Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12080

Technical Note

Hysteroscopic hydrotubation of the equine oviduct

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Summary

Reason for performing study: Diagnostic techniques for oviductal obstruction in the mare are limited and development of a more reliable and direct method to assess oviductal patency is needed.
Objective: To evaluate the feasibility of hysteroscopic selective hydrotubation of the equine oviduct via videoendoscopy in standing mares.
Methods: Using a catheter inserted under endoscopic guidance into the uterotubal junctions of 10 mares, 5 ml of indigo carmine solution (4 mg/ml) was flushed into the oviduct. After introduction of the dye, peritoneal fluid was obtained via abdominocentesis. A colpotomy was also performed to allow introduction of a videoendoscope into the abdominal cavity to assess the presence of dye visually at the ovarian end of the oviduct.
Results and conclusions: In 15 of 20 (75.0%) attempts, the catheter was successfully inserted into the uterotubal junction, and dye was observed at the ampulla, fimbria, overlying the ovary or surrounding tissue. In 2 mares, the videoendoscope could not be manipulated to identify the uterotubal junction. Only one of 2 oviducts was flushed in an additional mare because insufflation of the uterus could not be maintained. The colour of the dye was evident macroscopically and spectrophotometrically in 4 of 8 mares from which peritoneal fluid was successfully collected.
Potential relevance: The equine oviduct can be evaluated by hysteroscopic selective hydrotubation.

Keywords: horse; oviduct; endoscopy; hydrotubation

Introduction

The equine oviduct and uterus are connected at the uterotubal junction (UTJ), a papilla-like structure. This junction is composed of smooth muscles that form a narrow entrance from the oviduct into the uterus. Because of this anatomical structure, access to the oviduct *in vivo* is limited and diagnostic techniques, such as salpingography, and therapeutic options for disorders involving the oviduct involve laparoscopy or laparotomy [1–7]. Although oviductal abnormalities that result in infertility in the mare are considered to be relatively rare, there are reports of retention of collagenous masses in the ampullary-isthmic junction [8–10]. Because the majority of these masses are significantly smaller than the inner diameter of the oviduct, it was suggested they could not interfere with passage of fertilised ova into the uterus. However, Liu *et al.* [11] have described large masses that occupy the entire lumen of the oviduct and could thus result in infertility. In order to identify blocked oviducts, either starch granules and phenosulfonphthalein [1], or fluorescent microspheres have been sprayed onto the surface of ovaries or into follicles [2] directly or laparoscopically. Oviducts presumed to be blocked have also been flushed via the ampulla [1–4,6,7] or the UTJ through a ventral midline laparotomy with the mare under general anaesthesia [5]. Although oviductal masses were removed successfully via laparotomy, the technique is invasive, costly and includes the risks associated with general anaesthesia.

Attempts have been made to cannulate the equine oviduct from the uterotubal entrance or via the ampulla in the heavily sedated standing mare; however, this procedure was reported to be difficult due to its papilla-like structure, its position in the tip of the uterine horn and its narrow opening [3,4,12,13]. Recent reports suggest that the ampulla can be catheterised [4,7] or a triacetin gel containing prostaglandin E_2 can be placed over the ampulla via a laparoscopic approach [6]. In the former technique, either a dye or microscopic fluorescent beads were infused in the ampulla and the marker was subsequently collected from the uterus. In the latter method, the gel containing prostaglandin E_2 purportedly dilated the oviductal canal and thereby enabled oviductal masses to pass into the uterus. It has been suggested that removing oviductal masses by the ampulla to isthmus approach may be more difficult, as the luminal diameter of the isthmus is significantly smaller than the ampulla [13].

A technique known as hysteroscopic selective hydrotubation is performed to diagnose and treat oviductal diseases in women. A stain or contrast medium is injected into the UTJ through a catheter placed hysteroscopically [14–17]. In the present study, hysteroscopic selective hydrotubation was attempted with a flexible videoendoscope in 10 heavily sedated standing mares. Success at cannulisation, observation and collection of dye from the abdominal cavity and complications associated with this novel technique are described.

Materials and methods

Two maiden and 8 barren Thoroughbred mares, aged 4–22 years, were examined in 2000 and 2001 (Supporting Information Item 1). Barren mares had foaled in the previous season and had no reproductive abnormalities on breeding soundness examination. The procedure was performed during oestrus (n = 3), dioestrus (n = 4) and anoestrus (n = 3). The mares were restrained in stocks and given 350 mg xylazine and 5 mg butorphanol tartrate i.v. This was repeated as needed during the procedure. The mares' tails were bandaged and the perineal areas cleaned. An operator wearing a sterile surgical glove over a long sterile sleeve inserted a 160 cm flexible endoscope (Olympus CF230L)[a] into the uterus. The endoscope was disinfected by an endoscope steriliser (Olympus EW-10)[a] before the procedure. The videoendoscope was used in combination with a videoprocessor (Olympus CLV-U20)[a] and a light source. The endoscope was manipulated through the left uterine horn to the UTJ while the uterus was insufflated by continuous filtered air from the endoscope. After confirming the orifice of the UTJ, a catheter made for the selective hydrotubation in mares was extruded from the working channel of the endoscope.

The catheter was a 200 cm polyethylene tube (1.7 mm outer diameter) with a 22 gauge 4.45 cm injection catheter attached at one end and a 20 gauge 3.8 cm needle connected to a 5 ml syringe at the opposite end. The handle of a guide wire for human angiography (0.46 mm diameter, 220 cm long, RF-GA18263)[b] was inserted into the catheter (Fig 1). This component of the catheter was sterilised with ethylene oxide gas.

The guide wire was pushed a few millimetres out through the tip of the catheter and both catheter and guide wire were inserted into the orifice of

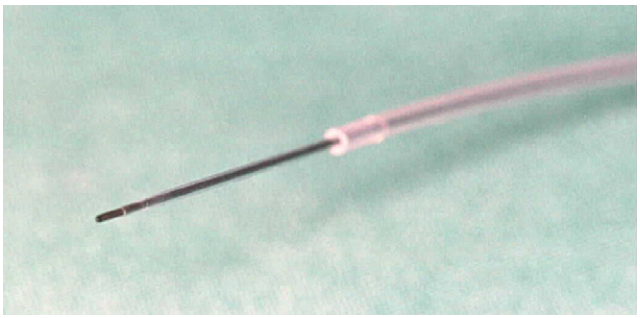

Fig 1: Close up view of the catheter. A polyethylene tube with an attached 22 gauge injection catheter and guide wire inserted into the lumen of the catheter was used in this study. A few millimetres of the guide wire are visible.

the UTJ by carefully manipulating the endoscope. The guide wire was then removed (Fig 2). Indigo carmine solution (5 ml, 4 mg/ml, Indigocarmine Injection)[c] was injected into the oviduct (Fig 2), and the catheter was removed from the endoscope. The endoscope was withdrawn caudally into the uterine body and redirected into the right horn and the procedure was repeated with a new catheter and a new sterilised guide wire. In 3 mares (5 oviducts) the catheter could not be inserted into the UTJ, so the indigo carmine solution was sprayed onto the UTJ and the results were compared with the UTJ cannulated mares.

Peritoneal fluid samples were collected with ultrasonographic guidance (SSD 500, 7.5 MHz)[d] before and 30 min after the procedure in all mares. In 2 mares, fluid was not obtained, and so one litre of lactated Ringer's solution (LRS) was infused into the abdomen through a 16 gauge catheter inserted through the ventral midline. Fifteen minutes later, peritoneal fluid was collected in a 10 ml clear centrifuge tube. The peritoneal fluid was centrifuged (1000 ***g***, 10 min) and its colour was examined macroscopically. In 6 mares, peritoneal fluid samples obtained before and after the procedure were examined with a spectrophotometer at 500, 550, 600, 650 and 700 nm; 612 nm is the wavelength that causes maximal absorption of the indigo carmine dye [18–20].

To determine the location of the dye within the abdomen, a 7 cm colpotomy incision was performed in mares after epidural anaesthesia with 10 mg of xylazine diluted in 10 ml of saline. Approximately 60 min after the initial infusion of the dye into the UTJ, the colpotomy incision was made with a scalpel on the vaginal fornix 5 cm ventral to the external cervical os at the 7 o'clock position. A second sterilised flexible endoscope (Olympus PCF200)[a] was inserted into the abdomen through the colpotomy incision and both oviducts were inspected directly.

Results

The UTJ of both oviducts was catheterised successfully in 7 mares (14 oviducts) and of the right oviduct in another (*Mare J* Supporting Information Item 1). In the latter mare, the procedure was performed during oestrus and was not completed because the uterus could not be sufficiently insufflated with air due to the inability of the cervix to remain closed. It was not possible to insert the catheter into the UTJ of 2 mares (*Mares C* and *F*; Supporting Information Item 1), because the UTJ moved from the centre of the endoscopic view, making it impossible to manipulate the endoscope appropriately. Overall, 15 of 20 UTJs were catheterised successfully in the 10 mares.

Indigo carmine dye solution was observed on the ampulla, fimbria, over the ovary and surrounding tissue on endoscopic examination (Fig 3) in all successfully cannulated oviducts, but not in mares that had the dye infused over the UTJ. More than 3 ml of peritoneal fluid was obtained in 6 mares (*A*, *C*, *F*, *G*, *H* and *I*) and a few drops was recovered in *Mare J*. Peritoneal fluid could not be obtained in 3 of the 10 mares (*B*, *D* and E). Peritoneal fluid was recovered from *Mare D* after LRS was infused into her abdomen; this was not possible in *Mare E* that had one litre of LRS infused into the abdomen. *Mare B* did not receive the lavage.

In the 2 mares (*C* and *F*) that had indigo carmine solution sprayed on both UTJs, the dye was not observed macroscopically in the peritoneal fluid, or on the left ampulla, left ovary or mesovarian in *Mare J*, which had dye sprayed on the UTJ. Of the 6 mares in which cannulation of one or both oviducts and peritoneal fluid collection were successfully performed (*Mares A*, *D*, *G*, *H*, *I* and *J*), indigo carmine dye was detected macroscopically in 4 mares (*Mares A*, *D*, *G* and *J*; Fig 4). In these cases, spectrophotometry revealed a peak at approximately 600 nm (Fig 5). In the remaining 2 mares (*Mares H* and *I*), the dye was not identified clearly in peritoneal fluid by spectrophotometry.

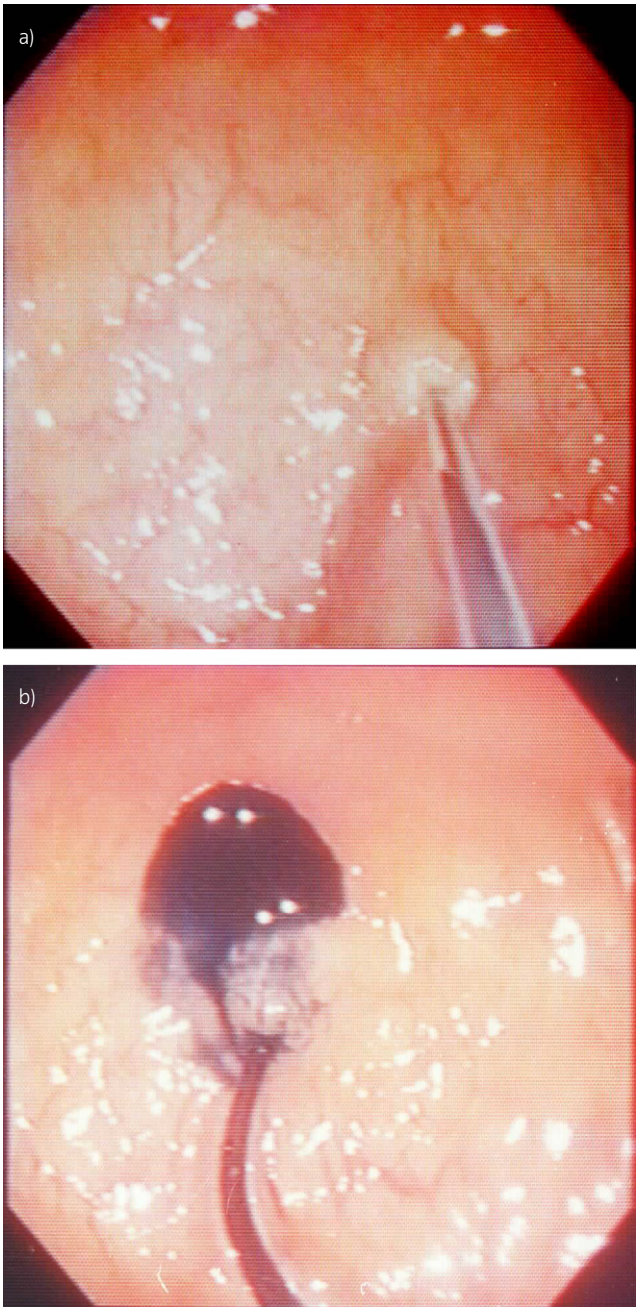

Fig 2: (a) The catheter with the guide wire was inserted a few millimetres into the uterotubal junction. (b) The indigo carmine solution was infused into the uterotubal junction, and back flow of the dye solution was observed.

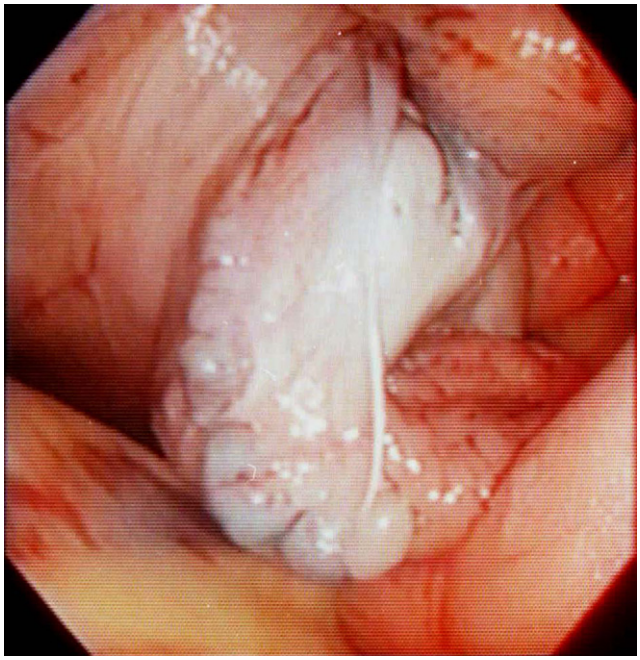

Fig 3: Direct endoscopic observation of the left ampulla and fimbria in *Mare A* after the oviduct was flushed with the indigo carmine solution. The blue dye was observed through the transparent wall of the ampulla.

Discussion

Catheterisation of the UTJ was performed successfully on 15 of the 20 attempts. While additional investigations need to be performed in subfertile mares, this is a novel method for studying oviductal patency. Previous reports indicated that catheterisation of the oviduct from the uterus was extremely difficult [3,12]; however, in the author's experience this was true only if the uterus was over insufflated. Over insufflation causes the UTJ to move from the centre of the endoscopic view, making it impossible to manipulate the endoscope appropriately. Therefore, it is advisable that the endoscope be passed through the uterine horn to the UTJ under minimal expansion of the uterus. When the procedure was performed in 2 mares during oestrus, insufflated air leaked from the uterus through the open cervix impairing visibility and the catheterisation was not completed in 3 of the 4 oviducts. Thus this technique should be performed during dioestrus or anoestrus.

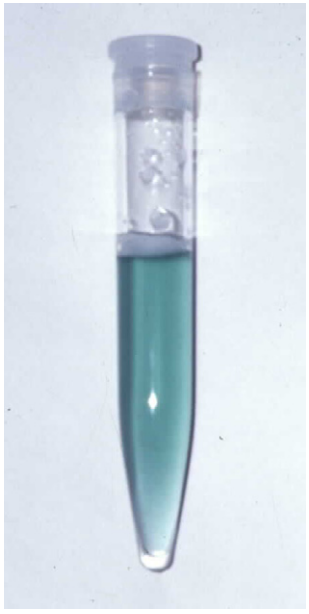

Fig 4: Collected abdominal fluid from *Mare A*. An obvious blue colour was present in the fluid.

In order to confirm the passage of the indigo carmine solution, direct observation of the area was performed using a flexible endoscope. This procedure is not necessary if the dye can be collected by abdominocentesis. None of the mares used in this study had any evidence of peritonitis for at least a month after the flexible endoscope was passed into the abdomen. Based on recent reports indicating that ovaries and oviducts can be observed by laparoscopy [3,4,6], that approach would be preferred to the flexible endoscope used in the present study, if the equipment and expertise are available. In all instances in which the catheter could be inserted into the UTJ, the indigo carmine solution passed through the oviduct. Therefore, if there is no significant luminal obstruction in the oviduct, fluid should pass through the oviduct and into the abdominal cavity. The technique described in this paper could be a potential diagnostic tool to identify the presence of masses that obstruct the oviduct lumen. However, fluid would bypass masses that do not completely block the oviduct lumen. Further research is needed to clarify the relationship between the passage of fluid and the existence of such masses in subfertile and fertile mares. In the majority of previous studies, attempts have been made to flush the oviducts from the ampullary to the isthmus end of the oviduct [1,2,4–7]. However, it may be more difficult to remove oviductal masses using that approach, as compared with the approach used in the present study, because the luminal diameter of the isthmus is significantly smaller than the ampulla [13]. It would appear to be more appropriate to flush the oviduct from the UTJ. Because back flow of the indigo carmine solution was observed in all cases in the present study, it is not known if the pressure was sufficient to remove all obstructions and further investigation will be necessary to assess the ability of this procedure to remove oviductal masses.

Laparoscopic techniques for investigating oviductal patency using an infused dye or fluorescent beads have been recently described. Köllmann *et al*. [4] successfully catheterised the ampulla and injected sterile methylene blue solution in 7 of 11 cases. In 5 of the 7 cases, the injected fluid was identified in the uterus by hysteroscopy post operatively. Arnold and Love [7] catheterised the ampulla and injected fluorescent beads in 31 of 32 oviducts in 16 healthy dioestrus mares. Beads of both colours were retrieved from uterine lavage fluids in 6 of 16 mares at 24 and 48 h post catheterisation, while *post mortem* examination revealed plug formation that precluded bead transport in 15 oviducts. Unfortunately, fertility data were not available so whether the masses interfered with normal oviduct function is not known.

In the present study, it was not possible to collect peritoneal fluid from 3 mares. In 2 of these mares, one litre of LRS was infused into the abdomen in an effort to obtain peritoneal fluid; however, it could not be ascertained how much solution should be used for this purpose. Furthermore, if the indigo carmine solution passed through the oviduct, the colour was not evident macroscopically or by spectrophotometry in 4 of 8 mares. In addition, in one mare, the absorption spectrum appeared to be higher before indigo carmine solution was flushed through the UTJ than after. In that instance, a few drops of peritoneal fluid were collected before and after the solution was injected, suggesting that the small volume offluid was insufficient to allow accurate measurement of the colour by spectrophotometry. Additional studies are needed to determine the amount of the dye solution that should be injected, the volume of the fluid that should be infused into the abdominal cavity, and the optimal interval between infusion of this fluid and collection of the peritoneal fluid. Finally, in the present study the same dye solution was used to flush both oviducts. Therefore, it was not possible to determine which oviduct or back flow was the origin of the dye solution that appeared in the peritoneal fluid. It would be advisable to use dyes with different absorption spectra to differentiate the passage of fluid through the oviducts. If the relationship between the colour of the peritoneal fluid and the passage of the dye through the

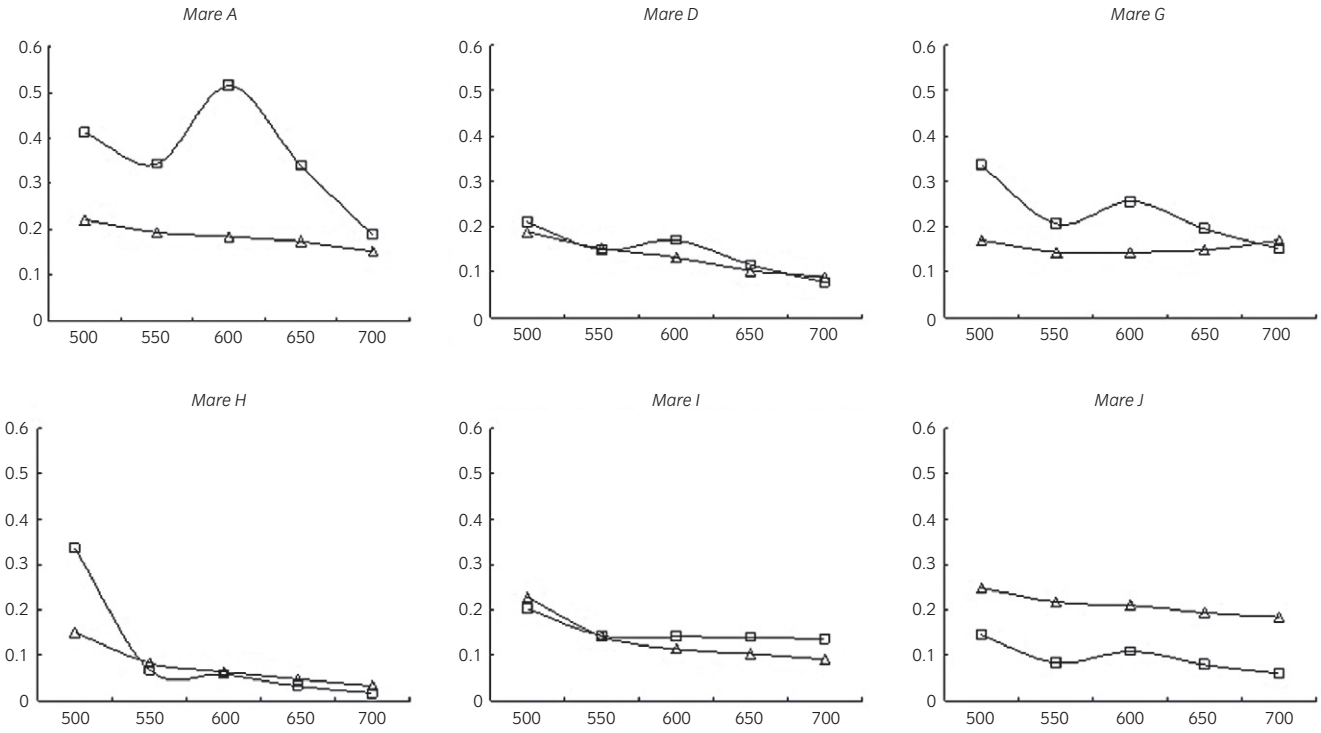

Fig 5: Absorption spectra at 500–700 nm before (triangle marker) and after (square marker) the oviducts were flushed with the indigo carmine solution in 6 mares. The X axis represents wavelength (nm), and the Y axis represents absorption spectrum.

oviducts can be established more accurately, this technique will be a practical alternative to previously described techniques.

Author's declaration of interests

No conflicts of interest have been declared.

Sources of funding

None.

Acknowledgements

The author would like to thank Michelle M. LeBlanc for assistance with the manuscript.

Manufacturers' addresses

[a]Olympus, Tokyo, Japan.
[b]Terumo, Tokyo, Japan.
[c]Daiichi Sankyo, Tokyo, Japan.
[d]Aloka, Tokyo, Japan.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supporting Information Item 1: Clinical details of 10 mares in this study.