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Diagnosis and effects of urine contamination in cooled-extended stallion semen

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ABSTRACT

Urospermia is known to affect semen quality in many mammals, including stallions. Determinations of semen pH and creatinine and urea concentrations have been used to diagnose urine contamination in raw stallion semen. Unfortunately, practitioners suspecting urine contamination in cooled-shipped samples have no proven means to confirm the presence of urine. Therefore, the objectives of this study were (1) to assess the effects of urine contamination on sperm motility of extended fresh and cooled-stored stallion semen, (2) to evaluate the usefulness of semen color, odor, pH, and creatinine and urea concentrations for urospermia diagnosis, and (3) to evaluate the accuracy of a commercial blood urea nitrogen test strip in diagnosing urine contamination in extended-cooled stallion semen. Thirty-seven ejaculates were obtained from 11 stallions with no history of urospermia before division into 5 mL aliquots, and contamination with stallion urine. Each resulting sample was assessed for sperm motility, color, odor, pH, creatinine, and urea nitrogen concentration using both a semi-quantitative test strip (Azostix), and a quantitative automated analyzer before and after cooling for 24 hour. Sperm motility parameters, pH, and creatinine and urea concentrations were analyzed using mixed models. Urine contamination decreased total and progressive motility in all samples before and after cooling ($P < 0.05$). Mean control total motility was 80% at 0 hour and 67% at 24 hours, whereas urine-contaminated samples ranged from 30% to 71% at 0 hour and 27% to 61% at 24 hours. Control mean urea (29 mg/dL) and creatinine (0.6 mg/dL) concentrations were significantly different ($P < 0.05$) from all urine-contaminated samples (158 mg/dL and 11.6 mg/dL, respectively) at 0 hour. Similarly, control mean urea (8 mg/dL) and creatinine (0.9 mg/dL) concentrations were significantly different than all urine-contaminated samples at 24 hours. Odor assessment presented moderate sensitivity (65%) and high specificity (100%), while color assessment presented low sensitivity (47%) and moderate specificity (79%) for urine in extended semen. Azostix strips were highly sensitive (95%) and specific (97%). Assessment of color, odor, and pH are not reliable methods to diagnose urine in experimentally contaminated cooled-stored stallion semen. Sperm motility parameters (in raw and cooled semen) are significantly reduced by the presence of urine in a concentration dependent. The results of the present study indicated that determination of urea and creatinine concentrations can be used to diagnose urospermia and that Azostix can be used as a point care method for diagnosing urine contamination in extended cooled stallion semen.

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1. Introduction

Contamination of semen with urine (urospermia) is a well-recognized ejaculatory dysfunction that affects the

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quality of raw semen and may affect fertility in stallions [1–3]. The effects of urine on semen quality appear to be mediated by an increase in semen pH and osmolarity [4]. In one report at a referral hospital, urine contamination was the second most common ejaculatory dysfunction in stallions [5]. Contamination of the semen with urine can occur at any time during ejaculation and can manifest as a continual or intermittent problem with an unpredictable pattern [6–9]. Urospermia has been associated with many conditions ranging from neoplasia or fractures that interfere with normal lumbosacral neurological pathways, to osteomyelitis, herpes-virus 1, sorghum toxicosis, cystitis, hyperkalemic periodic paralysis (HYPP), or idiopathic causes [1,6,7,10]. Overall, idiopathic causes appear to be the most prevalent [3,8].

Different management practices have been attempted to manage stallions presenting with recurrent urospermia, from encouraging stallions to urinate before collection or breeding, to urinary bladder catheterization before collection, fractionated semen collection with open artificial vagina, or pharmacologic intervention with drugs such as imipramine hydrochloride to improve bladder sphincter control [1–3,6,9]. However, the multifaceted and unpredictable occurrence of urospermia makes management and treatment difficult [2,7], and pharmacologic interventions such as bethanechol, imipramine, or furosemide are not always effective [11].

Although it has been shown that extending semen appears to mitigate the effects of urine contamination on sperm motility [4], the effects of urospermia on the motility or fertility of cooled stallion semen are not documented. A large amount of urine contamination can be easily detected in raw semen by evaluation of odor and color [3,7], but diagnosis of urine contamination in extended cooled-shipped semen can be challenging. In practice, urine-contaminated ejaculates may be shipped because of lack of knowledge of the personnel collecting and processing the semen or for fraudulent reasons without disclosure to the practitioner breeding managing the mare. Thus, objective means to detect urine contamination in cooled-shipped semen are warranted from both the practical standpoint and legal standpoint (e.g., grounds for another semen collection and shipping). Creatinine (>2 mg/dL) and urea (>25–30 mg/dL) concentrations have been suggested to be useful to diagnose urine contamination in raw stallion semen [3,12,13], and the strip-paper has been used as a screening tool for urine contamination in raw semen, where urea levels greater than 39 mg/dL indicate urospermia [12]. However, it remains unknown whether urine contamination can be properly diagnosed in cooled-extended semen using common means to assess urine contamination. We hypothesized that urine contamination affects sperm motility in extended cooled-stored semen in an amount-dependent manner and that measuring creatinine and urea can accurately diagnose urospermia in extended cooled-stored semen but not color, odor, or semen pH assessments. The objectives of this study were (1) to assess the effects of different amounts of urine contamination on sperm motility of extended fresh and cooled-stored stallion semen; (2) to evaluate the usefulness of semen color, odor, pH, creatinine and urea concentrations for diagnosis of urine contamination in fresh extended and extended-cooled stallion semen; and (3) to evaluate the accuracy of a commercial blood urea nitrogen test strip in diagnosing urine contamination in extended-cooled stallion semen.

2. Materials and methods

2.1. Animals, urine, and semen collection

Eleven reproductively healthy light breed stallions (four Standardbreds, four Quarter Horses, and three Thoroughbreds), averaging 9 years old (range 5–15 years old), with no known history of urospermia were enrolled in this study. All stallions were handled in accordance with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research, in accordance with the University of Illinois Institutional Animal Care and Use Committee protocol #1400.

All animals were housed at the same research facility in small turnout runs and fed similar grass and/or alfalfa hay diets. Free catch urine samples were obtained from three of the enrolled stallions, pooled and frozen at −20 °C until further use. Each stallion was collected on a dummy phantom, with a teaser mare present in the breeding shed using a Missouri model artificial vagina (Nasco Fort Atkinson, Wisconsin). Stallions were collected at 2 to 3-day intervals over a 2-week period in November of 2014 until a total of 37 ejaculates were obtained. The artificial vagina was lubricated (Priority Care1, First Priority Inc., Elgin Illinois) and fitted with a clean inline sperm filter (Har-Vet, Spring Valley, Wisconsin) for each semen collection.

2.2. Raw semen evaluation and processing

Immediately after semen collection, each ejaculate was assessed for initial motility using Computer-assisted sperm analysis (CASA) using default settings recommended by the manufacturer (Spermvision, Minitube of America, Verona, Wisconsin) for equine sperm. Semen was extended with a milk protein–based extender (INRA 96 IMV; Maple Grove, Minnesota) to 25 million sperm/mL. A small aliquot (10 μL) of extended semen was placed on a heated slide with a coverslip, and assessed using CASA. Parameters analyzed included total percent of sperm motility, progressive sperm motility, sperm velocity parameters (VCL, curvilinear velocity [μm/s]; VAP, average pathway velocity [μm/s]; and VSL, straight-line velocity [μm/s]), and coefficients (LIN, linearity [%], STR, straightness [%], and WOB, wobble [%]) were assessed for all samples using CASA.

Sperm concentration in raw semen was analyzed in standard fashion using a spectrophotometer according to the manufacturer's recommendations (Equine Densimeter; Animal Reproduction Systems, Chino, California). Measurement of semen pH was attained using a hand-held pH meter (LAQUA Twin; Horiba Instruments, Irvine, California). The sample well was filled until the bottom of the chamber was covered, and a reading was recorded when a stable measure was achieved. A standard buffer solution (pH = 7) was used to calibrate the pH meter immediately before each analysis. The loading chamber was thoroughly

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