

Complications associated with the use of intrauterine glass marbles to suppress estrus in a Standardbred mare

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Abstract

An 11-year-old maiden Standardbred mare was presented to The Ohio State University Veterinary Medical Center (OSU-VMC) Theriogenology service for treatment of endometritis and breeding management. It was suspected that pieces of a marble, placed in the uterus to suppress estrous behavior, remained in the mare's reproductive tract following removal. The clear glass marble was reported to appear "flakey" upon removal and had been present for an unknown period of time. Reproductive ultrasonography showed luteal tissue, endometrial edema, hyperechoic free intrauterine fluid and small echogenic fragments in the endometrium. Hysteroscopy showed free glass shards in the uterus. Treatment of the endometritis included: acetylcysteine, uterine lavages, ceftiofur, and ecbolics. Intensive breeding management over two estrous cycles and two embryo recovery attempts were unsuccessful. This report describes potential complications with the use of an intrauterine glass marble and provides additional evidence that serious complications such as embedded glass shards in the endometrium, persistent endometritis and infertility may occur. Glass marbles are not recommended for estrus suppression in mares.

Keywords: Mare, estrus suppression, marble, endometritis

Background

Estrus suppression in mares, particularly performance mares, is desirable for owners who report that their mare is difficult to manage when signs of behavioral estrus are present. Some owners report their mare may experience ovarian or back pain during estrus, which is perceived to affect the mare's performance. Some signs of behavioral estrus include leaning toward the stallion, lifting of the tail, squatting stance, clitoral eversion, and urinating.¹ However many times the mare's performance issues may not be related to estrus in the mare, because these signs fail to occur about every fourteen days and do not last for six days. In addition, in mares that are not under lights, the signs persist when the mare is in anestrus in the winter. Thorough examination of the mare by the veterinarian, and the chief complaint is important to determine whether the mare's behavior is reproductive in origin, or if these perceived signs are related to other causes.¹

There are numerous documented methods for suppressing estrus including: administration of exogenous progesterone or progestins, induction of a prolonged luteal phase, suppression of the follicular activity of the ovaries, and ovariectomy.^{1,2} Of these four possible methods to suppress estrus, administration of exogenous progesterone/progestins appears to be used most frequently.² Oral administration of altrenogest, a synthetic progestin, is considered by most veterinarians to be the "gold standard" for preventing behavioral estrus in mares, but the expense of the medication and the frequency at which it must be administered are just two of its potential drawbacks for owners.

Due to the drawbacks of progesterone/progestin administration, some owners may elect to suppress estrus by prolonging the luteal phase, which results in continued secretion of progesterone, which inhibits estrus behavior. There are numerous methods for prolonging the luteal phase such as the use of exogenous oxytocin, or human chorionic gonadotropin (hCG).¹ One method involves the use of an intrauterine device (IUD) in the form of a glass ball or marble, placed into the uterus immediately after ovulation.²⁻⁵ Common sizes reported in recent literature include 25mm and 35 mm diameter glass marbles³ while another study used a 20mm polypropylene ball filled with water.⁴ In the 2003 study by Nie et al, 25mm and 35mm glass marbles were placed in the uterus immediately following ovulation. In that study, 50%, or 6/12 mares spontaneously expelled the 25 mm marble. None of the 35 mm marbles were expelled. Their results indicated that in the mares which did not expel their glass marbles (18/24), luteal secretion of progesterone persisted for 35 days for 39% (7/18) of mares. In the control mares prolonged luteal function was noted in 13% (4/32) of mares. They also found that of those seven mares showing persistent luteal function, four showed persistent luteal function following the first normal estrous cycle after marble placement. The remaining three mares

showed persistent luteal function following multiple normal estrous cycles.³ These results appear to indicate that use of the IUD glass marble is somewhat efficacious for causing persistent luteal function and estrus suppression. However, the application of intrauterine marbles produced varying results in other studies. In a study conducted by Rivera del Alamo et al, 75% of mares showed prolonged luteal phases. The IUD was smaller (20mm), made of polypropylene, and filled with water.⁴ Argo and Turnbull investigated IUD glass marbles in pony mares. Mares in both the study group and control group showed normal estrus behavior throughout the duration of the study. However, even though the study failed to show any measurable change in estrus behavior, owner perception of improved behavior was greater than 60%.⁵

Recently, there have been multiple reports of complications related to the use of intrauterine glass marbles. In 2015, Turner et al reported fragmentation of a glass marble in the uterus of a mare after placement of two glass marbles in her uterus. Following uterine lavage and treatment, the pieces of the marble were removed from the uterus but the mare failed to become pregnant after multiple breeding attempts.² Diel de Amorim et al reported pyometra, infertility, and embedded glass in the endometrium of mares who had intra-uterine glass marbles(s) for longer than one year.⁶

Another report by Freeman and Lyle described a case of intermittent signs of colic in a mare, associated with the presence of two round glass marbles in the uterus. After cervical dilation, the two marbles were removed from the uterus. Follow-up with the owner revealed that the mare had not had any signs of colic during the 12 months following marble removal.⁷

Here we report the complications related to the use of a single glass marble in the uterus of a mare. These complications include: marble fragmentation and embedded glass fragments within the endometrium, endometritis, and infertility.

Case presentation

An 11-year-old maiden Standardbred mare was referred to OSU-VMC) for evaluation of a potential uterine foreign body from a previously placed uterine marble used to suppress estrus. The mare's primary care veterinarian removed the marble one month prior to presentation and the marble was reported as appearing "flakey" by the owner. For an undetermined period of time, the mare was observed urinating frequently, straining to urinate, and producing either small quantities or nothing at each urination. At presentation, her physical examination parameters were within normal limits and she appeared adequately hydrated.

Transrectal ultrasonographic examination of the reproductive tract revealed medium sized follicles ranging in size from 20-27mm and a corpus luteum on both ovaries. A moderate amount of uterine edema (grade 2 of 3) was noted despite the mare being in diestrus, and the cervix was closed at the time of examination. A moderate amount (ranging from 1-5 cm in depth) of mildly hyperechoic (grade 3) uterine fluid was visualized and multiple multifocal hyperechoic areas were visualized in the uterine lumen on ultrasonography. Digital palpation of the cervix revealed a 2cm x 1cm linear diverticulum within the body of the cervical lumen. Both the inner and outer cervical os were tightly closed.

The mare was sedated with 8 mg detomidine hydrochloride (Dormosedan®; Zoetis Inc. Kalamazoo, MI) and hysteroscopy was performed in an effort to remove the hyperechoic structures believed to be remnants of the uterine marble. Following sedation, purulent material and mucous were identified on endoscopic examination, but the glass fragments could not be detected due to a large amount of fluid. Following the initial examination, 30 milliliters of 20% acetylcysteine (20% acetylcysteine solution, USP, Hospira, Inc., Lake Forest, IL) was infused into the uterus to help to break up the mucopurulent material that was present. Approximately three hours later, a uterine lavage was performed using three liters of lactated Ringer's solution (LRS). A sample collected from the uterine lavage was submitted for antimicrobial culture and sensitivity testing. Results showed a heavy pure growth of *Staphylococcus warneri*. sensitive to cephalosporins. Cytology of the efflux revealed a high number of degenerative neutrophils with intracellular bacteria. The mare was treated with 6.6 mg/kg ceftiofur crystalline free acid (Excede®; Zoetis, Florham Park, NJ) given intramuscularly. No episodes of straining to urinate were noted during her hospitalization and urine produced was normal in color and quantity. It was determined that the previous urinary issues noted by the owner were related to estrus behavior.

The following day, a second uterine lavage was performed also using three liters of LRS. At this lavage, numerous glass fragments were retrieved along with the efflux (Figure 1). Following the lavage, 20 international units of oxytocin were administered and repeated every six hours to promote uterine clearance.

On the fourth day of hospitalization, an additional uterine lavage with LRS was performed in an effort to remove any remaining glass fragments. No additional fragments were recovered but on ultrasonographic examination, multiple hyperechoic areas were still visualized within the uterine lumen (Figure 2). A second hysteroscopic examination was elected to attempt to directly visualize and remove the remaining glass pieces, but despite the lack of intrauterine fluid, they could not be seen. Since they could not be visualized, it was assumed the hyperechoic areas seen within the endometrium on ultrasound examination were pieces of glass from the marble that were embedded in the uterine endometrium.

A decision was made to attempt to breed the mare following the removal of the uterine debris and treatment of endometritis.

On the fifth day of hospitalization, two preovulatory follicles (37 and 36mm) were visualized on the left ovary, and the mare was administered 1.8 mg of deslorelin acetate (SucroMate™; Thorn BioScience, LLC, Louisville, KY) IV to induce ovulation. Approximately six hours later, uterine lavage was again performed and the mare was artificially inseminated using cooled semen. On the sixth day of hospitalization, ultrasound revealed multiple large follicles on the right ovary. Uterine lavage was again performed to remove inflammatory debris, and she was administered 250 micrograms of cloprostenol sodium (Estrumate®; Schering Plough Animal Health, Union, NJ) to promote uterine clearance. The following day, ovulation of two follicles was confirmed via ultrasound.

Repeated transrectal ultrasonographic examinations were performed throughout the mare's hospitalization to monitor ovarian structures, uterine edema, uterine fluid, cervical tone, and to evaluate the multiple multifocal hyperechoic areas in her uterus. Uterine lavage was performed as needed according to uterine fluid accumulation. Intramuscular injections of 6.6 mg/kg ceftiofur crystalline free acid were performed every 96 hours during hospitalization. Additional intramuscular injections of oxytocin were administered every 4-6 hours following uterine lavage. Prednisolone acetate (PrednisTab®, Phoenix, Clipper Distributing Company, LLC, St. Joseph, MO), 0.1 mg/kg by mouth every 12 hours, was administered on days 5-9 of hospitalization in an effort to decrease post-breeding uterine inflammation. Intrauterine fluid was noted for three days following ovulation, gradually decreasing in volume through response to lavage and ecbolic therapy. On the tenth day of hospitalization, the mare was discharged to the owner with a recommendation to have an ultrasound performed 14 days following insemination to evaluate the mare for pregnancy and the presence of twins. There was no free intrauterine fluid noted at this time. The owner was advised that the mare's prognosis for fertility was guarded at the time of discharge, due to the prolonged fluid accumulation after breeding.

Thirteen days following discharge, the mare presented again to The OSU-VMC for suspected endometritis and uterine fluid accumulation noted at the time of her pregnancy evaluation. She was found to not be pregnant by her primary care veterinarian with a shortened inter-estrus interval. A reproductive examination showed that the mare had a 31mm follicle on the right ovary and one 42mm dominant follicle and a 27mm follicle on the left ovary. A marked amount (4-5cm in depth) of moderately hyperechoic (grade 3) uterine fluid was also seen on ultrasound. Uterine lavage with three liters of LRS was performed and the fluid collected appeared cloudy. A sample of the uterine fluid was submitted for culture and sensitivity testing and results showed no bacterial growth at that time. Numerous neutrophils were seen on cytology. Following uterine lavage, the mare was administered 2500 IU hCG IV to induce ovulation. She was also administered 6.6 mg/kg ceftiofur crystalline free acid for presumptive endometritis, ecbolics, and oral phenylbutazone (2.2 mg/kg) for mild trauma sustained during transport. Phenylbutazone was continued for four days for pain management. Two days following presentation, an anovulatory follicle was noted on ultrasound and it was decided that she should not be inseminated at that time.

Hospitalization was continued in an effort to breed on the next estrous cycle with the plan to enroll the mare in an embryo transfer protocol to a recipient mare. On day thirteen of hospitalization, a large, 40 mm follicle was visualized by ultrasound. A marked amount of uterine fluid (4-5cm in

depth) was also seen on ultrasound. Uterine lavage was performed using four liters of LRS, followed by artificial insemination using cooled semen. She was also administered oxytocin and oral prednisolone acetate. Lavage, ecbolic therapy, and prednisolone were continued as needed to remove uterine fluid and debris. Ovulation was confirmed with visualization of a corpus luteum using ultrasound 48 hours later. Seven days following artificial insemination, the mare began oral altrenogest (Regu-Mate® 0.22%; Intervet, Inc. Millsboro, DE) at a dosage of 0.044 mg/kg. On day seven following ovulation, an embryo recovery procedure was performed but no embryo was recovered.

The mare was administered 250ug cloprostenol intramuscularly in order to advance the onset of next estrus for a final attempt at embryo transfer immediately after uterine lavage. The mare was monitored via ultrasound until a preovulatory follicle was noted and administered 1.8 mg deslorelin acetate IV to induce ovulation. She was inseminated again with cooled semen from the same stallion. After breeding uterine lavage was performed using six liters of LRS. Oxytocin and prednisolone were administered at previously recorded doses for uterine clearance and mitigation of inflammation. Prednisolone was administered twice daily for six days following insemination and oral altrenogest was administered daily at the labeled dosage. She was flushed seven days after ovulation, and no embryo was recovered. The mare was then discharged.

Outcome

During hospitalization at OSU-VMC, the mare was bred three times on three consecutive cycles via artificial insemination. Unfortunately, pregnancy was never achieved despite intensive breeding management. Prognosis for fertility was guarded due to the presence of a cervical diverticulum, persistent uterine fluid accumulation, post-breeding endometritis and embedded shards of glass in the mare's endometrium. After the mare's second visit and failure of embryo recovery on two consecutive cycles, it was recommended that the owner consider oocyte transfer as a final option to achieve a foal.

Discussion

Intra-uterine glass marbles are not approved medical devices. As seen in this case, one of the disadvantages of intrauterine glass marbles is fragmentation. In 2013, Vanderwall described reports of spontaneous fragmentation of a glass ball in the uterus.⁸ Dascanio also indicated that a low quality glass ball could fracture, leaving glass shards present in the uterine lumen and making recovery of the remaining pieces of glass difficult.⁹ One recent report evaluating five mares showed 5/5 (100%) of mares had their glass marbles present for longer than one year and of those five mares, two (40%) had chronic endometritis, three (60%) had pyometra, and three (60%) had fragmented marbles with glass shards embedded in the endometrium.⁶ In another recent study, fragmentation of two intrauterine glass marbles was reported in a mare that presented to the authors for removal of both glass marbles.² One marble was found to have "pitting" on the marble surface, while the second marble was found to be shattered. Following repeated uterine lavage, multiple glass shards were recovered, but collection of every piece of glass could not be definitively determined. The authors indicated that additional pieces could have been embedded in the uterine wall. It was presumed that repeated contact between the marbles caused the fragmentation.² In the case of the mare in the current report; only one intrauterine glass marble was placed in the uterus and subsequently removed by the primary care veterinarian. The owner's report that the marble appeared "flakey" is consistent with the "pitting" reported by Tuner and associates.² Following repeated uterine lavage, numerous glass shards were recovered, but multiple hyperechoic areas continued to be visualized on ultrasonography, indicating foreign bodies such as glass shards were embedded in the uterine endometrium. These glass shards if embedded in the endometrium, could act as a nidus for inflammation and decrease fertility. Glass is a porous substance and body fluids and inflammatory debris likely act to break down the surface of the glass. This would lead to fragmentation of the surface and the release of glass shards into the lumen that may then become embedded in the endometrium over time.

After culture and cytology of the purulent discharge, a bacterial infection was identified as *Staphylococcus warneri*. This species of bacteria is often considered a contaminant, however with the heavy pure growth and positive cytology seen, it is believed that this was in fact a true infection. It is possible the bacterium could have been introduced during manual marble removal. While reports

describing uterine infection following placement or long term retention of an intrauterine glass marble are limited, reports have been made describing the possibility of intrauterine glass marbles causing pyometra.¹⁰ In one case report, a mare was found to have pyometra following presentation for foul smelling uterine discharge. A glass marble was found in the uterine lumen that the owners indicated had been present for at least two years. The authors suspected the marble acted as a nidus for infection following diestrus. The authors did not evaluate long-term fertility in that case.¹⁰ Additionally, Nie et al describe infusing ticarcillin sodium into the uterus following placement of an intrauterine glass marble to prevent bacterial infection and endometritis.³ In that study, some uterine fluid accumulation was seen following glass marble placement and removal, but none of the mares in the study were shown to develop pyometra or endometritis. These data indicate placement of an IUD glass marble poses some risks as this procedure has the potential for introducing bacteria into the uterus.

Cervical incompetence is one predisposing factor to persistent fluid accumulation as well as reduced myometrial contractions, poor lymphatic drainage, and degenerative uterine changes.¹¹ During placement or removal of an intrauterine glass marble, the cervix may be damaged if it is not relaxed, such as during diestrus, or if proper medications have not been administered to induce cervical relaxation. Lacerations, or other injury to the cervix, are possible and can lead to cervical adhesions when healing. When the cervix is adhered to another tissue layer, it becomes more difficult, or impossible, to relax appropriately.¹² During the initial examination, it was noted that the mare in our report had a cervical diverticulum upon palpation. The cause of the diverticulum could not be established, but the presence of the diverticulum could lead to impairment in cervical function.

Transrectal ultrasonography of the uterus is an extremely valuable tool for estimating quality and quantity of fluid in the uterine lumen.¹³ The mare in this case report was monitored daily as needed for evaluation and treatment. In this case, the mare usually presented with trace amounts of hypoechoic uterine fluid before breeding and moderate to large amounts of slightly echoic fluid after breeding. Post-breeding uterine fluid accumulation is a physiologic inflammatory response that may help to clear the uterus of foreign material such as excess spermatozoa, seminal plasma and bacteria.^{11,14} However, persistent post-breeding uterine accumulation has been associated with a decrease in fertility after natural mating or artificial insemination of fresh semen.^{15,16}

Chronic uterine fluid accumulation after breeding is typically managed with uterine lavage and ecboic therapy to remove inflammatory products. Ecboic therapy such as oxytocin increases myometrial contractions and aids expulsion of material.^{17,18} The aim of therapies in the immediate period after breeding is to reduce infection and inflammation to create a uterine environment capable of supporting pregnancy and prevent further contamination. Spermatozoa are safely in the oviduct within four hours of breeding and are protected from inflammatory products in the uterus and/or uterine treatments by the utero-tubal junction.¹³ In this case, uterine lavage was instituted prior to insemination and again, along with ecboic therapy, following insemination. This was in an effort to clear the uterus of fluid and any foreign material present, allow the semen to migrate to the oviduct, and aid in decreasing inflammation of the endometrium in preparation for pregnancy. However, in this case, the mare did not become pregnant despite the appropriate measures taken.

Embryo transfer now lends itself to obtaining foals from performance and show mares, multiple foals from the same mare in one year, foals from mare with non-reproductive health or musculoskeletal problems, and foals from mares with reproductive problems.¹⁹ Embryo transfer may be a good alternative for the mare in this case if she is found to be unable to carry a foal to term. Glass shards embedded in the endometrium may function as a nidus for inflammation and/or infection and affect the mobility phase of early gestation or implantation.² However, if the oocyte is able to be fertilized in the oviduct, embryo transfer into a recipient mare with a normal, healthy uterus may be an attractive alternative in this case.

Carnevale and coworkers analyzed the effects of repeated inseminations and embryo transfer attempts on uterine health and found an association with increased chronic inflammatory changes. These authors concluded that mares were susceptible to the additive effects of repeated uterine insults. In clinical practice, there is substantial evidence that repeated breeding and embryo transfer attempts yield an increased likelihood of acute bacterial endometritis, in addition to chronic inflammation.²⁰ There is a concern that glass shards embedded in the endometrium and repeated attempts at embryo transfer may lead to additive effects and decrease fertility in this mare. However, in this case, chronic

endometritis has already been documented. Assisted reproductive procedures may be necessary to allow this mare to reproduce.

Oocyte transfer can be used to bypass many of the fertility problems that render an older mare unsuitable as an embryo transfer donor. These include failure of ovulation, chronic endometritis, pyometra, cervical scarring or severe lacerations and unexplained infertility.²¹ In this case, repeated breeding and embryo transfer attempts were unsuccessful, but the mare continued cycling normally. Oocyte transfer into a recipient mare with a normal, healthy uterus may allow this mare to reproduce since embryo transfer was unsuccessful.

In conclusion, this case supports that severe complications such as chronic endometritis and embedded shards of glass leading to infertility, may occur when an intrauterine glass marble is inserted into a mare's uterus to suppress estrus. Intra-uterine placement of glass marbles is not recommended for estrus suppression in mares.

Learning points

Intra-uterine marbles are not medical devices and are not currently recommended for estrus suppression in mares.

Complications of intra-uterine marble use include loss, fragmentation, chronic inflammation, endometritis, embedded shards of glass in the endometrium and infertility.

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Figure 1. Multiple glass fragments retrieved from the uterus of an 11 year old Standardbred mare via uterine lavage following marble removal; no. 22 scalpel blade provided for size.

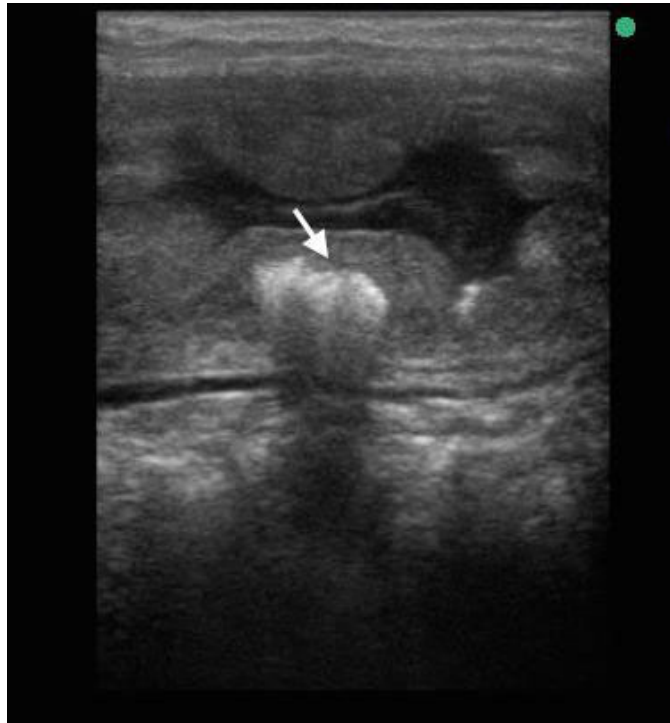


Figure 2. Ultrasonographic image of the uterus of an 11 year old Standardbred mare showing hyperechoic areas consistent with embedded glass fragments in the endometrium.

