has been used successfully on several stallions whose semen required the removal of seminal plasma prior to cooling.

1. Immediately after determining sperm concentration, slowly dilute the entire volume of gel free semen 1:1 with skim-milk glucose extender containing antibiotics. Care should be taken to ensure that the extender is at 35-37° C prior to dilution.

2. Pour the extended semen (40mL per tube) into pre-warmed, sterile 50mL conical-bottom centrifuge tubes.

3. Load the tubes into a centrifuge with a swinging bucket-type rotor. It is advisable to pre-warm the stainless-steel holders to prevent cold shock of the spermatozoa.

4. Centrifuge the semen at approximately 300x g for 12-15min.

5. Following centrifugation, aspire the top 30mL of supernatant and discard.

6. Add 10mL of fresh extender to the remaining concentrated spermatozoa and re-suspend the pellet by gently mixing. The temperature of the extender should be similar to that of the

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sperm pellet (approximately 20-25°C).

7. To calculate insemination dose volume, assume 75% recovery of spermatozoa. Therefore, if 75% of the spermatozoa were recovered and then diluted to equal the original volume of the get-free semen, the extended concentration is equal to the original concentration x 0.75. For example, an ejaculate of 60mL of gel-free semen with a concentration of 80×10^6/mL (total sperm=4800×10^6) is diluted 1:1 with skim-milk-glucose. The extended concentration is now 40×10^6/mL and the total volume of extended semen is 120mL. The extended semen is divided equally (40mL each) into three 50-mL tubes and centrifuged. Following centrifugation, 30mL is aspirated from each tube, leaving 10mL of concentrated spermatozoa per tube. To each tube, 10mL of fresh extender is added resulting in 20mL extended semen per tube or 60mL total volume. Assuming a 75% recovery of spermatozoa, the total number of spermatozoa in the 60mL is $4800 \times 10^6 \times 0.75 = 3600 \times 10^6$. The extended concentration is then 60×10^6/mL. The volume required to achieve an insemination dose of 2000×10^6 total sperm or 1000×10^6 motile sperm (for a sample with 50% progressive motility) is 33mL.

8. The extended semen is placed in Whirl-paks and cooled in the Equitainer. It is important to ensure that the components of the Equitainer that come in contact with the sample (ballast bags and isothermolizer) are removed from the incubator in advance so that they are at the same temperature as the re-suspended semen sample.

Care should be taken to avoid damaging the spermatozoa by too vigorous a centrifugation. Spermatozoa should be concentrated into a soft slurry rather than a hard pellet. If the pellets are hard, dark, and difficult to re-suspend, reduce the force or time of centrifugation slightly. Although strong centrifugation will increase recovery, the spermatozoa recovered are likely to be damaged.