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## Urospermia in horses

Urospermia em cavalos

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### Abstract

Contamination of the raw semen with urine can result in irreversible damage to the semen. The sensitivity of spermatozoa to osmotic damage differs markedly between species. Urospermia has been associated with intermittent subfertility and infertility in the horse. The cause may involve failure of the bladder sphincter occlusion and a functional disorder in the components of the autonomic sympathetic and parasympathetic autonomic nervous system that control urination, erection, and ejaculation. Few studies have been done reporting the effects of urospermia on changes in pH, osmolality and motility of equine semen, but it has been reported in the literature that a urospermic condition leading to a hyperosmolar solution is detrimental to sperm motility in horses.

**Keywords:** Urine, equine, semen, motility

### Resumo

A contaminação do sêmen bruto com urina pode resultar em danos irreversíveis ao sêmen. A sensibilidade dos espermatozoides ao dano osmótico difere marcadamente entre as espécies. A urospermia tem sido associada à subfertilidade intermitente e infertilidade no cavalo. A causa pode envolver falha na oclusão do esfíncter da bexiga e um distúrbio funcional nos componentes do complexo sistema nervoso autônomo simpático e parassimpático que controlam a micção, a ereção e a ejaculação. Poucos estudos foram feitos relatando os efeitos da urospermia sobre as alterações no pH, osmolalidade e motilidade do sêmen de equino, mas foi relatado na literatura que uma condição urospermica que leva a uma solução hiperosmolar é prejudicial à motilidade dos espermatozoides em cavalos.

**Palavras-chave:** Urine, equino, sêmen, motilidade

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## **Introduction**

Urospermia is the contamination of semen with urine (BLANCHARD et al., 1990) and the contamination of ejaculates with urine occurs occasionally during collection of semen in many species (ALTHOUSE et al., 1989); in stallions can occur sporadically or consistently (McDonnell, 1992). Urospermia manifests itself individually in different stallions, ranging from stallions that present normal copulation with ejaculation to an abnormal pattern of copulation with or without occasional ejaculation (LOWE, 2001). A yellow / amber color of the ejaculate suggests urospermia (KENNEY et al., 1983).

The contamination of the raw semen with urine can result in irreversible damage to the semen (VOGE et al., 2016) and the deleterious effects of urine on sperm are due to changes in pH, increased osmolality and toxic effects of urea and other components of urine (GRIGGS et al., 2001; WIRTU et al., 2008).

The sensitivity of spermatozoa to osmotic damage differs markedly between species, which determines the ability of cells to regain motility after osmotic stress (GOMES-ALVES et al., 2014).

Urine contamination is detrimental to semen motility (KIM, KIM, 1998), acrosome integrity of the membrane and reduces its fertilization capacity (SANTOS et al., 2011), preventing its use in artificial insemination, thus reducing fertility of the stallion (VOGE et al., 2016).

Urospermia has been associated with intermittent subfertility (HOYOS SEPÚLVEDA et al., 1999) and infertility in horses and other species, including man (GRIGGERS et al., 2001). Subfertility is thought to be caused by a direct adverse effect of urine on sperm and indirect adverse urine change in vaginal and endometrial pH of the mare (BLANCHARD et al 1996). Consequently, pregnancy rates decrease due to the toxic effects of urine on sperm function (McCUE, 2014).

Although there are cases where mixing of semen with urine can be avoided, the clinician should often manage artificial insemination with contaminated semen (VOGE et al., 2016). The importance of how to reverse the harmful effects of urinary contamination on semen (GRIGGERS et al., 2001), as well as the need for further studies on the effects of urospermia on fertility and on the quality improvement of equine urosperm semen samples (ELLERBROCK et al. al., 2016) justifies the investigation. The objective was to evaluate the viability of the semen contaminated with urine through the evaluation of sperm motility, function and morphology.

## **Literature review**

### **Causes of urospermia**

The causes of urospermia in stallions are not well defined. The pathology may develop as an isolated disorder or may be associated with other primary neurological abnormalities, such as equine protozoal myeloencephalitis, equine herpesvirus 1, and sorghum toxicosis, as well as clinical conditions such as periodic hyperkalemic paralysis, cystitis and urolithiasis. Urospermia can also develop secondary to fractures, osteomyelitis and neoplasia. In addition, some cases do not appear to be associated with a specific pathological condition and may be behavioral in origin (BLANCHARD et al., 1990, MAYHEW et al., 1990; TURNER et al., 1995; McCUE, 2014).

Urospermia may also occur as reproductive dysfunction in cases of equine tail injury (TUTKO et al., 2002).

Urospermia is mentioned as an ejaculatory disorder caused by failure of the bladder neck at the time of ejaculation (VARNER et al., 2000). Inadequate closure of the sphincter may result from mechanical causes, such as fibrosis, or from neurogenic causes (TURNER et al., 1995; VARNER et al., 1991). This may be a transient condition in excessively excited stallions during the first ejaculations. However, in most cases of transient or permanent urospermia, spermatozoa are easily observed (ESTRADA et al., 2003).

It is possible that, under certain circumstances, the hypogastric sympathetic nerve fibers innervating the ejaculatory system and the bladder are stimulated simultaneously, resulting in urine during ejaculation (LOWE, 2001). As the control of urination, erection and ejaculation is orchestrated by parasympathetic and sympathetic activity, any injury to components belonging to the autonomic nervous system can result in urospermia (HOYOS SEPÚLVEDA et al., 1999).

Semen contaminated by urine (inside the urinary bladder) may also occur during retrograde ejaculation (BRINSKO, 2001).

### **Pathogenesis of urospermia**

Little information is available on the pathogenesis of urospermia (MAYHEW et al., 1990), but may involve failure of bladder sphincter occlusion and a functional disorder in components of the sympathetic and parasympathetic autonomic nervous system complex that control urination, erection, and (LEESTER et al., 1990).

The various sites of potential lesions that may interfere with ejaculation and cause the appearance of urine in the ejaculate include afferent and efferent nerves that innervate the structures of the bladder and urethra, ascending visceral afferent and proprioceptive pathways, and efferent motor neuron pathways autonomous and somatic descending within the spinal cord and brain stem. These pathways and their associated reflexes, voluntary and involuntary activity are very complex (MAYHEW, 1990).

Spinal cord disorders were not specifically incriminated in causing urospermia, but were associated with abnormal urination patterns and therefore, a complete evaluation of the horse's gait should be performed. The specific stage of the neurological examination that must be performed in some detail is the evaluation of the signs obtained from the tail, the anus and the rectum. The assessment of tail tonus and anal reflex should be evaluated critically. The evidence of muscular atrophy and localized sweating in the gluteal and perineal region should be examined. This is because lower motor neuron disturbances in this region are the most likely neurological cause of urination during ejaculation. This may be associated with 1) a lower motor neuron lesion that affects lumbar sympathetic output to the hypogastric nerve by controlling alpha-adrenergic muscle tone or 2) a sacral lesion that affects the somatic efferent outlet to the pudendal nerve of cholinergic receptors, maintains urethral striated muscle tone (MAYHEW, 1990).

However, most cases of urospermia in stallions are idiopathic in nature (TURNER, 2007).

### **Clinical signs of urospermia**

A stallion with persistent urination during ejaculation does not show other abnormalities of the urogenital system. It is observed that urine is released in ejaculation on several occasions. The position of the horse while urinating should be normal. The endocrine status of the stallion does not reveal abnormalities as demonstrated by normal serum, testosterone concentration at rest, and by a normal testosterone stimulation test (TURNER et al., 1995; COX, 1975).

As part of a thorough behavioral assessment, the horse can be filmed in its stall for a period of 24 hours. The horse's posture during urination and the frequency and flow of urination may be normal. Frequency, duration and control of the penis are also normal. Frequency and posture of defecation are equally normal. If the stallion leans on the bay wall in an unusual posture, it suggests a slight neurological deficit. Urinary incontinence may not be observed during the 24 hour period at which it is filmed or at any time during its stay (TURNER et al., 1995).

The stallion's libido is good. He develops and maintains an erection and mounts normal, but can not mate well during push. During the first collection of semen, the ejaculate of the stallion is normal, but the ejaculation is immediately followed by urination, which contaminates ejaculation with approximately 20 mL of urine and 20 mL of sand as urine sediment. This causes the ejaculate to appear opaque and fetid. The number of normal motile sperm cells is reported as being less than ideal (KENNEY et al., 1993).

An hour later, a second ejaculate can be collected by the use of an artificial vagina in an attempt to obtain semen jets without urine and determine at which point during ejaculation the urine is emitted. All jets of this fractured ejaculate may be grossly normal, appearing translucent white without obvious urine sediment. The initial motility may be 40% total and 30% progressive (TURNER et al., 1995).

The seminal vesicles, ampulla and prostate are normal on palpation by the rectum (LEEDERTSE et al., 1990). The urinary bladder, accessory sex glands, caudal aorta and iliac arteries should be examined by palpation and ultrasonography by the rectum; no abnormality should be apparent. The bladder may not be manually evident from the rectum, which indicates the presence of any functional sphincter. Rectal palpation and ultrasonography should be performed again and, despite the apparently normal voluntary urination behavior, urine and sediments may remain in the bladder, suspecting a detrusor sphincter muscle dyssynergy (TURNER et al., 1995).

A small amount of urine crystals can be detected during semen evaluation, visible in a microscope smear (LEEDERTSE et al., 1990).

A complete neurological examination does not reveal apparent deficits beyond the urinary tract. Specifically, cranial nerve function, hind limb function, anal tone, anal reflex, tail tone, and defecation are considered normal. There are no areas of cutaneous hypoalgesia (TURNER et al., 1995).

Stallions with less sacral development may be impotent due to incomplete and sterile erections due to urospermia (DEBOWES, 1990).

## **Diagnosis of urospermia**

Urospermia is more likely to occur with stallions that can not ejaculate readily (McKINNON, 2013) and the diagnosis is based on physical characteristics, such as urine odor, increased volume (HOYOS SEPÚLVEDA et al., 1999) and color ranging from yellow to amber (KENNEY et al., 1983) or pale yellow to dark yellow color (ALTHOUSE et al., 1989; BALL, 2008).

Color is influenced not only by urine contamination, but also by the concentration of semen, the diluent used, and the cleansing of ejaculation. Failure to properly wash the penis and remove smear prior to collection can result in ejaculation debris and a color change (ELLERBROCK et al., 2016).

Likewise, odor is a poor diagnostic tool for small amounts of urinary contamination, but is highly specific for moderate to high amounts of urine contamination in raw and refrigerated semen. In addition, the odor may be influenced by the stallion, diluent used or failure to evaluate odor after collection (ELLERBROCK et al., 2016).

The level of contamination of urine semen can also be evaluated by the pH value of the ejaculate and results higher than 8.0, and may even reach 8.5, indicate the presence of urine in the sample (CARY et al., 2004; JASKO, 1992).

However, the color, odor and pH of semen are not reliable tests for detecting small amounts of urinary contamination in widely cooled samples, but a commercial test strip of urea nitrogen can detect the presence of small urine concentrations in samples of semen crude and with moderate degrees of urine contamination in samples prolonged cooled (ELLERBROCK et al., 2016).

In other cases, urospermia may be more difficult to detect and requires the use of urea or creatinine assays (BALL, 2008).

Urea and creatinine are well-known markers for the detection of urine in the ejaculate (MRÁCKOVÁ, ZAVADILOVÁ, SEDLINSKÁ, 2015). Biochemical analysis reveals high concentrations of urea and creatinine in seminal plasma and decreased sperm concentrations in the ejaculate (VOSS, McKINNON, 1993).

Concentrations of urea above 5 mmol / liter or creatinine above 177  $\mu$ mol / liter in semen are said to be diagnostic (BOYLE, 1990).

However, urea nitrogen and / or creatinine determinations require expensive laboratory equipment, trained technicians, and time, and therefore do not have immediate value to the clinician in the field (ALTHOUSE et al., 1989).

The identification of a rapid and reliable method to determine the presence of urine in ejaculates would be beneficial for immediate diagnosis. A test could be used as a screening procedure for further extensive and costly further analysis of collected samples. In addition, the analysis of urinary contamination in ejaculates not routinely implemented in ultrasound examinations and daily protocols of semen evaluation could be performed easily and without cost. Such testing could also provide additional information about a particular animal and a particular ejaculate in question (ALTHOUSE et al., 1989).

Equine semen samples can be analyzed using the Azostix, Multistix or uric acid test kit. The Azostix diagnostic test contains a reagent strip that is sensitive to urea nitrogen. All urine / semen and control samples are gently shaken several times before testing. The Azostix reagent strip is then immersed in the urine-contaminated control or sample and immediately removed. The strip is held

horizontally and rinsed with distilled water. Subsequently, visual examination for a color change is done at 5-second intervals for 1 minute (ALTHOUSE et al., 1989).

A change in color (yellow to green) is observed within 10 seconds in the ejaculates that had urea nitrogen concentrations above 39 mg / dL (ALTHOUSE et al., 1989).

The Azostix manufacturer recommends that you wait at least 60 seconds to read the test strip against the scale provided on the back of the container. Azostix is highly sensitive and specific when used to diagnose urine contamination in cold semen, regardless of the percentage of urinary contamination (ELLERBROCK et al., 2016).

Another diagnostic test, Multistix, contains 10 reagent strips that can detect glucose, bilirubin, ketones, specific gravity, blood, pH, protein, nitrites, urobilinogen and leukocytes in a sample. Multistix is tested by immersing the reagent strip in the control or in the urine-contaminated sample and then removed. The strip is held in a horizontal position to prevent mixing of chemicals from the adjacent reagent areas. Multistix is examined for a color change at 15-second intervals for 1 minute and then at 30-second intervals for a further 3 minutes (ALTHOUSE et al., 1989).

The Azostix and Multistix tests successfully detect urine in the equine ejaculate (ALTHOUSE et al., 1989) and are highly specific and sensitive methods to evaluate urinary contamination in semen of raw, diluted and cooled stallion (C et al., 2016).

For the detection of urinary contamination in semen, the uric acid test can still be performed. This test is performed by adding 0.3 mL of the control or the urine-contaminated semen sample to a vial containing the premixed phosphotungstic acid test reagents and a color buffer. The flask is shaken gently and examined for a color change at 15-second intervals for 1 minute and subsequently at 30-second intervals for an additional 3 minutes (ALTHOUSE et al., 1989).

The semen should not contain calcium carbonate crystals or other mineral sediments. The osmotic pressure of seminal plasma contaminated with urine is increased due to osmotically active constituents of urine (HOYOS SEPÚLVEDA et al., 1999).

A rectal examination should include assessment of rectal tone and residual urine volume after urination and full palpation of pelvic contents with special attention to the ventral surface of the sacrum where fractures can be detected. With lesions of the sacral nerve root, an abrupt loss of rectal tone can be palpated when the hand is moved from the proximal to the distal rectum (MAYHEW, 1990).

For stallions that apparently can not ejaculate or ejaculate containing lower seminal volumes or lower than expected numbers of spermatozoa, obtaining a urine sample after ejaculation through bladder catheterization is a simple diagnostic procedure which can be used to investigate the possibility of retrograde ejaculation (BRINSKO, 2001).

## **Treatment of urospermia**

A few studies have reported on urospermia and its treatment in stallions (GRIGGERS et al., 2001) and most cases are idiopathic in nature, limiting therapeutic options (VOGE et al., 2016). Targeted therapies to reduce urinary contamination include reducing the amount of urine in the bladder prior to reproduction, collecting only the semen-rich portion of the ejaculate using an open-



ended artificial vagina, or pharmacological treatment to increase closure of the cervix bladder during ejaculation (TISCHNER et al., 1974).

Among these limited therapeutic options available for the treatment of urospermia in stallions, none were consistently satisfactory. Encouraging the horse to urinate immediately prior to ejaculation has been suggested (LEENDERTSE, et al., 1990) and techniques to induce micturition in stallions include lodging in a recently laid bed, stool placement of another stallion in the stallion of the affected stallion and the use of diuretics (LEENDERTSE, et al., 1990; HELD, et al., 1992). Fractionation of ejaculate has also been suggested as a means of managing urospermia. Theoretically, urine-free ejaculation could be obtained by collecting only urine-free jets (VARNER, et al., 1991; NASH, et al., 1980). This technique may be satisfactory if urine contamination occurs only in the final ejaculation jets. Unfortunately, the pattern of urinary contamination during ejaculation is not always repeated in an affected individual (TURNER et al., 1995).

The affected stallion can be housed in the stall of another stallion that still contained a fecal pile of that other stallion. Initially, the affected stallion seems very anxious to be in the stall of another stallion. However, after about 3 minutes, the horse smells like the other stallion's fecal pile, perches on it and urinates on the other stallion's fecal stack (McCUE, 2014).

The stallion will then be immediately taken to the breeding shed and the semen collected without contamination with urine. The cycle of moving the stallion to another stall and allowing it to urinate should be repeated three times on all other days. Normal ejaculation in the absence of urinary contamination will occur during each collection procedure and will be normal since then (McCUE, 2014).

Another management technique that can be employed to encourage a stallion to urinate prior to semen collection is to remove the stallion from its normal stall, clean the stall, lay fresh bed, and then return the horse to its stall. Most stallions will urinate within a few minutes to mark their territory (McCUE, 2014).

Management of the stallion during the remaining breeding period implies delaying mating until immediately after urination. This can be achieved by administering a diuretic followed by exposure to urinary stimuli 30 minutes later. This includes stallion walking around a pasture with a mare in the estrus, whistling for the stallion or shaking the drinking water while it is in its stall. When the stallion has urinated, the mare is brought (LOWE, 2001).

Twelve hours after mating, mares are reexamined by ultrasonography for evidence of uterine fluid not present before mating and possibly associated with urination during intercourse. If present, the mare is treated with a high volume uterine lavage with 3 L of isotonic saline (0.9% NaCl) infused through a uterine lavage catheter and applied 20 IU of oxytocin IM. The mare is then reexamined 2 hours later (LOWE, 2001).

Treatment of urospermia stallions includes diuretics,  $\alpha$ -adrenoceptor agonists,  $\beta$ -adrenoceptor antagonists, tricyclic antidepressants, parasympathetic muscarinic receptor antagonists, muscarinic parasympathetic receptor agonists and hormones. These drugs are intended to (1) promote evacuation of the bladder prior to ejaculation; (2) increase the tone of the external urethral sphincter during ejaculation to avoid contamination of the semen by urine; or (3) altering the production of urine (HOYOS SEPÚLVEDA et al., 1999).

The use of diuretics may be helpful if the stallion completely empties its bladder prior to ejaculation, but may result in a large volume of diluted urine deposited in ejaculation that would be detrimental to fertility (GRIGGERS et al., 2001).

Tricyclic antidepressants, such as imipramine, were used as therapy for urospermia in stallions in an effort to increase bladder sphincter tone (McDONNELL, 1992) and the external urethral sphincter during ejaculation (TURNER et al., 1995).

The owner should administer 500 mg of imipramine in ration 2 to 3 hours prior to collection of semen and collect semen immediately after the stallion has urinated. If the first ejaculate has gross urine contamination, a second ejaculate should be collected 10 to 15 minutes later (TURNER et al., 1995).

Imipramine also appears to increase the contractility of the bladder neck during urine output. The mechanism of action is not fully understood, but apparently tricyclic antidepressants and their metabolites promote  $\alpha$ -adrenergic activity by inhibiting norepinephrine reuptake (McDONNELL, 1992). On the other hand, a test with three stallions exhibiting urospermia showed no beneficial effect of imipramine on the subsequent biochemistry of seminal plasma, including urea and creatinine concentration (HOYOS SEPÚLVEDA et al., 1999). These medications can also be used to decrease the frequency of retrograde ejaculation (BRISKO, 2001).

In an additional attempt to control urospermia, administration of phenylpropanolamine, another  $\alpha$ -adrenergic agent, was administered (0.35 mg / kg) twice daily for 14 days with no apparent improvement (TURNER et al., 1995).

As urination during ejaculation is usually intermittent in stallions, success is often elusive (VOSS, McKINNON, 1993). The response to treatment should be evaluated individually (HOYOS SEPÚLVEDA et al., 1999).

Semen diluents are commonly used in equine AI to prolong semen viability and inhibit bacterial growth, but the use of a semen diluent may also be beneficial in relieving the negative effects of urine on sperm motility. The recovery of progressive sperm motility, reduced by urinary contamination, depends on the diluent used to dilute the semen (GRIGGERS et al., 2001).

When urine contamination is substantial, the semen should be mixed with an equal volume of semen diluent and centrifuged for 10 minutes at 400 x g. Centrifugation will separate seminal plasma, sperm and any urine sediment (DASCANIO, WITONSKY, 2005).

While centrifugation may be expected to provide an additional benefit by reducing the amount of urine contaminating the semen, centrifuging the ejaculate and resuspension with the crude diluent have no advantage over the simple addition of diluent. It is possible that the exposure of spermatozoa to a higher concentration of urine crystals, which would be centrifuged in the sediment along with the sperm, could cause additional damage to the cells and inhibit any beneficial effect of the centrifugation. In addition, a potential disadvantage of centrifugation is the possible loss of some viable spermatozoa in the discarded supernatant if the procedure is not performed correctly; for example, rapid removal of the supernatant after completion of the centrifugation process. Although centrifugation is a valuable technique to remove undesirable seminal plasma for some infertile stallions, it is still unclear in cases of urospermia (GRIGGERS et al., 2001).

In cryopreservation of semen in many species, centrifugation of ejaculates is necessary to remove seminal fluids, increase semen concentration and recover samples contaminated with urine (ALVAREZ et al., 2012).



When these treatments are unsuccessful, the minimization of the toxic effects of urine on the semen is attempted. Treatment of semen contaminated with urine by cushioned / resuspension centrifugation may improve some of the adverse effects of urospermia on fertility, maintaining sperm membrane integrity, but stallions vary in their response to these treatments (VOGE et al., 2016).

### **Effects of urospermia on equine semen**

Few studies have been done reporting the effects of urospermia on changes in pH, osmolality and motility of equine semen, but it has been reported that a urospermic condition leading to a hyperosmolar solution is detrimental to sperm motility in horses (GRIGGERS et al. 2001). It is believed that spermatozoa can undergo training in media with higher ionic strength. Thus, without adjustment of the ejaculate, the spermatozoa in semen contaminated with urine can undergo premature acrosome formation and reaction, resulting in decreased fertility (DASCANIO, WITONSKY, 2005).

The addition of diluted urine (obtained after administration of furosemide) to semen to produce a 5% (v: v) mixture has little effect on osmolality or pH, increasing from 312 to 316 mOsm and 7.71 to 7.80, respectively, and do not significantly affect motility immediately after treatment or after 1 hour. In the present study, it was found that the volume of urine collected by catheterization increased significantly.

Addition of 50% diluted urine or any amount of urine collected by catheterization resulted in an immediate reduction of initial progressive motility from 116% to 64%. The effect of adding 33 or 50% catheterized urine resulted in an immediate reduction to 5% and 0% progressive motility, respectively. There is no clear indication whether acidic or basic conditions are more detrimental to the motility of equine semen, but urine is detrimental to semen motility in almost all cases. Only when a small amount (5%) of diluted urine was added to ejaculation, there was no apparent effect on motility. Any amount of "normal" urine or greater amounts of diluted urine reduces motility (GRIGGERS et al., 2001).

Other preliminary studies have shown that the addition of urine to the semen prior to the addition of diluent resulted in a marked reduction in sperm motility (VOGE et al., 2016), affecting the initial motility and semen motility after 24 hours of cooling, even when there is a small amount of urine. Urine contamination has a more pronounced effect on the reduction of sperm motility in the raw semen compared to refrigerated and diluted stored samples. Addition to semen with a commercial milk-based semen diluent can correct the damaging effects of a hypertonic solution and alkaline pH. However, even a small amount of urine contamination (less than 10% of the total semen volume) has deleterious effects on semen motility and may go unnoticed by semen processing and transport ( ELLERBROCK et al., 2016).

Centrifugation of semen contaminated with urine and resuspension with new amounts of diluent does not improve the above motility. The skim milk diluter restores better motility than the egg yolk extender at a ratio of 1: 3 immediately after addition and in any ratio when centrifuged. In addition, the skim milk extender continues to provide superior motility as compared to the egg yolk extender under these circumstances 1 hour after centrifugation and resuspension (GRIGGERS et al., 2001).

Semen DNA quality decreased as urine contamination increased. This effect was immediately apparent (T0), but became more pronounced with increasing storage time of the cooled semen (VOGE et al., 2016).

The effects of urospermia on the motility or fertility of cooled stallion semen were not documented. In practice, urine-contaminated ejaculates may be marketed because of the lack of knowledge of personnel who collect and process semen or for fraudulent reasons without disclosure to the breeding practitioner who manages the mare. It remains unknown whether urine contamination can be adequately diagnosed in prolonged-cooled semen using common means to assess urinary contamination (ELLERBROCK, et al., 2016).

### **Effects of urospermic semen on mare**

Urinary contamination of semen can also cause decreased conception rates due to possible inflammation induced in the uterus by urinary components. The effects of urine on the endometrium were extrapolated from the effects found in mares with vesico-vaginal reflux (DASCANIO, WITSKY, 2005).

Urine in the uterus may result from vesico-vaginal reflux and subsequent flow forward through an open cervix and may also occur from contamination by a stallion having urospermia. (GRIGGERS et al, 2001). In addition, urine in the uterus creates chemical endometritis and, if it persists, will interfere with the maintenance of pregnancy. Urine in the uterus of the mare is identified by hyperechoic accumulations most commonly at the cornual junction and does not easily creep with the catheter. It seems that the echogenicity of urine is a combination of crystals and mucus. Urine in the uterus should be removed with voluminous lavage (McKINNON, McCUE, 2013).

Ultrasonography of the mare after the service is a useful technique for the diagnosis of urospermia in natural mare creations. Normally, they have a hyperechoic accumulation of urine crystal sediment in the body of the uterus, which, although similar to the appearance of air, does not have the typical air refraction (McKINNON, 2013).

### **Final considerations**

Finally, there is still a need to evaluate the endometrial effects of mares inseminated with samples of urospermic semen from stallions, as well as the performance of *in vivo* fertility tests.

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