Original Research

Laparoscopic Ovariectomy in Standing Mule Mares

Lucio Petrizzi [a], Giulia Guerri [a], Paola Straticò [a, *], Amedeo Cuomo [a], Cecilia Vullo [b], Ippolito De Amicis [a], Domenico Robbe [a], Vincenzo Varasano [a]

[a] University of Teramo, Teramo, Italy
[b] University of Messina, Messina, Italy

A B S T R A C T

Mules are hybrids bred from the mating of a jack donkey and a horse mare, known for their strength and resistance and still used to work in agriculture. Although they have been for long considered sterile, evidence of estrus cycle has been demonstrated together with abnormal behavior related to ovarian activity. In this study, a bilateral standing laparoscopic ovariectomy technique using the LigaSure technology was applied in 10 mare mules for treating unwanted behavioral patterns. The technique was effectively performed on these animals avoiding the risk of general anesthesia, and the use of the LigaSure technology allowed good hemostasis and reduced surgical time. Owners declared to be satisfied with the resolution of the behavior.

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1. Introduction

Mules (*Equus mulus*) are hybrids resulting from a cross between a mare (*Equus caballus*) and a jack donkey (*Equus asinus*); the female mule is commonly referred to as a molly or mule mare. Mollies are bred because of their resistance, adaptation to several types of weather conditions, and a comfortable riding [1,2]; little information is available on their ovarian activity and reproductive features.

Mules are usually considered sterile, and the reason for infertility has been investigated by several authors [3–9]. It has become clear that some mule mares possess a fully functional hypothalamic—pituitary—ovarian axis that allows them to display estrus behavior and ovulation [8].

Besides the hormonal issue, due to the different number of chromosomes between the horse and the donkey, mules often have a mosaic karyotype, some of their cells carrying 62 chromosomes and some 63. When karyotype shows 63 chromosomes, the pairing of homologues before the first meiotic division is impeded, and this

results in infertility. From 1845, when the first paper about a conception and foaling of a mule mare was presented [9], at least 60 reports about fertile mule mares were published. However, in many of these cases, the exact karyotype was not reported, so mosaicism could be suspected [8].

Generally speaking, estrus indicates that an ovarian follicle is under development and that ovulation will occur; but this biological rule varies in mule mares [10]. Some mule mares show estrus behavior without any fully developed follicle, and others do not ovulate even in the presence of fully developed follicles [10].

The evidence of ovarian cyclicity in mule mares was demonstrated long time ago [11], and both follicles and corpora lutea were identified in ovaries of fetal mules [12]. More recently, evidences of regular estrus cycles and description of gross anatomic features of mule ovaries were provided, together with a report of the birth of two donkeys after successful embryo transfer in mule mare recipients using a nonsurgical approach and a spontaneous estrus cycle [13].

Estrus-associated behavior has been well documented in mule mares and shown to affect performances [14,15]. Typical behavioral signs of estrus are performance reduction and temperament variations; these can include attitude changes, reluctance to work, tail swishing, difficulty in training, squealing, horsing, excessive urination, aggression toward other mules or horses and less commonly toward humans, overt anxiety, kicking, and biting [15–17].

In a recent survey about the owners' perception of mollies' behavior, 50% of respondents replied that their molly had behavioral changes that interfered with their use during estrus. In the same survey when owners were asked to rate how much the behavioral changes interfered with the mollies ability to perform, 48% answered that it was mildly affected, 30% moderately affected, and 22% severely affected. Thirty-five percent of the respondents said that for this reason, they would consider having ovariectomy performed on their molly [17].

Ovariectomy can be performed with different techniques [18–21], but the laparoscopic techniques, first described in the 1990s [22,23], are the most commonly used in horses; their advantages are reduced morbidity and mortality rates, historically associated with laparotomic ovariectomy [4,24], minor surgical trauma with smaller flank incisions, and direct visualization of abdominal viscera [4,22,23,25,26].

The aim of this study is to describe (1) the abdominal laparoscopic anatomy of the internal reproductive tract together with ovaries gross anatomy and dimensions and (2) the technique of a standing laparoscopic ovariectomy, in working mule mares. The postoperative effect on behavior of ovariectomy in mule mares was also investigated.

The hypothesis is that standing laparoscopic ovariectomy would be a safe technique for eliminating unwanted estrus behavioral changes in working mule mares, preventing interference with exercise and performances.

2. Materials and Methods

Adult mule mares were selected for this study among a population of working mules (wood carriage along forest paths), mixed in breed and age.

Criteria of selection were clinical and ultrasonographic evidence of estrus cycle, associated with recurrent misbehavior referred from the owner (attitude changes, reluctance to work, poor performances, tail swishing, difficulty in training, squealing, horsing, excessive urination, aggression toward other mules or horses, anxiety, kicking, biting).

To assess the presence of ovarian activity and define the stage of the cycle itself, two transrectal ultrasound examinations 10 days apart from each other were performed, using a 5 MHz linear probe (Mindray DP-6600 Portable Ultrasound).

In order to reduce the ingesta and the space occupied by the intestine within the abdomen, the mares were fed with pelleted food twice a day, starting 2 days prior to surgery; 24 hours before surgery, food was completely withdrawn. Water assumption was not restricted.

Just before surgery, an 18 G jugular catheter was aseptically placed over the left jugular vein, and a broad-spectrum antibiotic therapy (ampicillin 20 mg/kg and gentamicin 8 mg/kg intravenously) together with flunixin meglumine (1.1 mg/kg intravenously) was administered.

Patients were premedicated with acepromazine (50 μg/kg intramuscularly) and sedated with romifidine (50 μg/kg intravenously). Ten minutes after sedation, a bolus of butorphanol was given intravenously (50 μg/kg), and a continuous infusion of romifidine was initiated (50 μg/kg/h) 10 min from the opioid injection [27].

After standing restraint in a wooden stock, a rectal examination was performed to evacuate the rectum from feces and check the internal anatomy before laparoscopy. A silicone Foley catheter (10 Fr × 30 cm) was used to catheterize the urinary bladder before the beginning of surgery. Then both paralumbar fossae were prepared and draped for aseptic surgery.

Anatomic landmarks were identified over the paralumbar fossae, and 20 mL of 2% lidocaine hydrochloride was injected at the level of each expected laparoscopic portal to achieve local subcutaneous, intramuscular, and subperitoneal anesthesia, for a total of three portals on each side. The first portal was located on the left side halfway a horizontal line connecting the last rib to the ventral border of the tuber coxae, and two other portals were placed 4–5 cm dorsal and ventral to the first [23].

After creating a 1.5 cm vertical stab incision through the skin and fascia with a scalpel blade, a laparoscopic cannula with a blunt trocar (11 mm diameter, 30 cm long) was introduced through the abdominal wall; then a 30°, 10 cm diameter, 58 cm long laparoscope was inserted through the cannula. At this stage, the abdomen was insufflated with CO_2 to reach an intra-abdominal pressure of 12–15 mm Hg. Then with the same technique, the other portals were created in the flank.

Once the ovarian bursa and the ovary were identified, a pair of laparoscopic grasping forceps were introduced through the ventral instrument portal to reach and grasp the ovary. Holding the ovary, the ovarian pedicle was infiltrated with 10–20 mL of 2% lidocaine through a laparoscopic needle. Then the laparoscope was moved to the dorsal portal, and a cutting and vessel-sealing device (LigaSure) was introduced through the central portal to transect the mesovarium.

Having excised both ovaries and verified the correct hemostasis, the gonads were removed widening the distal portal for a few centimeters, on each side.

The distal portal of the abdominal wall was closed suturing the fascia and the muscle with a polyfilament absorbable suture material (Polysorb, USP 1) in a simple continuous pattern. The proximal portals were sutured in one layer, involving the cutis, with disposable skin staples.

Surgical time, from the first skin incision until the application of the last skin staple, was recorded.

For each ovary, the time of use of the LigaSure forceps was also recorded.

A stent bandage was applied on each flank covering the skin incisions, and mule mares were brought back to the stable. Flunixin meglumine was administered once a day (1.1 mg/kg IV sid) in the 2 days following surgery.

Six hours after the end of surgery, feeding was reintroduced, and 5 days after surgery, mule mollies were discharged from the clinic. No operative or postoperatory complications were recorded.

A long-term follow-up was obtained by telephonic interview with owners, which included assessment of (1) resolution of estrus-related misbehavior and reluctance to work (original reason for surgery), (2) time to return to the expected activity, (3) incision site appearance, and (4) overall owner satisfaction.

3. Results

Ten mollies were included in the study, ranging from 3 to 5 years old (3.6 years ± 6 months), weight ranging between 450 and 573 kg (476.5 kg ± 55.5).

They all showed recurrent misbehavior during working activity that was estrus-related as confirmed by transrectal ultrasonography that revealed ovarian activity (Figs. 1A and 1B). The estrus-related misbehavior was characterized by restlessness, difficult handling, reluctance to work, frequent urination, difficult acceptance of the burden, kicking and biting of other mules, and excessive tail whipping.

In all cases, bilateral laparoscopic ovariectomy was successfully performed in standing position with the technique described. The laparoscope provided an excellent visibility for the ovary and mesovarium (Fig. 2), with magnification of the structures.
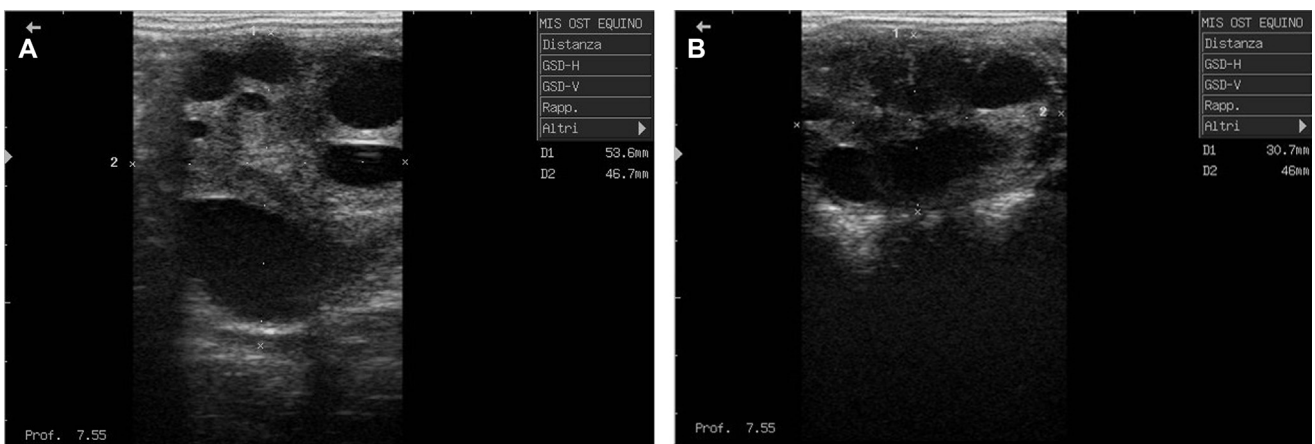
Fig. 1. Longitudinal (A) and transverse (B) images of left ovary of a mare mule showing multiple follicles.

Abdominal laparoscopic anatomy did not differ from horse, with a cylindrical uterine body showing a dorsal concavity toward the tips of the horns, due to an increase in the length of the mesometrium. Ovaries were suspended by the mesovaiurm to the sublumbar region near the fifth lumbar vertebra. Differently from the jenny, the ligament of the salpinx did not cover the lateral aspect of the ovaries that were more accessible during surgery.

The time of use of the LigaSure forceps (Fig. 3) to obtain coagulation and complete excision of the ovary was between 4.51 and 6.41 min (5.58 ± 0.72 min on the left side, 5.04 ± 0.88 min on the right side). The total time for a monolateral ovariectomy was between 13 min and 18.30 min (17.4 ± 1.25 min on the left side, 14.17 ± 1.04 min on the right side).

The quality of hemostasis obtained was excellent (Fig. 4). No intraoperative complications were observed.

The ovaries had an elongated kidney shape. The left ovary (Figs. 5A, 5B, and 5C) weighted 28.7 ± 8.38 g and measured 4.14 ± 0.51 cm in length, 3.13 ± 1.46 cm in width. The right ovary (Figs. 6A, 6B, and 6C) weighted 27.3 ± 10.6 g and measured 3.63 ± 0.5 cm in length, 2.37 ± 0.92 cm in width.

During the postoperative period (between second and third day after laparoscopy), 8/10 animals developed a mild emphysema on both flanks around the distal portals (that were previously enlarged to remove the ovaries) that regressed spontaneously after 1 or 2 days; one molly showed a mild inflammation on the right distal laparoscopic portal, with moderate subcutaneous edema and pain at palpation that regressed with NSAIDs therapy. No abnormalities in clinical parameters were detected. Five days after surgery, all the animals were clinically sound when discharged from the hospital.

The time to follow-up ranged between 90 and 540 days, with a mean follow-up time of 160 days. All owners were satisfied with the procedure, the associated resolution of misbehavior and the appearance of the incision sites. The mean time to return to the expected activity was about 60 days. They also declared they would consider laparoscopic ovariectomy again in the future for other mares with misbehavior.

4. Discussion

Bilateral laparoscopic ovariectomy can be effectively performed in mollies using a technique that substantially resembles the one used in the horse mare [28] without major complications.

The selection of the mule mollies was based primarily on the owner's complaint about abnormal behavior during working activities. As in equine mares [15,16,29–31] and in agreement with a previous study on mules [13], the most often referred misbehavioral patterns were reluctance to work, restlessness, difficult handling, frequent urination, mouthing, and excessive tail whipping.
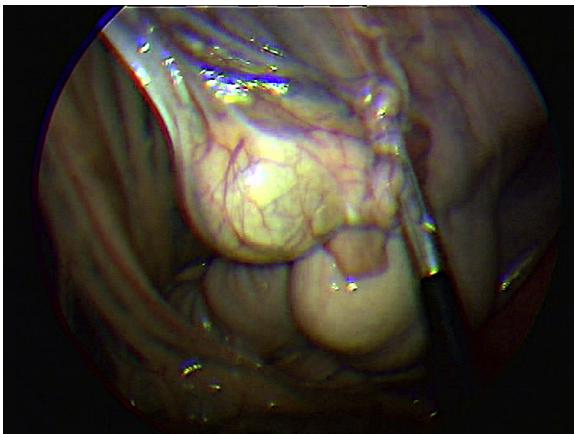

Fig. 2. Laparoscopic image of the left ovary after application of grasping laparoscopic forceps.
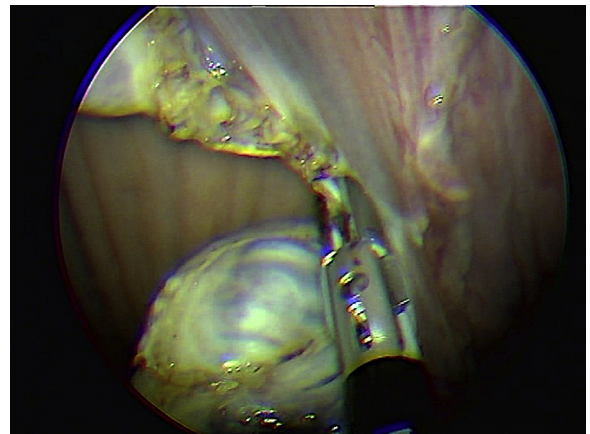

Fig. 3. Laparoscopic image during dissection of the mesovarium using the LigaSure vessel sealing system.
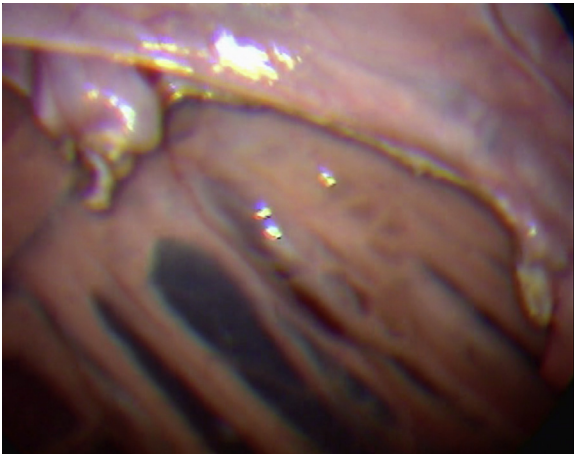
Fig. 4. Laparoscopic image of the left mesovarium after ovarian transection. Secure hemostasis was correctly achieved.

Although a hormonal evaluation was not performed to assess the endocrine array of the animals, a history of recurrent abnormal behavior was the main criterion for selection; in these animals, the transrectal ultrasound examinations allowed to identify follicles and corpora lutea, whose diameter reached values suggestive of ovulation [32].

Romifidine has been described to be highly effective for sedation during standing procedures in horses because, compared to xylazine and detomidine, it produces less ataxia at equi-sedative doses [33,34]. Moreover, in horse, its association with butorphanol is related to better quality of sedation and analgesia [35–38]. Clinical evaluation of the sedative effect of romifidine at different dosages in mules has been described [39], although pharmacokinetic study was not performed. Romifidine was chosen for the sedation protocol because of its clinical reported effects and for a prospective study about pharmacology of the drug in mules. The protocol provided suitable conditions for standing laparoscopy in mules producing adequate sedation and analgesia for laparoscopic procedures.

Compared to a celiotomic approach [22,28,40–43], laparoscopy allowed an excellent visualization of the abdominal cavity and particularly of the ovaries and mesovarium, in molly mares as in horse mares and jennies, excluding the risks of general anesthesia and anesthetic recovery [41].

A particular challenge in this study was related to the different individual conformation of the mules' flank that rendered of paramount importance an accurate evaluation of the sites for portal positioning, in order to avoid damages to the internal structures. Furthermore, short flanks can lead to difficulties in manovrating instruments and in obtaining a successful triangulation, because of less surgical workspace with possible interference during the procedure. The left lank approach was sometimes difficult due to the spleen that appeared to be displaced backward compared to the horse. In order to overcome the lack of space in the flank for portal positioning, the 17th intercostal space could be considered in further studies.

The LigaSure technology allowed a good hemostasis [25], coagulating, and cutting of the mesovarium in a shorter time compared to horse mares [26,28] and jennies [43,44]. A reduced thickness and length of the mesovarium, due to the animal size, can explain a faster procedure in mule mares, but, to the authors's knowledge, these measures of mesovarium were never evaluated in equids. In a study by Aziz and colleagues, in jennies, an instrument provided with a loop-shape thermal wire allowed isolation, coagulation, and cutting of each ovary in 2.8 min [43].

The average dimensions of the excised ovaries in our study were similar to those reported for jennies [45]; in relation to their body mass, jennies have an internal reproductive tract more developed than mollies [43,45], although smaller than horse mares [26,45].

Also the phase of the estrus cycle and the presence of ovarian pathologies can possibly affect the time to perform the dieresis of mesovarium and the application of the LigaSure forceps [26]. Another factor known to be affecting surgical time is the confidence of the surgeon with the procedure [42,46].

In this study, no major complications were encountered, during or after surgery. Differently from other reports describing the occurrence of chemical peritonitis due to CO_2 insufflation [47,48], we did not observe any rise of rectal temperature.

As in other species [46,49], all owners declared to be satisfied with the procedure, both in relation to the regression of the misbehavior that was the original demand for surgery and under a cosmetic point of view.

Despite the evident advantages of the technique, laparoscopic ovariectomy requires specific and expensive instrumentation; LigaSure is defined by the manufacturer as a single-use instrument. The economic issue can be a reason for a limited use of this technique for treating working animals, although the increased interest in mules and their use are heading to an increasing concern on estrus-related behavior and associated problems. Moreover,
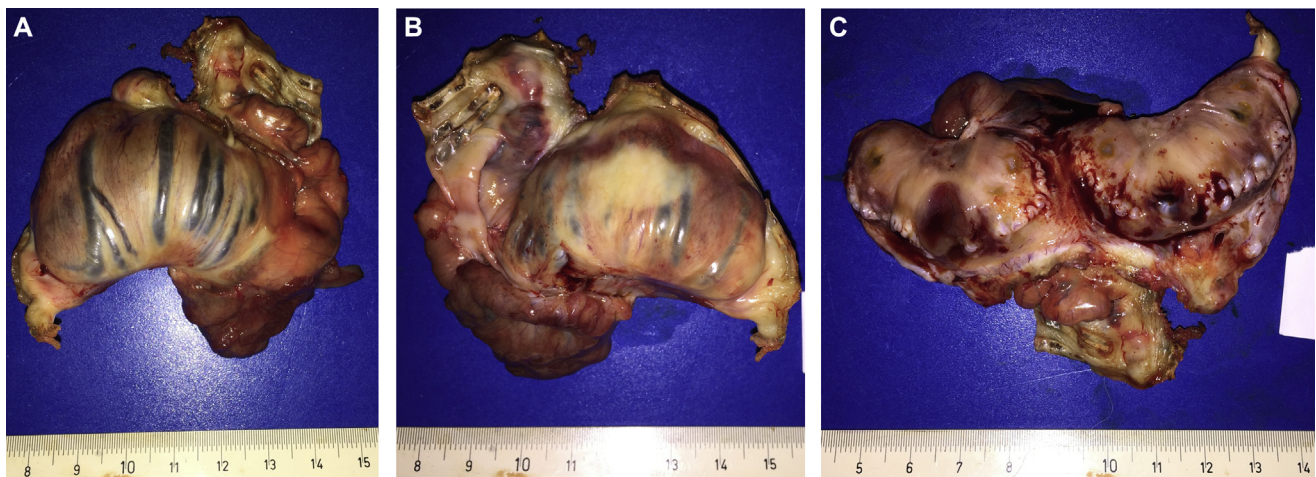

Fig. 5. (A and B) Longitudinal aspect of both sides of the left ovary after removal; (C) cut surface of the same ovary that shows an irregular pattern suggesting ovarian activity.
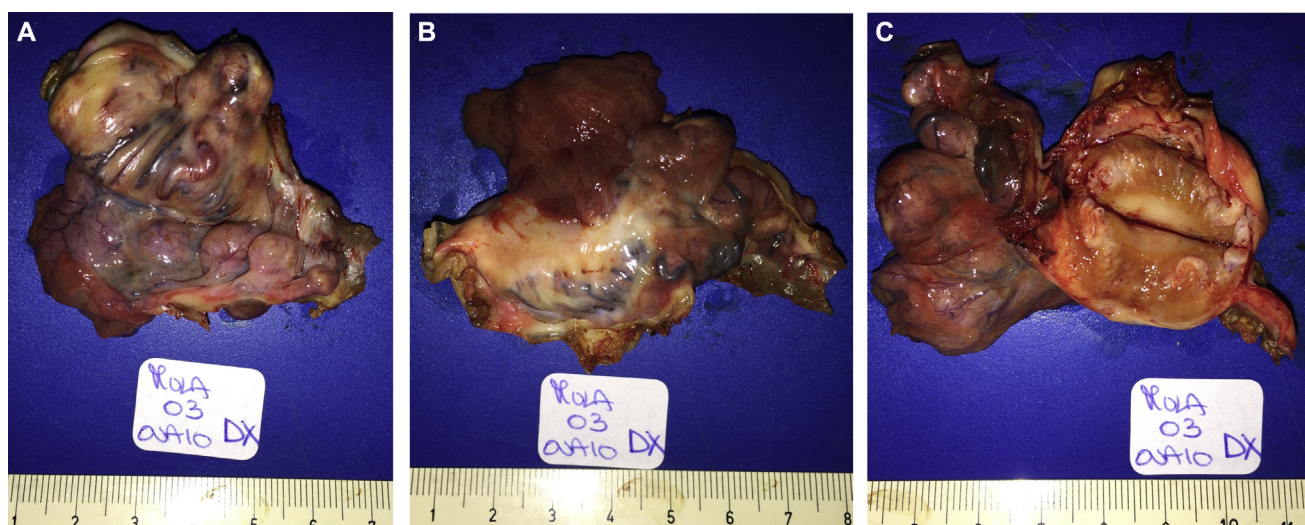
Fig. 6. (A and B) Longitudinal aspect of both sides of the right ovary after removal; (C) cut surface of the same ovary that shows an irregular pattern suggesting ovarian activity.

vaporized hydrogen peroxide (VHP) has been described to be effective in sterilizing single-use devices allowing the cost reduction [28,50]. Differently from the horse mare, whose reproductive integrity can be preserved for breeding purposes [17], ovariectomy can be a more likely option in mules, compared to the hormonal treatment.

5. Conclusions

It can be concluded that bilateral laparoscopic standing ovariectomy with LigaSure technology can be successfully used to treat estrus-related misbehavior in mollies, reducing the risks associated with general anesthesia and conventional laparotomic approaches, the total surgical time, and providing optimal hemostasis. Differently from mare owners, mule handlers appreciate laparoscopic ovariectomy, as a permanent treatment for estrus-associated misbehavior that allows easier handling throughout the breeding season.

Financial disclosures

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