

Use of a new artificial vagina without a liner reduced sperm motility in a stallion

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Abstract

A 24 year old Tennessee Walking Horse stallion was presented at beginning of breeding season for a sudden decrease in sperm motility noticed 1 hour after semen was collected with a new artificial vagina. Stallion was healthy and previous breeding soundness examinations indicated no signs of subfertility. Collection procedure, processing temperature, semen extender addition, and seminal plasma exclusion were examined as possible causes. None identified the reason for reduced motility. When the same artificial vagina, with or without a liner, was used to collect semen from this stallion and 2 other stallions, sperm motility increased for all 3 stallions when a liner was used. Therefore, the new artificial vagina was regarded as the cause of decreased sperm motility.

Keywords: Semen, stallion, artificial vagina, sperm motility

Background

Decreased sperm motility in stallions has been attributed to disease, age,¹ season,² hot weather,³ or stress induced by physical activity.³ Many other factors can contribute to a sudden decline in sperm motility, e.g. freezing or cooling procedures for preservation.³ We investigated the reason for an abrupt decrease in sperm motility in a healthy stallion that had normal sperm motility in the previous season.

Case Presentation

A 24 year old Tennessee Walking Horse stallion with no signs of disease and with normal fertility parameters in the previous breeding season had a sudden decrease in sperm motility (Table 1). Semen was collected in a new artificial vagina (AV) using an established (18 years) protocol. Breeding soundness examination did not identify any anomalies related to decreased motility. Sperm concentration, motility, and morphology were assessed immediately after collection. All parameters were within normal values (concentration: 150 - 190 x 10⁶/ml, motility 50 - 70%, morphology 60 - 71% normal sperm); however, 1 hour after sperm collection, sperm were immotile.

Table 1. Semen parameters in a stallion during 2012 and 2013 breeding seasons.

Date	Volume (ml)	Concentration (millions/ml)	Total sperm/ejaculate (billions)	Morphology (%)	Motility (%)
2/15/2012	60	280	16.8	55	82
2/17/2012	60	250	15	72	94
2/20/2012	50	273	13.5	74	82
2/22/2012	60	355	21.3	60	94
3/11/2013	75	190	14.2	60	70
3/13/2013	90	162	14.6	70	50
3/14/2013	50	173	8.7	60	50
3/15/2013	90	150	13.5	71	50

Causes for a decrease in sperm motility were investigated. Temperature was measured with an infrared thermometer (IRT207, General) in every step of semen processing and no changes occurred that could be related to a decrease in motility. Various semen extenders were used [(E-Z Mixing Original Formula (OF), E-Z Mixing Cool-Store-Transport (CST), and E-Z Freezing Modified French (MFR5)]

with no improvement in sperm motility. Semen was extended 1:1 with E-Z Freezing Modified French (MFR5); then, seminal plasma was removed by centrifugation (600 x g for 6 minutes) and the pellet resuspended with extender to eliminate possible detrimental effects of seminal plasma on sperm viability.⁴ This procedure did not improve sperm motility. Finally, it was decided to collect the stallion with and without a disposable plastic AV liner to determine whether direct contact of semen with the AV was deleterious. Sperm motility was maintained when a liner was used, but a sharp decrease occurred when a liner was not used. To verify if these results were specific to this stallion or were repeatable in other stallions, the same AV was tested, with and without a liner, in 2 other stallions (a 4 year old Appaloosa and a 11 year old American Paint Horse). Sperm from Stallion 2 were immotile even at 0 hours and Stallion 3 sperm were immotile at 1 hour (Figure). Presence of a spermicidal component in the newly purchased AV was suspected.

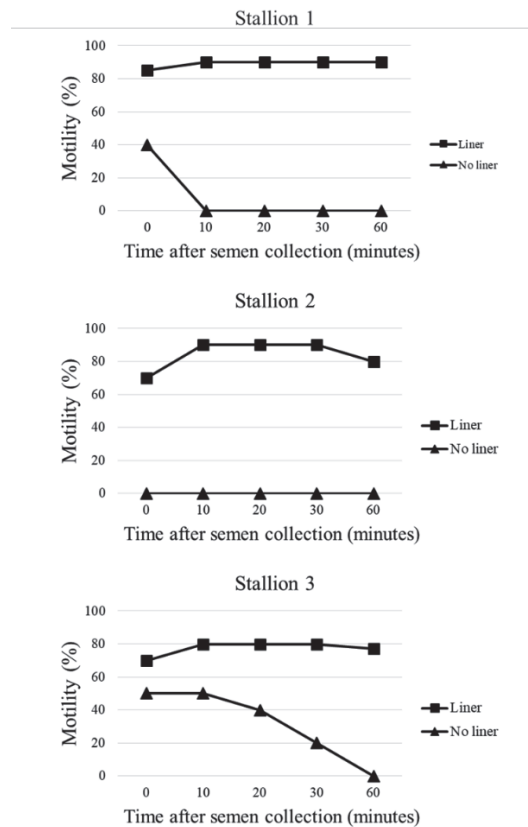


Figure. Sperm motility over time (from 0 to 60 minutes) of ejaculates from 3 stallions collected with and without a liner in the artificial vagina.

Differential Diagnosis

Chronic motility reduction has been reported in both young (< 3 years) and old (> 14 years), stallions,¹ during warmer seasons,^{2,3} or due to stress caused by physical activity.³ Infectious diseases (e.g. equine arteritis virus disease)⁵ or testicular trauma⁶ can adversely affect sperm motility. Iatrogenic spermicidal toxins or various handling methods can cause a sharp decrease in quality of semen parameters. Based on differences in semen motility when collecting with versus without a plastic AV liner, we inferred that the AV had component(s) that adversely affected sperm motility.

Treatment

Samples were collected from 3 stallions using the same AV previously described,⁷ both with and without a liner, and were evaluated for motility over time. The same extender was added to each sample following initial evaluation. After 1 hour, motility of each sample, collected using a liner was not affected. In samples collected without a liner, motility decreased to 0% within 1 hour following collection. When collected with a liner, semen motility was unaffected. Therefore, we inferred that the newly purchased AV caused decreased sperm motility.

Outcome

When a liner was used in AV, collected semen had expected sperm motility in all 3 stallions (Table 2). After 24 and 48 hours of cooling, sperm were immotile in semen collected without a liner (Table 2).

Table 2. Sperm motility of cooled extend semen 0, 24, and 48 hours after collection in 3 stallions.

Time (hours)	Stallion 1		Stallion 2		Stallion 3	
	Liner	No liner	Liner	No liner	Liner	No liner
0	85	40	70	0	70	50
24	70	0	45	0	50	0
48	60	0	10	0	15	0

Discussion

Sperm motility can be affected by season, cleaning protocol of AV and penis, poor management and semen handling practices, noninfectious and infectious diseases, and chemical residues in equipment used.^{2-4, 8-13} Reproductive pathology was not noted in these 3 stallions. Sperm motility was similar when tested without seminal plasma in these 3 stallions. Effect of season was unlikely to be a factor, as this apparent problem was noticed during the breeding season.

In the present case, the AV was suspected to be the reason for sudden change in sperm motility. Semen collection from 2 other stallions with and without a liner in AV confirmed that the AV was the most likely cause. Liners are not commonly used when a single AV is used for a particular stallion during a breeding season. Changes in materials to make a new AV by manufacturers could be detrimental to sperm and could go unnoticed until multiple mares are diagnosed nonpregnant. Testing a new AV is necessary to avoid misdiagnosis.

To our knowledge, this is the first case report of a spermicidal effect caused by an AV. A limitation of the study was not being able to determine the specific cause of reduced motility, whether it was due to a cleaning procedure or some toxic material in the rubber. Ideally, a semen sample collected with a different AV (routinely used for collection and proven nontoxic) without a liner would have made this argument (new AV as the cause) stronger. If both AVs had a similar problem with these stallions, then it could be due to some toxic residue in the AV after cleaning. However, if another AV yielded a normal sample compared to the new AV in question, then it could be attributed to some toxic material in the rubber. This approach is suggested for future investigation.

Learning points

- Artificial vaginal surface can be toxic to sperm.
- Surface of a new AV may contain toxic material that can render sperm immotile.
- Verify safety of a newly procured AV by testing semen quality immediately and 1 hour after collection.

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