Strategies for Processing Semen from Subfertile Stallions for Cooled Transport

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KEYWORDS

• Equine • Stallion • Semen • Processing • Centrifugation

KEY POINTS

- Simple dilution of semen in extender is generally satisfactory for cooled transport of semen if certain guidelines are applied.
- Subfertility following insemination with cool-transported semen can be associated with different inciting factors.
- Concentration of sperm in semen can be achieved by filtration or centrifugation procedures. Currently, centrifugation is most commonly applied.
- Centrifugal fractionation of semen (also termed density gradient centrifugation) can be used to enhance sperm quality but recovery rates can be low, thereby necessitating low-dose insemination techniques for breeding purposes.

GENERAL GUIDELINES FOR CENTRIFUGATION OF SEMEN

A variety of centrifuge types can be used for centrifugation of semen; however, it is generally recommended to centrifuge semen in a centrifuge fitted with a swinging rotor so that the tubes are held in a horizontal position during the centrifugation process (**Fig. 1**). It is also sensible to secure a centrifuge with adapters that can accommodate either 50-mL or 15-mL centrifuge tubes. Centrifuges come in all shapes and sizes, and rotor radiuses (radii) can vary considerably among these units. Centrifugation speeds can be standardized for all centrifuges by using relative centrifugal force (or gravitational force [*g*]) to normalize centrifugation speeds among instruments, as opposed to revolutions per minute (RPM). Conversion tables are available for this purpose, as are Web-based conversion programs. Conversions can also be manually calculated by the following formula: $\text{RPM} = \sqrt{\left(\frac{g}{[0.0000112 \times r]}\right)}$, in which g = relative centrifugal force

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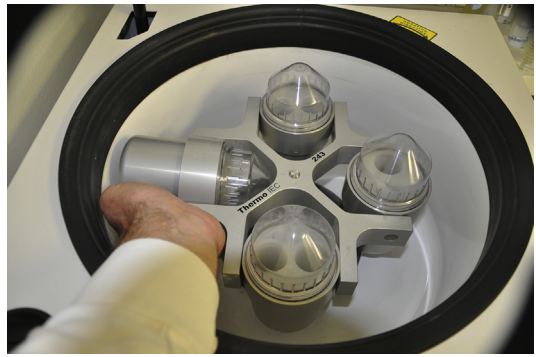

Fig. 1. Centrifuge with the adapter rotated into a horizontal position to demonstrate the mechanics of a swinging rotor during centrifugation. The rotational radius of the rotor can also be determined by measuring from the center of the rotor attachment to the bottom of the adapter when it is held in a horizontal position.

and r = rotational radius (in cm). Therefore, if a centrifuge rotor has a rotational radius of 15.5 cm and the centrifuge speed is designated to be 1000 × g, one would solve for RPM as follows:

$$\text{RPM} = \sqrt{\left(\frac{1000}{[0.0000112 \times 15.5]}\right)}$$

$$= \sqrt{\left(\frac{1000}{[0.0001736]}\right)}$$

$$= \sqrt{5760368.664}$$

$$= 2400$$

CUSHIONED CENTRIFUGATION OF SEMEN

As construed from the general principles previously noted for processing semen, concentrating sperm in samples to be prepared for cooled shipment may be necessary to meet the criteria of for maximum seminal plasma concentration (20%) and minimum sperm concentration (25×10^6/mL). This can be achieved by filtration to separate sperm from seminal plasma in raw or extended semen, or by centrifugation of extended semen. Currently, centrifugation of semen is the most commonly applied technique for this purpose. The goal of centrifugation is to maximize sperm recovery rate while avoiding injury to sperm during the centrifugation process. Understandably, an increase in centrifugation time and/or g-force yields an increased sperm recovery rate but it can also lead to decreased sperm quality associated with the amplified mechanical forces of centrifugation and excessive packing of the sperm. Under ideal circumstances, centrifugation would result in a 100% sperm recovery rate with no resulting damage in sperm quality. A variety of centrifugal times and forces have been applied in an attempt to achieve this goal; however, these protocols often lead to a 15% to 20% loss in sperm numbers that could otherwise be used for breeding purposes.