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Broad uterine ligament plication by toggle use during laparoscopic surgery

[*Plicatura do ligamento largo do útero com utilização de* toggle *por videocirurgia*]

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ABSTRACT

The aim of the present study is to describe the surgical technique and complications of uterine elevation by laparoscopic surgery, using the broad uterine ligament plication with toogles. Five mares of different breeds, weight and age were selected, presenting poor perineal conformation and pendulous uterus. The surgical technique was performed with a toogle applicator, 0.6mm sterile nylon thread and toogles, using three portals on each side of the flank. After one month, a new laparoscopy was performed, with only one portal on each side, to observe uterine positioning and healing. The average surgical time reached in this study was 82 minutes per procedure, and the uterus of all mares was successfully raised, remaining in a more horizontal orientation and presenting satisfactory healing. There are only two articles published with uteropexy in mares, where the proposed techniques take longer, which consequently increases the surgical risk.

Keywords: Horse; infertility; reproduction; videolaparoscopy; uteropexy

RESUMO

O objetivo do presente estudo é descrever a técnica cirúrgica e as complicações da elevação uterina por cirurgia laparoscópica, utilizando-se a plicatura do ligamento uterino com aplicação de toogles. *Foram selecionadas cinco éguas de diferentes raças, peso e idade, as quais apresentavam baixa conformação perineal e útero pendular. A técnica cirúrgica foi realizada com aplicador de* toogles, *fio de* nylon *estéril de 0,6mm e* toogles, *sendo utilizados três portais de cada lado do flanco. Após um mês, uma nova laparoscopia foi realizada, com apenas um portal de cada lado, para observar o posicionamento uterino e a cicatrização. O tempo cirúrgico médio alcançado neste estudo foi de 82 minutos por procedimento, e o útero de todas as éguas foi elevado com sucesso, permanecendo em uma orientação mais horizontal e apresentando cicatrização satisfatória. Existem apenas dois artigos publicados com a utilização da uteropexia em éguas, porém nestes as técnicas propostas demoram mais, o que, consequentemente, aumenta o risco cirúrgico.*

Palavras-chave: cavalo, infertilidade, reprodução, uteropexia, videolaparoscopia

INTRODUCTION

Mares can physically perform uterine cleaning, whether by natural breeding or artificial insemination (Veronesi *et al*., 2006; Rebordão *et al*., 2014). In order for the proper occurrence, the uterus must be anatomically positioned in the abdominal cavity. Mares with ventral uterus are not able to properly perform, the physiological cleaning, as consequence of that, intrauterine fluid may accumule. (Veronesi *et al*., 2006; Brink *et al*., 2010). The Delay Uterine Clearance (DUC) is defined by the inability of the uterine cleaning.

Other factors may stimulate the occurrence of cleanse inability, in addition to the changes in anatomical position, including uterine lymphatic system modification and motility. In such cases,

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the remaining fluid effectiveness cleaning is affected and a endometritis state can be installed, resulting in a pregnancy impossibility (Brink *et al*., 2010), it is also a severe, fibrotic and irreversible uterus condition, responsible for reproductive problems and economic losses, since the economic return of equideoculture depends directly on the reproductive performance of these animals (Rebordão *et al*., 2014; Christoffersen *et al*., 2015; Maia *et al.*, 2019).

A surgical correction of uterine positioning in the abdominal cavity, called uteroprexy, is among the endometritis treatments. This procedure can be performed by videosurgery and corresponds to fix the broad ligament of the uterus to the abdominal wall, repositioning it horizontally, in order to correct its position (Brink *et al*., 2010; Corsalini *et al.*, 2016).

This study was conducted with mare exhibiting inability to perform uterine cleaning, following mating and the main aim was to perform and describe the surgical technique and complications of uterine elevation by laparoscopic surgery, using the broad uterine ligament plication with toogles.

MATERIAL AND METHODS

The Federal University of Paraná approved this study, under protocol number 009/2017, 07/03/2017 by the Animal Use Ethics Committee of the Agrarian Sciences Sector. The project execution for this work to be possible was supported by the ICETI. Five mares from different horse breeds, aged and weighing between 14 to 25 years and 91kg to 555kg were suitable for this study, qualified as being under the aforementioned condition. The inclusion criteria was defined by mares whose uterus were ventrally positioned in the abdominal cavity, also known as abnormal uterine conformation or pendulous uterus. The evaluation method included the inspection of the vulvar conformation was by visual and rectal palpation. The procedure allowed to detect the uterus weigh and position, by the possibility of being filled and ventrally inclined. The animals included in this study were from three different horse breeds, two were mixed-breed, two thoroughbred and one mini horse. Perineal conformation and vaginoscopy were performed, as reproductive evaluation and uterine biopsy, on five selected mares prior to the surgical procedure. The biopsy was executed in a horse stock.

A sterile forceps for endometrial biopsy was introduced into the uterus through the vagina, passing through the cervix with the aid of one of the evaluator's hands. At the same time the operator assisted the maneuver by rectal palpation using the contralateral hand. Once the sample was obtained, it was immersed in Bouin's liquid in previously identified containers, where they remained for 24 hours and then transferred to containers containing 10% formaldehyde solution. All were sent for histopathological examination. The animals were not submitted to any type of sedation before or during the procedure.

The five mares underwent a 36-hour food fasting in preparation for laparoscopy, but the water remained *ad libitum*. About 30 minutes before the surgical procedure, the stool was removed manually from the rectal ampoule, bilateral abdominal flank plus the region of the last three intercostal spaces clipping were performed.

Animals were sedated with 10µg/kg of detomidine (Dormiun V, detomidine, Agener União, Brazil) and methadone (Mytedom, methadone, Cristália, Brazil) 0.05mg/kg administered intravenously. Anesthetic maintenance was performed with continuous infusion of detomidine (Dormiun V, detomidine, Agener União, Brazil), starting at a dose of 10µg/kg/h and titrating every five minutes, for the degree of the animal sedation, remaining throughout the procedure at a rate between 1 and 10µg/kg/h.

In the horse stock, the animals were kept in a quadrupedal position. The mares had their urethra catheterized with urethral catheter number 18 and their tail bandaged with 15 cm crepe bandage after sedation. Antisepsis was performed in the hairless area, followed by fixing and positioning the surgical drapes at four distinct points at its margins. Where the backaus clamps were placed, 5mL of 2% lidocaine (2% Xylestesin, 2% lidocaine, Cristália, Brazil) without vasoconstrictor was previously infiltrated.

For the first portal placement through the endotip (11 mm), infiltrative local blockade was performed by the same manner as for the placement of backaus. After blockage, a 2 cm vertical skin incision was made, 2cm after the last rib, in the line of the coxal bone, between the cranial and caudal aspect of the coxal tuberosity.

Following the placement of the endotip, the cavity was inflated with a maximum pressure of 12 mmHG CO^2 and then the uterus, ovaries, oviduct and bladder were inspected with the 0° laparoscopic camera. A second 11 mm cannula was inserted into the abdominal cavity also with previous local block through a second vertical incision of 1 cm, created 3 cm caudally and 1 cm dorsally to the central portal. This portal has been introduced in order to obtain a direct view Thereafter, the third and last portal was introduced, also guided by direct viewing and with previous local block, by a trocar and cannula of 5mm, about 3cm ventral and 1 cm caudal to the second portal. The final configuration of the portals is shown Fig. 1.

Figure 1. Positioning of the portals during laparoscopy. (A) Central portal (11mm), inserted 2 cm after the last rib, in the line of the coxal bone, between the cranial and caudal face of the coxal tuberosity, (B) Second portal (11mm), inserted about 5cm of flow to the central portal,(C) Second portal (11mm), inserted about 5cm of flow to the central portal.

After the portals fixation, 20mL of 2% lidocaine (Xylestesin 2%, 2% lidocaine, Cristália, Brazil) was applied to the broad ligament of the uterus through the second portal before initiating the application of the alternator.

The toggles were made of polyamide, cut and drilled by hand, and the holes were made with a 40x12mm needle, about 2cm long, 3.2mm wide and 1.5mm thick (Fig. 2).

Five minutes after the blockage of the broad ligament of the uterus block, the toggle applicator (Fig. 3 and 4) was introduced through the second portal and also applied to the broad ligament of the uterus by transfixing it.
Before abdominal cavity insertion, a nylon wire (0.6mm in diameter) was passed through the middle of the toogle by its hole. At the end of the wire, a knot was made, which fixed it to the toggle, since the knot did not pass through the hole of this. Once the tools were applied, the wire was pulled manually outside the portal.

Throughout the application of the toggles, the babcock forceps were introduced through the second or third portal to assist in the elevation of the uterus in its ventral portion, in order to contribute to suture traction and support and reducing the risk of rupturing the thread. To finish the plication, a last toogle was loosed placed on the wire, inside the abdominal cavity. After the last toggle, a metal clip was applied to the 0.6mm nylon wire, with no need for a knot at the end of the procedure.

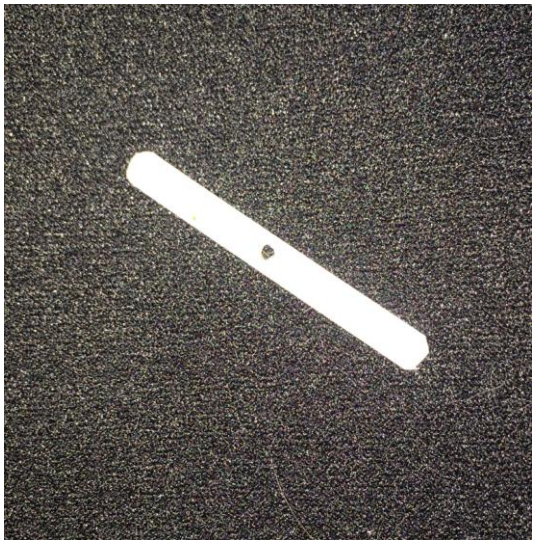

Figure 2. Toggle - Polyamide lever 2cm long, 3.2mm wide and 1.5mm thick.

Figure 3. Toggle applicator produced in stainless steel. Leaked tube with cutout at the tip for wire fitting, 0.5mm wall thickness, 50cm long and 5mm in diameter. Inside is another stainless steel cylinder with 3.5mm to push the "toggle".

Figure 4. Toggle applicator tip. Leaked tube with cut-out at the tip for wire fitting, 0.5mm wall thickness, 50cm long and 5mm diameter. Inside is another stainless steel cylinder with 3.5mm to push the "toggle".

To avoid oviduct obstruction, the toogles were placed about 5cm away from them. The same procedure was repeated on the contralateral side. All instruments were removed and the abdominal cavity deflated. The skin and subcutaneous tissue were sutured with nylon 0 thread, simple interrupted pattern. The animals received: flunixin meglumine (Flunixina, Flunixin meglumine, UCBVET, Brazil) 1.1mg/kg intravenously every 24 hours for five days; penicillin G benzathine, penicillin G procaine and dihydrostreptomycin sulfate (Penfort PPU, Penicillin G benzathine, penicillin G procaine and dihydrostreptomycin sulfate, Ouro fino, Brazil) 30,000 IU/kg intramuscularly every 48 hours, totaling three applications; and omeprazole (Omeprazol, Drogavet, Brazil) 20g per animal orally every 24 hours for five days. The surgical wound was daily cleaned with 2% chlorhexidine (Riohex 2%, Chlorexidine, Rioquímica, Brazil) and Penicillin G benzathine, penicillin G procaine and dihydrostreptomycin sulfate ointment (Ganadol, Penicillin G benzathine, penicillin G procaine and dihydrostreptomycin sulfate ointment, Zoetis, Brazil).

The sutures were removed ten days after the surgical procedure. After 30 days, laparoscopy and the biopsy was repeated. For the laparoscopy the same sedation protocol used in the first procedure was used. The second procedure was performed only to observe the healing and positioning of the uterus in the abdominal cavity, and the average execution time was approximately 15 minutes for each side of the flank. Simple statistical analysis was performed by calculating mean, median and standard deviation of age, weight and time of bilateral surgery of each procedure. To analyze the data found in the classification of uterine biopsies performed before and after surgery, the Mann Whitney Test was applied.

RESULTS

Before the surgery, five mares were diagnosed by rectal palpation presenting flaccid uterus, ventrally directed and supported by the urinary bladder. The time of each procedure, summing left and right, counting from the time from the placement of the first portal to the skin and subcutaneous sutures were 98, 88, 82, 67 and 75 minutes respectively, as Table 1. The procedure average time was 82 minutes.

Table 1. Animals identification for laparoscopy time, when performed with toggle plication on both mares sides.

Animal	Surgical Time – Left Side	Surgical Time – Right Side
Mare 1	47min	51min
Mare 2	42min	46min
Mare 3	40min	42min
Mare 4	35min	32min
Mare 5	35min	40min

*min - minutes

The tables below represent the findings on uterine biopsy before (Table 2) and after the surgical procedure (Table 3).

The results of the biopsy classification performed in this project, according to Kenney (1978). Statistical evaluation of uterine biopsy classification data was analyzed by the Mann Whitney test with none statistical change ($p > 0.05$). According to Kenney (1978) the histopathological classification of the endometrium is subdivided into: I (Normal, mild, sparingly spread inflammation or fibrosis), IIA (Mild stray inflammation, mild fibrosis, reproductive endometrial atrophy), IIB (Moderate scattered inflammation, moderate fibrosis) and III (Severe irreversible changes including fibrosis and inflammation). Of the five animals present in the study, two (Mare 2: classification I; Mare 3: classification IIB) remained with the same classification before and after the procedure. Mare 5 presented an inconclusive result due to the size of the fragment removed in the post-procedure biopsy and could not be evaluated. Mare 1 improved after the procedure from III to IIB) and Mare 4 went from IIB to III.

Table 2. Description of uterine endometrial biopsy found in research animals prior to surgery

Animal	Evidence of luminal content	Luminal epithelium	Inflammatory Changes	Degenerative Changes	Other cells present
Mare 1	Purulent exudate, abundant neutrophils and macrophages	Cuboid with occasional areas of pleomorphism	Mild diffuse lymphocyte and neutrophil infiltration	Absence of endometrial glands	Absent
Mare 2	Absent	Mostly cuboid, columnar aspect areas and pseudostratification	Absent (small foci of lymphocytes in all sample)	Presence of rare foci of mild fibrosis involving branches of endometrial glands	Occasional hemosiderocytes
Mare 3	Absent	Cuboid	Mild inflammation surrounding some glandular nests	Multiple foci of glandular nests. Some endometrial glands in glandular nests exhibit ectasia. Presence of lymphatic gaps	Absent
Mare 4	Absent	Cuboid	Mild diffuse monomorphonuclear infiltration	Incipient fibrosis surrounding branches of uterine glands. Rare glandular nests surrounded by very mild fibrosis. Presence of lymphatic gaps	Absent
Mare 5	Absent	Absent	Diffuse neutrophil infiltration	Dilated glands, many exhibit mild periglandular fibrosis. Apparent diffuse fibrosis	Absent

Table 3. Description of uterine endometrial biopsy found in post-surgical research animals

Animal	Evidence of luminal content	Luminal epithelium	Inflammatory Changes	Degenerative Changes	Other cells present
Mare 1	Absent	Bass columnar to cuboid	Presence of small and rare foci of lymphocytes, some neutrophil capillaries, observe some neutrophils through the luminal epithelium.	Decreased endometrial glands (mild to moderate atrophy), lymphatic gaps across endometrial and myometrium thickness	Absent
Mare 2	Absent	Columnar with pseudostratification	Absent	Some sets of endometrial glands exhibit incipient fibrosis without dilation	Rare hemosiderocytes and eosinophils
Mare 3	Absent	Low columnar, discrete multifocal pseudostratification	Absent	Multifocal periglandular fibrosis, moderate to severe intensity. Mild atrophy of the endometrium. Very mild and occasional dilation of endometrial glands with slight content retention	Absent
Mare 4	Absent	Cuboid	Mild diffuse lymphocyte infiltration	Mild periglandular multifocal fibrosis. Endometrium exhibits moderate atrophy. Presence of many lymphatic gaps reaching myometrium	Absent
Mare 5	-	-	-	-	-

One animal showed clinical signs compatible with intra-abdominal hemorrhagy (pale mucosa, elevated heart rate - 100 beats per minute) in the immediate postoperative period. The animal was continuously monitored and received an appropriate clinical management, becoming stable after 24 hours, without any advancing bleeding signs.

After 24 hours of surgery, three other mares exhibited pain, remained supine, and showed occasionally dug, apathy and with decreased appetite. The presence of an emphysema was observed in all animals used in the research, at the surgical access site, being naturally resolved. 30 days after surgery, a laparoscopy performed, observed that the uterus of all mares was higher compared to the preoperative evaluation, without touching the urinary bladder and the ovarian ligaments exhibited optimal healing. The complications of the second procedure, was possible to observe only in one animal, local pain, and in two, the presence of subcutaneous emphysema in the places of access to the abdominal cavity.

DISCUSSION

Endometritis is mainly caused by DUC and together with endometriosis, become the major responsible for reproductive disorders in the mares, generating economic losses. When its presence is detected, endometrial fibrosis may irreversibly occur, at the same time, getting worse by aging (Walter *et al*., 2012; Rebordão *et al*., 2014; Christoffersen *et al*., 2015; Reghini *et al*., 2016).

The procedures perform time decreased during its process and the average surgical reached 82 minutes. Brink *et al*. (2010) and Corsalini *et al*. (2016) obtained mean surgical times of 162 and 169 minutes, both above what the number observed in this study. It should be emphasized that the decreased surgical time is a major factor when considering a new technique and especially in videosurgery, one should take into account the

time spent on the learning curve, which means that, the more the procedure is repeated, the shorter the surgical time (Cezário de Melo, 2004).

The study describes a surgical uteropexy technique that improves uterine cleansing through drainage and uterine motility, benefiting endometrial health. Brink *et al*. (2010) believe mares that became infertile due to endometritis caused by uterine malformation, may recover fertility after corrective surgery. Although the uterine elevation technique has already been reported by Brink *et al*., 2010 and Corsalini *et al*., 2016, the subject still scarce. Some issues occurred during the procedure, such as the presence of bowel loops near the uterus and difficulty to penetrate certain uterus areas with the applicator. Brink *et al*. (2010) also reported major difficulties in penetrating the uterine wall with the suture needle.

This manuscript is the first using the plication of the broad uterine ligament with "toogles" in it surgical technique. According to Brink *et al*. (2010), uterine elevation may restore mares fertility, even with persistent endometritis, improving uterine cleansing. The candidates for this surgery are mares with endometritis or delayed uterine cleaning caused by poor perineal conformation and pendulous uterus. The toggle applicator used in this study was an instrument developed to improve the uterine lifting technique, easy to obtain and to access, being practical, fast and effective. This is possible to observe when the toggle applicator is a leaked tube, with cut-out at the tip for wire fitting and bizel tip. Therefore, the wire was manually pulled outside the portal, as the toggle applied. The babcock forceps were positioned in the ventral portion of the uterus to lift it, as traction assistance. The procedure made easier to the traction and support of the suture, not allowing the thread rupture.

The mare 5 uterine biopsy examination performed after the procedure was inconclusive, because it was not possible to remove a fragment of adequate size for the exam. Despite the fact that the result of the biopsy is unknown, postoperative uteroscopy revealed a significant improvement in the clinical position, and there was no presence of pus in the womb, as before the procedure, lefting just a small amount. And the animal had these characteristics before surgery.

The main advantages observed was reduced surgical time, when compared to the literature reviewed, resulting in reduced sedation time and lower risk of infection. The technique allows to easily handle the instrument within the abdominal cavity, with the absence need of needle and suture, resulting in a satisfactory healing without site adhesions. In one of the operated mares, clinical signs compatible with possible intra-abdominal haemorrhage were observed, possibly caused by some vessel of the muscle wall after removal of the accesses. After proper treatment, the animal was stable.

After 24 hours of surgery, three other mares exhibited pain, remained supine, and showed occasionally dug, apathy and with decreased appetite. The presence of an emphysema was observed in all animals used in the research, at the surgical access site, being naturally resolved. The complications observed in the study did not compromise the success of the procedure or the prognosis of the patients.

Thirty days after surgery, a laparoscopy performed, observed that the uterus of all mares was higher compared to the preoperative evaluation, without touching the urinary bladder and the ovarian ligaments exhibited optimal healing. The complications of the second procedure it was observed only in one animal local pain and in two presence of subcutaneous emphysema in the places of access to the abdominal cavity.

The limitations of this study include the low number of animals, the performance of a single post operative evaluation, the animals lost of contact with its owners. The animals used in the research were donated after the second procedure, there was no follow-up regarding fertility of mares before or after surgery. This study focuses only on improving the surgical technique.

CONCLUSION

The use of a toggle applicator for laparoscopic broad ligament plication of the uterus is a new method, not described in the literature, with great clinical potential, This technique also has the

potential to reduce surgical time compared to other techniques. The perceived complications were resolved quickly or spontaneously, not compromising the success of the procedure or the prognosis of the patient. Studies with larger numbers of animals, longer period evaluations, fertility evaluations, and comparison of techniques are recommended for a complete understanding of this alternative.

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