Instructions for use of Semen Separating Solution

Introduction

It is important to use only sperm-rich fraction (SRF) when diluting semen for an artificial insemination with chilled extended or frozen semen, since the inclusion of seminal plasma will, in most cases, decrease the survival time and fertility of the sperm. Sperm-rich fraction should be thick and white, with average volume of 0.5 to 2.0ml, depending on the size of the dog. The following technique should be used to separate sperm cells from the seminal plasma, especially if fractions were not separated well during the collection process. Although the volume at which a semen sample requires concentration is variable, use the following general guidelines: Small breeds >0.5ml; Medium breeds >1.0ml; Large breeds >1.5ml; Giant breeds >2.0ml.

Equipment required

- Clinical centrifuge
- Semen sample
- 15ml polystyrene centrifuge tube (supplied in the Synbiotics Collection Kit)
- Semen Separating Solution

Instructions

1. Break off the top of the pre-scored ampule of SSS. Draw up 0.75ml of solution and place in centrifuge tube.

2. Slowly layer the semen on top of the SSS to form two distinct layers. Although mixing of the layers is less desirable, it will still allow for separation with centrifugation.

   NOTE: Do not put more than 6ml of semen in one tube. If you have more than 6ml, prepare two tubes. Each ampule contains enough SSS to allow for this eventuality.

3. Centrifuge the semen and SSS at 2000RPM or 100-150G for 5 minutes. If your centrifuge is not graded in RPMs or Gs, use the "Urine" setting. This should produce a soft pellet of sperm cells at the bottom of the centrifuge tube. Spinning faster or longer than recommended may result in damage to sperm cells.

4. Using a pipette, remove the seminal plasma and SSS above the soft pellet. Remove as much as possible without disturbing the pellet.

5. Mix the pellet with the remaining SSS. Add Extender or Buffer as directed.