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Abstract

The effect of artificial vagina lubricants on stallion sperm motion measures and semen pH during cooled storage

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1. Introduction

Stallion semen is routinely collected with an artificial vagina (AV). Several non-spermicidal lubricants are recommended for stallion AV preparation, but little is known about how exposure of semen to these may affect sperm longevity of motility or viability.

Lubricants commercialized as non-spermicidal for stallion semen collection include, HR Lubricating Jelly® (Carter Products, Division of Carter-Wallace Inc., NY) (HR), Priority Care Non-Spermicidal Sterile Lubricating Jelly® (First Priority Inc., Elgin, IL) (PC), and KY Jelly® (Personal Product Company, Division of McNeil-PPC Inc., Skillman, NJ) (KY). One study showed that a 10 min exposure to 10% KY at 37 °C was detrimental to stallion sperm motility [1]. Furthermore, KY has been reported to adversely affect human sperm motility [2,3].

The effects of long-term exposure of AV lubricants on stallion sperm have not been reported. The purpose of this study was to evaluate the effects of commonly used lubricants on longevity of stallion sperm motion measures during cooled storage.

2. Materials and methods

Three ejaculates were collected from each of five stallions of known fertility with ages ranging 12–29 years. Semen was collected using a Missouri model (four stallions) or a

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Colorado State University model (one stallion) AV. Immediately prior to semen collection, the AV was lubricated with a minimum amount (approximately 15 ml) of PC.

The hamilton-thorn motility analyzer (Hamilton-Thorn Research, Division of Hamilton-Thorn Biosciences Inc., Beverly, MA) (HTMA) was used to determine initial sperm concentration. The ejaculate was then diluted to 25×10^6 sperm/ml in a skim milk–glucose extender with Ticarcillin disodium (1 mg/ml). Extended semen was aliquoted into seven treatments of equal volume in conical disposable 50 ml centrifuge tubes. Treatments included: no lubricant (control); and 5 and 25% (v/v) concentrations of HR, PC, and KY lubricants. Each sample contained either 375×10^6 or 475×10^6 total sperm for the 25 and 5% (v/v) lubricant treatments, respectively. Tubes were packaged into individual Equitainers (Equitainer, Hamilton-Thorn Research, Division of Hamilton-Thorn Biosciences Inc., Beverly, MA) for slow cooling to 4 °C and storage.

Sperm motion measures analyzed with the HTMA included: percent total motility (MOT) and progressive motility (PMOT) and straight-line velocity (VSL; μm/s), before cooling (0 h), and at 24 and 48 h after cooled storage. Extended semen pH was determined at 0 and 48 h for all treatments.

Table 1
Effect of artificial vagina lubricants on stallion sperm motion measures and pH during cooled storage

Time (h)	Lubrication treatment	MOT[a] (%, ±S.E.M.)	PMOT[b] (%, ±S.E.M.)	VSL[c] (μm/s, ±S.E.M.)	pH (±S.E.M.)
0	Control	73 ± 3.6^{d}	49 ± 4.4^{d}	93.1 ± 4.4^{d}	6.9 ± 0.05^{d}
	5% HR	$70 \pm 3.5^{d,e}$	$47 \pm 4.5^{d,e}$	$85.3 \pm 4.5^{d,e}$	6.9 ± 0.05^{d}
	5% Priority care	64 ± 2.9^{e}	40 ± 3.7^{f}	81.2 ± 4.3^{e}	$6.8 \pm 0.05^{d,e}$
	5% KY	$67 \pm 3.8^{d,e}$	$44 \pm 4.6^{e,f}$	$87.0 \pm 4.8^{d,e}$	5.8 ± 0.20^{f}
	25% HR	64 ± 5.8^{e}	41 ± 6.5^{f}	65.0 ± 6.3^{f}	7.0 ± 0.04^{d}
	25% Priority care	39 ± 4.0^{g}	21 ± 3.1^{h}	53.0 ± 4.2^{g}	6.7 ± 0.04^{e}
	25% KY	55 ± 4.2^{f}	33 ± 5.0^{g}	73.0 ± 5.9^{f}	5.2 ± 0.20^{g}
24	Control	53 ± 3.8^{d}	27 ± 3.1^{d}	61.0 ± 2.6^{d}	–
	5% HR	35 ± 3.7^{e}	13 ± 2.2^{e}	43.0 ± 2.1^{e}	–
	5% Priority care	33 ± 3.2^{e}	15 ± 2.2^{e}	49.0 ± 3.0^{e}	–
	5% KY	15 ± 1.9^{f}	4 ± 0.7^{f}	42.0 ± 2.1^{e}	–
	25% HR	1 ± 0.7^{g}	0 ± 0.1^{f}	9.0 ± 2.3^{g}	–
	25% Priority care	3 ± 0.8^{g}	1 ± 0.3^{f}	22.3 ± 4.5^{f}	–
	25% KY	3 ± 0.9^{g}	1 ± 0.2^{f}	27.0 ± 3.5^{f}	–
48	Control	53 ± 4.7^{d}	23 ± 3.3^{d}	54.0 ± 2.5^{d}	6.9 ± 0.07^{d}
	5% HR	23 ± 4.4^{f}	7 ± 1.9^{f}	$37.2 \pm 2.1^{e,f}$	7.0 ± 0.05^{d}
	5% PC	31 ± 3.2^{e}	12 ± 1.8^{e}	43.2 ± 2.1^{e}	6.9 ± 0.06^{d}
	5% KY	10 ± 2.0^{g}	$3 \pm 0.6^{f,g}$	32.3 ± 3.4^{f}	6.9 ± 0.05^{d}
	25% HR	1 ± 0.6^{h}	0 ± 0.1^{g}	14.0 ± 3.9^{g}	7.0 ± 0.04^{d}
	25% PC	2 ± 0.9^{h}	0 ± 0.2^{g}	12.0 ± 3.8^{g}	6.7 ± 0.05^{e}
	25% KY	1 ± 0.4^{h}	0 ± 0.1^{g}	3.1 ± 1.7^{h}	6.1 ± 0.12^{f}

$n = 15$ ejaculates. Treatment aliquots were diluted to 25×10^6 sperm/ml in skim milk–glucose extender and packaged in individual Equitainers for cooled storage. Columns within time with different superscripts (d, e, f, g, h) differ $P < 0.05$.

[a] Percent total motility.

[b] Percent progressive motility.

[c] Straight line velocity.

Means for motion measures and pH were compared among treatments at each time period by ANOVA and mean differences were separated using Tukey's test ($P < 0.05$).

3. Results

Results for motion measures and pH are summarized in Table 1. Immediate exposure to KY resulted in a marked decrease in pH ($P < 0.05$).

Before cooling, samples exposed to 5% KY and HR had similar MOT and VSL to control samples. Samples with 5% HR also had similar PMOT to control samples ($P > 0.05$). For all other treatments exposure to lubricant resulted in an immediate decrease in sperm motion measures when compared to control samples ($P < 0.05$).

Motion measures decreased over time for all treatments. At 24 and 48 h of cooled storage, all treatments yielded lower motion measures than control samples ($P < 0.05$). At 24 h, MOT and PMOT were comparable for 5% HR and PC ($P \geq 0.05$), but lower for 5% KY ($P < 0.05$). At 48 h, samples with 5% PC had the highest MOT and PMOT over all other treatments ($P < 0.05$). For all lubricants, the higher concentration resulted in decreased motion measures over time ($P < 0.05$).

4. Discussion

We tested the effects of exposure to two concentrations of commonly used AV lubricants on stallion sperm motion measures initially and during Equitainer storage. Whereas it is probably unrealistic that a stallion ejaculate will be exposed to a 25% (v/v) of an AV lubricant, we wanted to optimize detection of any adverse effects that these compounds may have had on sperm viability. Conversely, a 5% (v/v) concentration is not as unrealistic if an AV is excessively or repeatedly lubricated, as it may happen when working with a slow-breeding or novice stallion, or if a low volume ejaculate is yielded. In any case, high concentrations of any of the lubricants tested will markedly decrease sperm motility.

In this study, exposure to low concentrations of KY reduced longevity of stallion sperm motion measures during cooled storage, compared to the other commonly used AV lubricants. That is consistent with previous findings that KY has a detrimental effect on stallion and human sperm motility [1–3]. Furthermore, KY contains the bacteriostatic agent clorhexidine gluconate, which has been shown to be spermicidal in human studies [4]. While we cannot confirm that our findings were due to clorhexidine exposure, the marked decrease in pH observed with KY-treated samples may also explain increased loss of motility over time.

Based on our results we do not recommend KY as a routine AV lubricant for stallion semen collection. Based on this study, PC may be the best lubrication when collecting semen for long-term cooled storage. For any lubricant, the quantities used when preparing an AV should be limited to a minimum amount, so as to avoid sperm exposure to these compounds. This may also be achieved by lubricating the AV immediately prior to semen collection, by avoiding repeated lubricant application and by limiting spread of lubricant to the proximal one-third of the AV.

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