



Diagnosis and management strategies for urospermia in stallions

Diagnóstico e estratégias de manejo na urospermia em garanhões

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Resumo

Urospermia, contaminação do sêmen com urina durante a ejaculação, é considerada a segunda causa mais frequente de disfunção ejaculatória em garanhões. A presença de urina no sêmen pode afetar drasticamente a fertilidade através do pH alcalino e alta osmolaridade da urina, além de causar uma potencial inflamação uterina após a cobertura ou inseminação artificial. Esta condição pode estar associada a outras doenças como cistite, herpesvírus equino-1 e paralisia periódica hipercaleêmica; no entanto, causas idiopáticas são as mais comuns. Por esse motivo, o tratamento da urospermia pode ser frustrante e o prognóstico para a resolução completa dessa condição costuma ser desfavorável. Garanhões que sofrem de urospermia são frequentemente diagnosticados após resultados frustrantes de fertilidade e baixa qualidade do sêmen. O diagnóstico de urospermia é baseado na aparência amarela e odor típico, presença de cristais de urina, pH alcalino, além do aumento da creatinina e níveis de nitrogênio. O tratamento da urospermia é desafiador, já que a maioria dos casos é idiopática, limitando as opções terapêuticas. No entanto, quando uma condição primária é diagnosticada, ela deve ser tratada para tentar resolver a causa da urospermia. Na maioria dos casos, as terapias direcionadas à redução da contaminação da urina incluem a redução da quantidade de urina na bexiga antes da cobertura ou coleta de sêmen, tratamento farmacológico para auxiliar o fechamento do colo da bexiga durante a ejaculação, ou a coleta apenas da porção rica em esperma através da coleta fracionada do ejaculado. O método mais simples e estabelecido para o manejo da urospermia é encorajar o garanhão a urinar antes da coleta de sêmen ou cobertura natural. No entanto, quando a coleta de sêmen livre de urina não é possível, o sêmen contaminado com urina pode ser processado para minimizar os efeitos adversos do pH e da osmolaridade ao esperma. O sêmen contaminado com urina deve ser diluído com diluente à base de leite no intuito de diminuir os efeitos deletérios da urina sobre os espermatozoides. A centrifugação com gradiente de densidade a fim de selecionar os espermatozoides com características superiores também é uma alternativa nesses casos. Além disso, a criopreservação de sêmen contendo baixos níveis de contaminação com urina pode ser realizada.

Palavras-chave: Sêmen, urina, fertilidade, andrologia, equino.

Abstract

Urospermia is the contamination of semen with urine during ejaculation. Urospermia is the second most frequent ejaculatory dysfunction of stallions. It can drastically affect fertility mediated by alkaline pH, the high osmolarity of urine, and presumably excessive post-breeding inflammatory response. This condition can be associated with many other diseases (i.e., cystitis, equine herpesvirus-1, and hyperkalemic periodic paralysis); however, idiopathic causes appear to be predominant. For this reason, the treatment of urospermia can be frustrating, and the prognosis for complete resolution of this condition is often poor. Stallions suffering from urospermia are usually diagnosed after poor semen quality and fertility results. Diagnosis of urospermia is based on the yellow appearance, urine smell, urine crystal, alkaline pH, increased creatinine, and urea nitrogen levels. The treatment for urospermia is challenging. Most cases are idiopathic, thereby limiting therapeutic options. However, when a primary condition is diagnosed, it should be treated to solve the cause of urospermia. In most cases, therapies directed at reducing urine contamination include reducing the amount of urine in the bladder before breeding, pharmacological treatments to enhance bladder neck closure during ejaculation, or collecting only the sperm-rich portion of the ejaculate using an open-ended artificial vagina are used to manage stallions with urospermia. The simplest and most established method for managing urospermia is to encourage the stallion to urinate before semen collection or natural breeding. However, when the

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collection of semen free from urine is not possible, urine-contaminated semen can be processed to minimize the adverse effects of pH and osmolarity on the sperm. Semen contaminated with urine should be immediately extended with a milk-based extender to mitigate the deleterious effects of urine on the sperm. Single-layer gradient centrifugation can also be used to select sperm with superior traits for low urine contamination. In addition, semen cryopreservation can be performed in stallion semen with a low level of urine contamination.

Keywords: semen, urine, fertility, equine, andrology.

Introduction

Urospermia (urine contamination of the semen) is one of the most common ejaculatory dysfunctions affecting stallions (McDonnell 1992). It may occur continuously or intermittently with an unpredictable pattern (Varner et al., 1991, Turner et al., 1995, Lowe 2001). Urine contamination can affect stallion fertility (Varner et al., 1991). Unfortunately, some stallions may present urospermia throughout their entire breeding career, making breeding management for optimal fertility a true challenge (Turner et al., 1995, Dascanio 2014).

Detrimental effects of urine on sperm appear to be mediated by the alkaline pH, high osmolality, and possible high concentrations of urine crystals (Varner et al., 1991, Griggers et al., 2001, Dascanio 2014). Urine is also thought to interfere with fertility by inducing excessive post-breeding endometrial inflammation on mares bred by stallions suffering from urospermia (Dascanio 2014). As semen extension appears to mitigate the effects of urine on stallion sperm (Griggers et al., 2001; Ellerbrock et al., 2016), it is also thought that post-breeding endometrial inflammation can be mitigated by extending urine contaminated semen before artificial insemination; however, this has not been critically tested.

Urospermia has been associated with a wide range of conditions such as neoplasia or fractures that interfere with normal lumbosacral neurological pathways, osteomyelitis, equine herpesvirus 1, sorghum toxicosis, cystitis, hyperkalemic periodic paralysis, or idiopathic causes (Naylor et al. 1999; Turner 2007, Dascanio 2014). Overall, idiopathic causes appear to be the most prevalent, challenging our ability to treat or manage the condition (Turner 2007).

Despite several clinical practices to prevent urospermia, many stallions may still present urospermia, even after repeated attempts to obtain semen samples free of urine. Different procedures have been attempted to reduce the amount of urine contamination with variable results (Varner et al., 1991, Hoyos et al., 1999, Dascanio and Witonsky 2005, Turner et al., 1995). Unfortunately, genetically and economically valuable stallions may present ejaculates recurrently or constantly affected by urine contamination, which causes significant economic losses to the industry. Therefore, this manuscript aims to discuss the pertinent literature concerning urospermia in stallions and contrast it with the authors' experiences diagnosing and managing the condition in clinical practice.

Overview physiology of ejaculation

The etiology of urospermia is not fully understood (Varner et al., 1991; Turner, 2011). However, it is known that any functional disturbance of the complex nervous mechanism controlling ejaculation (e.g., emission and ejaculation) can lead to contamination of semen with urine (Leendertse et al., 1990; Mayhew, 1990). Urospermia may involve failure to close the internal bladder sphincter due to mechanical deficit (e.g., carcinoma) or injury on the autonomic parasympathetic and sympathetic systems controlling urination, erection, and ejaculation (Hoyos Sepulveda et al., 1999).

Emission, which is the transport of sperm from the tail of the epididymal to the ampulla by contraction of the ductus deferens and accessory glands before ejaculation, occurs as the result of a thoracolumbar reflex arc; simultaneously with emission, there is a contraction of the bladder trigone avoiding urine passage during the ejaculation (McDonnell 1992, Turner, 2011).

Ejaculation and emission are predominantly controlled by a sacral reflex of the pudendal nerve and an α -adrenergic sympathetic event; thus, lesions at these locations can lead to ejaculatory dysfunction (Mayhew, 1990). Although sensorial stimulation of the penis is the physiological trigger to induce ejaculation, it can also occur without this stimulus, as is the case of pharmacologically induced ejaculation (McDonnell and Odian, 1994).

The copulation and ejaculation in horses last about 35-40 seconds. During ejaculation, pulses can be felt at the shaft, medially at the penile urethra. The release of jets of semen accompanies the pulses of



the urethra. A total of 5-8 jets are typically felt in horses (Varner et al., 1987; Kareskoski et al., 2006), and the first three jets contain 80% of the total number of sperm ejaculated and represent <50% of the final volume (Varner et al., 1991). The following jets have more seminal plasma and less sperm due to the contribution of the accessory sex glands; thus, urine contamination can happen at the beginning, middle, or the end of ejaculation (Varner et al., 1991).

Etiology of urospermia

The majority of conditions causing urospermia are not specific to the urinary tract. Idiopathic causes are still the most prevalent association with cases of urine contamination of the ejaculate, and often the intermittent nature of the urospermia cannot be diagnosed. Of interest, often stallions with idiopathic urospermia have some level of urinary incontinence or bladder dysfunction (Turner, 2011). Many conditions such as neoplasia or fractures that interfere with normal lumbosacral neurological pathways, sorghum toxicosis, cystitis, equine herpes-virus 1, hyperkalemic periodic paralysis have been reported in association with cases of urospermia in stallion (Nash et al., 1980; Naylor et al., 1999; Lowe, 2001; Turner, 2011).

Sorghum grazing causes enzootic equine cystitis, hindlimb ataxia, and bladder paralysis (Adams et al., 1969). A combination of bladder paralysis and cystitis is thought to lead to urospermia. Stallions and geldings grazing sorghum present urinary incontinence and a history of urine dripping from the penis (Adams et al., 1969). Sublethal doses of cyanic acid contained in sorghum and its crosses can cause degeneration of the spinal cord and nerve fibers innervating the bladder, thus causing bladder paralysis (Adams et al., 1969).

The most apparent problem in semen contaminated with urine is the reduced sperm quality of the ejaculate or poor fertility of stallions bred by natural cover. The contamination can happen anytime before, during, or immediately after ejaculation (Varner et al., 1991). Even though the toxic effects of urine on sperm are not well known, it is suggested that urine contamination negatively affects sperm by alkaline pH and high osmolarity, and possibly high concentration of urine crystals (Griggers et al., 2001). It has also been recommended that detrimental effects of urine affect fertility in a dose-dependent manner (Ellerbrock et al., 2016; Ellerbrock et al., 2018). Urine contamination can vary from a few millimeters to >250 mL (Varner et al., 1991). However, the minimal amount of urine affecting fertility has not been determined.

Diagnosis

The presence of urine in an ejaculate can be suspected in a sample with an excessive volume, semen with a characteristic urine smell, and yellow-tinged color semen (Varner et al., 1991) (Figure 1). Experimentally, urine contamination and extension cannot be visually detected when small amounts of urine are present just when a large amount is present (Figure 2). The diagnosis of urospermia can be challenging in stallions presenting a small amount of urine in the ejaculate and those breeding mares by the natural cover (Varner et al., 1991; Turner, 2011; Ellerbrock et al., 2016).

The urospermia should be immediately evident with semen collection when a large amount of urine is present; however, stallions may present intermittent urospermia, or a variation in the amount of urine into semen makes the diagnosis more difficult (Turner, 2011; Ellerbrock et al., 2016). Thus, a diagnosis of urospermia may take serial semen collections on different days.

A large amount of urine can be easily detected by visual inspection of the gross appearance (e.g., yellow to amber color) and odor of the semen (Ball, 2008; Ellerbrock et al., 2016; Voge et al., 2016). In cases of mild contamination, it is often possible to detect changes in the odor of the semen, and test analysis detecting an increased pH (7.7 or above) may be suggestive of the presence of urine or incomplete ejaculation (Varner et al., 1991; Hurtgen, 1992). However, in cases with minor gross urine appearance in which only a small amount of urine might be contaminating the ejaculate, additional laboratory tests such as creatinine and urea are required to detect the presence of urine in the semen (Varner et al., 1991). Creatinine concentrations >2.0 mg/dL and urea nitrogen >30 mg/dL are indicative of urospermia (Varner et al., 1991).

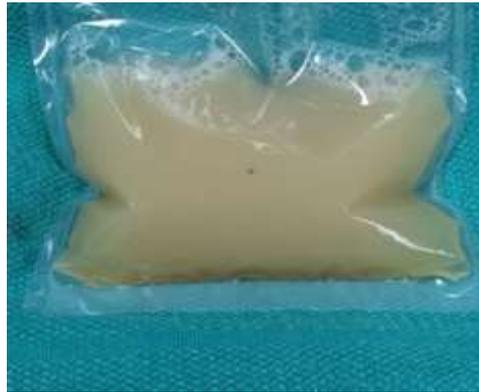


Figure 1. Urospermia obtained from a stallion affected with hyperkalemic periodic paralysis. The characteristic, yellow-tinged color, large volume ejaculated, and urine odor could be continuously detected in the ejaculates of this stallion.

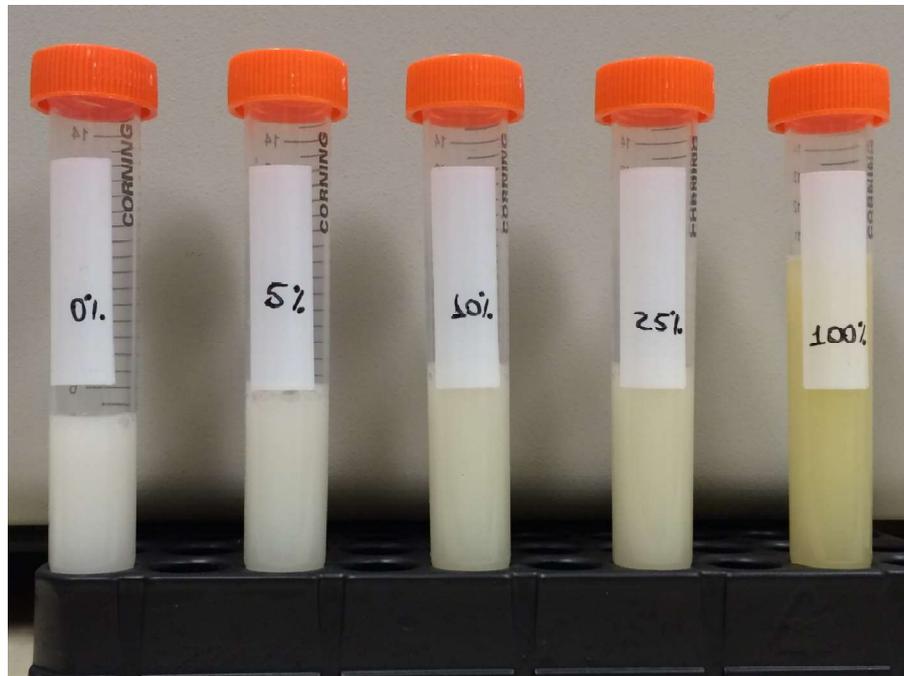


Figure 2. Urine contamination and extension of stallion semen. Small amounts of urine cannot be easily detected, only when large amounts are present.

For rapid diagnosis of urine in semen, or in the absence of a diagnostic laboratory, clinicians can use commercially available strip tests used to detect the presence of blood urea nitrogen in small animals (Azostix[®]) or strip tests for urine analyses (Multistix[®]) (Althouse et al., 1989; Varner et al., 1991; Ellerbrock et al., 2016). However, care must be taken when using these horse-side tests, as false-positive results are expected when the manufacturer's recommendations are not strictly followed (Varner et al., 1991).

The Azostix test strip detects urea concentrations by color change (yellow to green) on the strip. The reagent pad should be fully immersed in the sample being tested. After 60 seconds, the pad should be rinsed thoroughly with distilled water and immediately directly compared to the color chart on the side of the Azostix bottle. No change in color corresponds as negative for the presence of urea. The four colors categories corresponded, from lightest to darkest, to (5–15 mg/dL), (15–26 mg/dL), (30–40 mg/dL), and (50–80 mg/dL). Azostix strips are graded as positive for urine contamination if the strip reads greater than 5 mg/dL in the raw and 5 mg/dL in the extended cooled semen samples (Ellerbrock et al., 2016). The Azostix test has been reported to be effective in detecting urine contamination in both raw or extended



semen samples (Althouse et al., 1989; Varner et al., 1991, Ellerbrock et al., 2016).

The Multistix diagnostic test contains ten reagent pads that can detect glucose, bilirubin, ketones, specific gravity, blood, pH, protein, nitrites, urobilinogen, and leukocytes in a sample. The nitrite pad had been reported to elicit a color change when semen is contaminated with urine (Althouse et al., 1989, Varner et al., 1991). Multistix is tested by immersing the reagent areas of the strip into the sample to be tested. The strip should be held in a horizontal position and examined for a color change. For example, the nitrite pad elicits a color change from yellow to radiant orange when urine is present in semen samples 3.5 minutes following immersion (Althouse et al., 1989, Varner et al., 1991).

Other methods of diagnostics that can suggest urospermia in stallions are the presence of urine crystal into semen and the mare's uterus after natural cover. The presence of urine crystals into semen can be detected during semen evaluation by microscopic examination and used as an indicator of urospermia. In some specific stallions breeding by a natural cover, urine crystals may be seen in the uterus of mares by transrectal ultrasonography as hyperechogenic accumulations. However, this diagnosis can only be ruled out when there are a considerable amount of urine crystals in the mare's uterus. It should not be used as a gold standard diagnostic method (Turner, 2011).

Once the stallion is diagnosed to be suffering from urospermia, an in-depth clinical examination should be pursued to determine if urospermia is associated with severe diseases. Observation of neurological or urinary tract disorders, such as dysuria, polyuria, and stranguria, can be helpful to diagnose cases of urospermia. In addition, it is recommended a complete neurological examination (e.g., anal sphincter tone and reflex, tail tone, cranial nerve function, limb function, defecation pattern, and frequency) of the stallion to rule out any neurological disorder that can be associated with this ejaculatory dysfunction (Varner et al., 1991; Turner et al., 1995; Turner, 2011). Even though this diagnosis can be challenging in some animals, it can be performed by careful clinical neurological evaluation or videotaping the stallion into the stall. It can be helpful to the diagnosis of the primary cause of urospermia.

Evaluation of the urinary tract should be performed to assess the bladder competence to evacuate urine and abnormalities in the bladder wall (e.g., lesions) and content (e.g., uroliths, inflammation) (Varner et al., 1991; Turner, 2011). The urinary tract can be evaluated by transrectal palpation and ultrasonography. A rectal examination should include evaluation of rectal tone and residual urine volume after urination and thorough palpation of the pelvic contents with particular attention to the ventral surface of the sacrum, where fractures may be detected (Varner et al., 1991; Turner, 2011). An abrupt loss of rectal tone can be palpated with sacral nerve root lesions as the hand is withdrawn from the proximal to the distal rectum (Mayhew, 1990). It should be performed before and after the voluntary urination; therefore, the ability to deplete urine can be evaluated. In addition, the ultrasound exam may reveal abnormalities in the bladder wall or the bladder content. To assess the bladder sphincter competence, the bladder can be pressured during rectal palpation. The amount of pressure required to induce urination can be used to indicate the sphincter's bladder competence. The bladder should not be expressed manually per rectum, which suggests the presence of some functional sphincter and bladder neck tone (Varner et al., 1991; Turner, 2011).

Bethanechol chloride can induce evacuation of the bladder (Varner et al., 1991; Hoyos Sepúlveda et al., 1999). This drug can be administered subcutaneously (0.07 mg/kg) followed by evaluation of the bladder content after urination, which occurs around 15 minutes after treatment (Varner et al., 1991; Hoyos Sepúlveda et al., 1999). A sterile urine sample should also be collected for urinalysis and urine culture. For this, a sterile catheter can be inserted after sanitation of the penis, and the samples must be sterilely collected and submitted for analysis.

Diagnosis of urine contamination in cooled-shipped semen

Urine contamination in raw semen can often be easily detected by visual inspection; however, diagnosing the presence of urine in extended cooled-shipped semen or present in small amounts can be challenging (Ellerbrock et al., 2016). In practice, semen contaminated with urine may be inadvertently processed and shipped out or for fraudulent reasons without disclosure to the practitioner breeding managing the mare (Ellerbrock et al., 2016;).

Although assessment of color and odor are not reliable methods to diagnose urine in extended cooled-stored stallion semen and alkaline pH is only suggestive, urea and creatinine concentrations can be used to determine the presence of urine in these semen samples (Ellerbrock et al., 2016). It is important to note that the concentration of urea and creatinine to detect urine contamination in extended semen differs



from in raw semen. Our study indicated a potential threshold for urea and creatinine concentrations in extended cooled samples should be greater than 12 and 1.3 mg/dL, respectively, to diagnose urospermia (Ellerbrock et al., 2016). When compared with the concentrations used to detect urine in raw semen (>30 mg/dL of urea and >2.0 mg/dL of creatinine), this difference can be explained by the dilution of semen with an extender. In addition, in practice, the urea blood nitrogen test strip Azostix can detect the presence of even small amounts of urine in extended cooled-shipped semen samples when the manufacture protocol is strictly followed (Ellerbrock et al., 2016).

Treatment and management of the stallion with urospermia

There is scarce literature regarding treatment for urospermia in stallions. When a primary cause of urospermia is identified, it should be treated to solve the problem and mitigate the urospermia (Varner et al. 1991). However, as previously discussed, most cases are idiopathic, thereby limiting therapeutic options (Leendertse et al., 1990; Lowe, 2001; Turner, 2011). Therapies directed at reducing urine contamination include reducing the amount of urine in the bladder before breeding, pharmacologic treatment to enhance bladder neck closure during ejaculation, or collecting only the sperm-rich portion of the ejaculate using an open-ended artificial vagina (Varner et al. 1991, Turner, 2011).

Changes in the management of the stallion are often involved in the treatment of stallions with urospermia. The simplest and most established method for managing urospermia is to encourage the stallion to urinate before semen collection or natural breeding (Leenderstse et al., 1990). Techniques for inducing urination in stallions include movement to a freshly bedded stall, pile old stall bed in the center of the stall, placement of feces from another stallion in the subject stallion's stall or moving the stallion to the stall of another stallion in which a fecal pile from that other stallion is visible, and also the use of diuretics (e.g., furosemide) can be an option (Varner et al. 1991, Turner, 2011). Some racehorses are trained to urinate on command, such as a whistle, as part of the anti-doping testing before racing; such training can benefit stallions suffering from urospermia. The use of diuretics may be helpful if the stallion completely voids his bladder before ejaculation but may alternatively result in a large volume of dilute urine being deposited in the ejaculate that would be detrimental to fertility (Griggers et al., 2001). These techniques can help reduce the frequency and severity of urospermia; however, they do not always result in a urine-free ejaculate. Many horses retain some variable amount of residual urine in the bladder after voluntary voiding that can still be expelled during ejaculation; stimulating voluntary urination before ejaculation remains central to the management of urospermia (Varner et al. 1991, Turner, 2011). The authors observed that changing the time of semen collection for midday can be beneficial for reducing the probability of having an ejaculate contaminated with urine when compared with early morning.

Drugs administered before semen collection to treat stallions with urospermia include diuretics, α -adrenoceptors agonists, β -adrenoceptors antagonists, tricyclic anti-depressants, muscarinic parasympathetic receptor antagonists, muscarinic parasympathetic receptor agonists, and hormones. These drugs are intended to (1) promote the evacuation of the bladder before ejaculation; (2) to augment the tone of the external urethral sphincter during ejaculation to prevent contamination of semen by urine; or (3) alter production of urine (Hoyos Sepulveda et al., 1999). Tricyclic antidepressants, such as imipramine, have been used as therapy for urospermia in stallions to increase the tone of the bladder sphincter (McDonnell, 1992) and the external urethral sphincter during ejaculation (Turner et al., 1995). Imipramine should be administered at 500-1000 mg/stallion orally 2 to 3 hours before semen harvesting. Also, the stallion can be observed for urination and semen collected immediately after the stallion had voided urine. Suppose the first ejaculate had gross urine contamination. In that case, a second ejaculate should be collected 10 to 15 minutes later (Turner et al., 1995) if the stallion has enough libido for a repeated semen collection in such a short interval.

Imipramine also appears to enhance contractility of the bladder neck during emission and has helped treat urospermia. The mechanism of action is not entirely understood, but apparently, tricyclic antidepressants and their metabolites promote α -adrenergic activity by inhibiting the reuptake of norepinephrine (McDonnell, 1992). Conversely, a trial with three stallions displaying urospermia demonstrated no beneficial effect of imipramine on ensuing seminal plasma biochemistry, including creatinine and urea concentration (Hoyos Sepulveda et al., 1999). In general, these pharmacological therapies should be combined with management to reduce the probability of urine contamination. The authors of the present review have excellent results in clinical practice with urospermia stallions by administering 1000-2000 mg of imipramine orally 2-3 hours before collection, coupled with strategies to stimulate the stallion to urinate before collection.



Another option in cases in which voluntary urination voiding or pharmacologic management is insufficient is to pass a catheter into the bladder for urine drainage and bladder flush (Turner, 2011). This method can also be used in cases where a urine-free ejaculate must be obtained in a single session (e.g., for semen freezing). After drainage of the urine, the bladder can be flushed with sterile physiological saline, Lactate ringer solution preferably, to ensure the complete emptying of the bladder and that any residual bladder contents will be expelled during ejaculation (Turner et al., 1995). In highly tractable animals, the procedure can be performed in the breeding shed after the stallion has obtained an erection, thus avoiding the need for sedatives or tranquilizers and allowing for hand-mating or semen collection to proceed as soon as the bladder is fully evacuated. In cases in which the stallion has to be sedated, α 2-adrenergic drugs (e.g., xylazine and detomidine hydrochloride) are preferred, and yohimbine hydrochloride at a dose of 0.03-0.1 mg/kg can be used after the procedure to revert the effects of sedation before semen collection (Grubb et al., 1997). Unfortunately, α 2-adrenergic drugs stimulate urine production. In addition, it is important to note that repeated catheterization of the bladder can predispose the stallion to bacterial cystitis and urethritis (Varner et al., 1991).

Fractionated semen collection is another approach that can be used to manage some stallions with urospermia (Nash; Voss; Squires, 1980; Varner et al., 1991). Stallions often ejaculate in 6-8 fractions, and the first three fractions are considered sperm-rich fractions (Kareskoski et al., 2006). In some stallions, using fractionated semen collection is possible to obtain urine-free semen (Nash et al., 1980; Varner et al. 1991, Turner, 2011). However, in practice, the variable pattern of urospermia can make this approach frustrating (Varner et al. 1991). The urine contamination can vary among animals and ejaculates (Varner et al. 1991). Although the contamination follows ejaculation in most stallions, it may also precede or coincide with ejaculation, making it challenging to manage these animals (Varner et al. 1991). A semen extender can also be placed in the collection bag, trying to minimize the adverse effects of urine on sperm. It is also important to carefully control the extender temperature not to cause cold shock to the sperms.

Pharmacological ejaculation has been suggested as a possible approach to obtaining semen free of urine from stallions suffering from urospermia. Although this approach has not been critically tested with this proposed yet, it has been reported to minimize contamination and improve semen quality in a stallion suffering from seminal vesiculitis (Cavalero et al., 2020). Furthermore, it has been suggested as during pharmacological ejaculation, there is no increase in abdominal or intra-vesicular pressure and thus may minimize pressure on the urethral sphincter and bladder neck at the point of ejaculation. However, it is essential to note that the success of pharmacological ejaculation is variable among stallions (0-50%) (McDonnell and Odian, 1994; Cavalero et al., 2019). Therefore, it is difficult to use this approach in standard management for semen collection and breeding, and cryopreservation of semen samples obtained using this approach should be considered.

Approaches to process urine-contaminated ejaculate

Often despite treatment and management strategies, stallions still yield ejaculates contaminated with urine; thus, strategies have been studied to manage such stallions. Sometimes stallions with high libido may provide an ejaculate contaminated with urine if semen is collected after a long trailer ride; semen of such stallions can be collected within one hour and yield a urine-free sample. However, if a similar clinical scenario happens in a low libido stallion, semen might not be successfully collected on the same day; thus, the practitioner needs to collect and process semen as described below.

As aforementioned, an excellent approach to start managing semen of stallions with urospermia is to minimize the direct effects of alkaline pH and high osmolarity of the urine contamination to the sperm (Griggers et al., 2001, Ellerbrock et al. 2016). Semen extension with a milk-based extender may mitigate the detrimental effects of urine (Grigger et al. 2001). Egg yolk-based extender resulted in inferior results than skim milk-based extender, indicating a negative interaction between egg yolk and urine (Grigger et al. 2001).

Removing the urine can potentially help to increase the longevity of contaminated semen. However, by the presence of urine crystals, standard centrifugation protocols might negatively affect sperm motility and viability after centrifugation (Griggers et al., 2001). Centrifugation of the urine-contaminated semen and resuspension with fresh quantities of either extender do not improve motility above simply adding an extender. Of interest, skim milk extenders restore motility better than the egg yolk extender at a 1:3 ratio immediately after standard centrifugation (Griggers et al., 2001).

An alternative to removing urine of stallion contaminated semen is to submit the semen, after

immediate extension in semen extender or if the extender was placed in the collection bottle, to a cushioned centrifugation ($1000 \times g/20$ min) and resuspension of the sperm pellet in fresh semen extender (Voge et al., 2016). It is suggested that the cushion centrifugation avoids the adverse effects of the concentration of urine crystal in the sperm pellet, as the urinary solids pass through the cushion and reach the bottom of the centrifuge tube, therefore, separating from the sperm fraction (Voge et al., 2016). However, in the author's opinion, the crystal can break the tension in the cushion solution and cause the deposition of urine crystals at the bottom of the tube (Figure 3). Removing the supernatant using SpermFilter has been proposed as another alternative to avoid the adverse effects of mechanical damage to the sperm contaminated with urine. However, in the authors' experience, this approach clogs the pores of the Sperm-filter and prevents filtration of the sample. It results in more profound exposure to urine, causing further sperm damage (Figure 4). This clinical observation needs to be critically investigated.

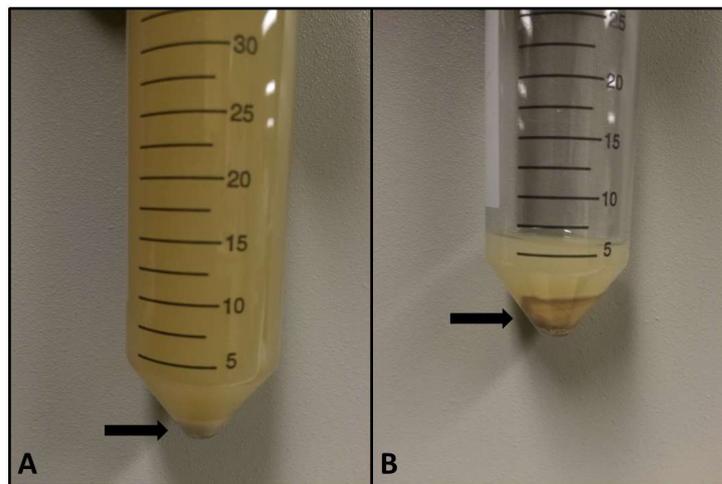


Figure 3. (A) Standard centrifugation (B) Cushion-centrifugation of stallion sperm suffering from urospermia. In both photographs, urine crystals can be observed at the bottom of each tube.



Figure 4. Sperm filter of semen from a stallion suffering from urospermia. The light yellow-tinged appearance denotes that this stallion had minor urine contamination.

Cryopreservation of urine-contaminated sêmen

Previously, it was assumed that semen from stallions presenting recurrent urospermia could not be frozen. Thus, a common standard practice was to discard the urine-contaminated ejaculate and re-collect the stallion to obtain a urine-free ejaculate (Ellerbrock et al., 2018). However, some valuable



stallions experiencing urospermia may also have additional problems such as orthopedic, neurological, or behavioral impairing their ability to be re-collected in an attempt to obtain a urine-free semen sample. Our recent studies showed that semen contaminated with 20% of urine, immediately extended in a milk-protein-based extender, and cushion-centrifuged could be frozen in a commercially freezing semen protocol (Ellerbrock et al. 2018). The same study demonstrated that semen contaminated with up to $\leq 20\%$ (v:v) of urine might be suitable for freezing, as post-thawed motility parameters and sperm viability were not different between the urine free- and urine contaminated-semen samples (Ellerbrock et al. 2018, Podico et al., 2020). However, semen samples with higher urine contamination (50%; v:v) had poor post-thawed motility and viability compared with the other groups (Ellerbrock et al. 2018).

Using gradient centrifugation to select sperm prior to freeze might be an alternative to improve the post-thawed sperm parameters of semen highly contaminated with urine (Ellerbrock et al., 2018). Gradient centrifugation was tested to improve post-thawed motility parameters of frozen urine-contaminated sperm in a recent study (Podico et al., 2020). The authors observed that semen selected by the gradient in non-contaminated and low-contaminated (20%; v:v) semen had an improvement on sperm motility after processing; however, highly this improvement was not observed in highly contaminated (50%, v:v) semen samples (Podico et al., 2020).

Final considerations

Urospermia can be present intermittently or continuously. It has been associated with numerous pathological conditions; however, most idiopathic cases do not have a known cause. Detrimental effects of urine on sperm are mediated by alkaline pH, high osmolarity, and concentrations of urine crystals. Urospermia is also thought to cause excessive post-breeding uterine inflammation in mares. Diagnosis of urospermia is based on the yellow appearance, urine smell, urine crystal, alkaline pH, increased creatinine, and urea nitrogen levels. Treatment and management for urospermia include stimulating the stallion to urinate before collection or administering drugs that close the bladder's trigone during ejaculation. Other desperate procedure includes catheterization and washing of the bladder before collection. Management of semen contaminated with urine can be extended with a milk-based extender to mitigate the deleterious effects of urine. Semen cryopreservation can be attempted in stallion semen presenting a low level of urine contamination. Single-layer gradient centrifugation can be used to select sperm with superior traits for low urine contamination.

Ethical statement

An ethical statement is not applicable in this review.

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