**Introduction**

Commercially available lubricants, labeled as non-spermicidal, are used to lubricate artificial vaginas prior to semen collection in stallions (Figure 1).

Improper type or amount of lubricant may affect stallion sperm quality, either after short exposure time or following cooled storage of extended semen.

**Experimental Aim**

Evaluate the effects of different commercial lubricants on an assortment of measures of sperm quality in stallions following:

1) Short-term exposure - 1 h (T1h) or
2) Long-term exposure - 24 h (T24h) of cooled storage.

**Materials and Methods**

Three ejaculates were collected from each of four stallions for the study using a Missouri model artificial vagina (AV, Nasco, Ft. Atkinson, WI, USA), which was lubricated with water-insoluble petroleum jelly (Vaseline™).

Prior to semen collection, semen extender (EquiPRO®, Coolguard®) was aliquoted into capped tubes, with extender containing no lubricant (Control), or 1% or 5% (w/v) of each of the following lubricants:

- **HR® Lubricating Jelly** (HR1, HR5, HR Pharmaceuticals Inc., York, PA, USA);
- **KY® Jelly** (KY1, KY5, Johnson and Johnson, New Brunswick, NJ, USA);
- **Therio-gel®** (TG1, TG5; Agtech Inc., Manhattan, KS, USA);
- **Priority Care®** (PC1, PC5; First Priority Inc., Elgin, IL, USA); and
- **Clarity®** (CL1, CL5; Aurora Pharmaceutical, LLC, Northfield, MN, USA).

Each tube was then placed on a mixer plate at 37 °C and gently inverted for 2 h prior to semen collection.

Gel-free semen (containing 30 x 10⁹ sperm/mL) was added to each tube, then samples were rotated gently at 37 °C for 1 h. Each treatment was then divided in two aliquots. One aliquot was subjected to immediate analysis (T1h), and one aliquot was placed in an Equitainer (Equitainer II™, Hamilton Research, Inc., South Hamilton, MA, USA) and evaluated after 24 h of cooled storage (T24h). Experimental endpoints were:

- Percent total sperm motility (TMOT);
- Percent viable acrosome intact sperm (VAI);
- Percent of sperm with abnormal DNA (COMP-α); and
- Percent of sperm with no or minimal DNA oxidative injury [8OHdG(-)]

Samples evaluated for abnormal DNA, lipid peroxidation and DNA oxidative injury were exposed to acid (HCl), ultraviolet light, or iron sulfate/hydrogen peroxide, respectively, as perturbations.

**Statistical Analysis**

Data were subjected to rank transformation, then analyzed using an ANOVA procedure within time (T1h, T24h). Means were compared using the Tukey’s studentized range test. Significance was set at P < 0.05.

**Conclusions**

Exposure of sperm to KY was detrimental to all sperm quality measures, except for 8OHDG. This may be due to the high non-viable sperm population that would not respond to the perturbation.

In general, exposure to 5% KY, PC, or TG lubricants yielded lower sperm quality, and the effect was most profound in KY. Most sperm quality measures were unaffected by different concentrations (1 or 5%) of HR and CL lubricants with values similar to control. Lubricant TG tended to yield lower values for sperm lipid peroxidation; however, TG increased sperm susceptibility to oxidative injury.

This study highlights the importance of using caution when selecting an artificial vagina lubricant for semen collection from stallions, even if lubricants are marketed as being safe for this purpose. Lubricants CL yielded high values for all 5 endpoints, whereas HR yielded high values for 4 endpoints; therefore, these lubricants might be the safest among those tested for collection of stallion semen.

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