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Title: Endoscopy Guided Photoablation of Endometrial Cysts using a 980 nm Laser with a Contact Fiber in Mares

Authors and Affiliations:

Jérôme Ponthier¹, Geoffroy de la Rebière², Alexandra Salciccia², Stéfan Deleuze¹

¹Equine Reproduction, Veterinary Teaching Hospital, Liège University, Liège, Belgium

²Equine Surgery, Veterinary Teaching Hospital, Liège University, Liège, Belgium

Corresponding Authors:

Jérôme Ponthier (Jerome.Ponthier@uliege.be)

Email Addresses for All Authors:

g.delarebieredepouyade@uliege.be

alexandra.salciccia@uliege.be

s.deleuze@uliege.be

Jerome.Ponthier@uliege.be

Author Questionnaire

- 1. Microscopy:** Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 14

Number of Shots: 29

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Stéfan Deleuze**: Reducing the number of intra-luminal cysts with laser under hysteroscopic control can improve fertility by reducing the delay for pregnancy confirmation and improving embryo migration between day 6.5 and 17.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
- 1.2. **Stéfan Deleuze**: Laser irradiation with a contact fiber results in smaller subsequent endometrial scars. Performing this procedure in a liquid environment avoids smoke emission or foam production inside the uterus during the procedure.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

- 1.3. **Geoffroy de la Rebière**: Diode laser is mainly used in horses to treat upper airways pathologies. It could also be used for other pathologies, such as some skin disorders or for facilitated ankylosis.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
- 1.4. **Jérôme Ponthier**: Before cyst ablation by laser, perform breeding soundness examination, including endometrial biopsy, to establish the prognosis and treat the underlying pathologies. If impossible, propose embryo transfer or ICSI.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.5. **Stéfan Deleuze**: Demonstrating the procedure will be Pascal Lejeune, an endoscopy specialized technician from our clinic. He will be working with Jérôme Ponthier.

1.5.1. INTERVIEW: Author saying the above.

1.5.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.6. This protocol is used for mares presented in the equine hospital and follows institutional animal care guidelines.

Protocol

2. During the Preceding Estrus and Diestrus

- 2.1. During the preceding estrus, perform a complete breeding soundness examination of the mare, including an endometrial biopsy, to ensure that the mare can potentially get pregnant after the procedure [1].
 - 2.1.1. Talent examining the mare.
 - 2.1.2. LAB MEDIA: Image of cytology and biopsy slides. NOTE: This was not uploaded, so can be skipped.
- 2.2. During the preceding diestrus, count, measure and map endometrial cysts in the uterus using trans-rectal [1] ultrasonography guided by palpation with a 7.5 or 5-megahertz linear rectal probe and calipers on the screen in a contention stock [2].
Videographer: This step is difficult and important!
 - 2.2.1. Talent performing ultrasonography.
 - 2.2.2. LAB MEDIA: JOVE61569-2.2.-a.jpg. NOTE: Can use any of the uploaded images (a – d), author did not specify which is preferred.
- 2.3. Intra-muscularly administer 250 micrograms of cloprostenol to induce estrus within 2 or 3 days at day 6 to 7 post-ovulation [1]. Perform routine daily ultrasonography and cervix palpation to observe estrus signs, including significant endometrial edema, antral follicle of 35 millimeters, or a softened and opened cervix [2].
 - 2.3.1. Talent administering cloprostenol.
 - 2.3.2. LAB MEDIA: JOVE61569-2.3.2.-a-uterus.jpg and JOVE61569-2.3.2.-c-ovaries.jpg. NOTE: Can use any of the uploaded images (a or b and c or d), author did not specify which is preferred.

3. Endometrial Cysts Photoablation

- 3.1. Make sure that anyone entering the operating room is wearing eye protection [1]. After emptying of the mare's rectum, wrap the tail [2] and clean the vulva aseptically using iodine-povidone [3].
 - 3.1.1. Talent entering the operating room with eye protection on.
 - 3.1.2. Talent wrapping the tail.
 - 3.1.3. Talent cleaning the vulva.
- 3.2. To ensure easy manipulation and comfort of the mare, intravenously administer 10 micrograms per kilogram detomidine and 0.1 milligram per kilogram butorphanol in

- the jugular vein with a 21-gauge needle [1]. Install the embryo collection catheter through the cervix [2] and inflate the balloon with 40 milliliters of air [3].
- 3.2.1. Talent administering detomidine and butorphanol.
 - 3.2.2. Talent installing the embryo collection catheter.
 - 3.2.3. Talent inflating the balloon.
 - 3.3. Infuse the uterus with 1.5 liters of sterile saline solution to inflate it [1-TXT], then deflate the balloon and remove the catheter [2]. Pass the endoscope aseptically through the cervix within a sterile sleeve [3]. *Videographer: This step is important!*
 - 3.3.1. Talent infusing the saline solution. **TEXT: 0.9% NaCl**
 - 3.3.2. Talent deflating the balloon.
 - 3.3.3. Talent passing the endoscope through the cervix.
 - 3.3.4. LAB MEDIA: Endoscopy video. **NOTE: This was not uploaded, so can be skipped.**
 - 3.4. Connect the 600-micrometer quartz fiber to a diode laser operating at 980 nanometers [1] and set the power at 20 to 25 Watts in continuous mode [2].
 - 3.4.1. Talent connecting the fiber to the laser.
 - 3.4.2. Talent setting the laser power.
 - 3.5. Pass the flexible quartz fiber via the biopsy channel of a 10-millimeter diameter video-endoscope [1] until 3 to 4 centimeters of free fiber can be identified on the screen [2]. A minimum of 10 millimeters from the end of the fiber should be visible [3].
 - 3.5.1. LAB MEDIA: JOVE61569-3.5.2.-a.avi. 0:26 – 0:30.
 - 3.5.2. Screen with the fiber visible.
 - 3.5.3. Appropriate length of fiber visible.
 - 3.6. Activate the laser to puncture the cyst, ideally at its apex. In some cases, multiple punctures at various locations are necessary to achieve complete voiding. The cystic fluid is then passively drained out into the uterus. After the cyst lining has collapsed around the fiber, deploy the laser until the membrane shrinks. *Videographer: This step is difficult and important!*
 - 3.6.1. LAB MEDIA: JOVE61569-3.6.1to3-a.avi. 1:00 – 1:40. *Video Editor: Speed up as needed.*
 - 3.7. **Alexandra Salciccia:** When acting in liquid medium, be sure that the fiber is in close contact with the cyst and verify that potential inner walls of the cysts are punctured.
 - 3.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

3.8. Perform transrectal ultrasonography of the uterus as many times as necessary during the procedure to confirm that the endometrial cysts have disappeared [1].

3.8.1. Screen showing that the endometrial cysts have disappeared.

4. Post-operative Treatment

4.1. Collect the liquid infused in the uterus before the endoscopic procedure through a sterile cuffed catheter [1], then flush the uterus with successive instillations of 1 liter of sterile isotonic solution until a clear liquid is collected [2-TXT]. After the procedure, administer general antibiotic treatment intra-muscularly twice a day for 3 days [3-TXT]. *Videographer: This step is important!*

4.1.1. Talent collecting the liquid through the catheter.

4.1.2. Talent flushing the uterus. **TEXT: 0.9% NaCl**

4.1.3. Talent administering antibiotic. **TEXT: 22,000 UI/kg penicillin-procaine suspension**

4.2. Administer 20 IU of oxytocin intra-muscularly at the end of the procedure [1]. On the next day, perform transrectal ultrasonography of the mare uterus. Intraluminal free liquid is frequently observed [2]. *Videographer: This step is important!*

4.2.1. Talent administering oxytocin.

4.2.2. LAB MEDIA: JOVE61569-4.2.-IntraluminalFreeLiquid.jpg.

4.3. As long as free intra-luminal fluid is observed by transrectal ultrasonography and until the end of the heat, perform uterine lavages daily. Sometimes, serum-like, limpid and yellow stained liquid is collected for several days [1].

4.3.1. Talent performing uterine lavage.

4.4. Perform trans-rectal uterine ultrasonography during the following diestrus to confirm disappearance of endometrial cysts and the absence of intraluminal free fluid [1].

4.4.1. Talent performing the ultrasonography and no cysts or intraluminal free fluid.

Results

5. Results: Endometrial Cyst Treatment

- 5.1. This protocol was used to treat cysts with diameters over 15 millimeters. Photoablation of the smaller cysts was also attempted if they were present. Generally, large cysts were at the bifurcation or the basis of the horns [1]. However, some of them were present in the body of the uterus and were also treated [2].
 - 5.1.1. LAB MEDIA: Figure 3.
 - 5.1.2. LAB MEDIA: Figure 2.
- 5.2. Parietal cysts were more difficult to identify because of their localization and their smaller size. Only the smallest parietal cysts were impossible to treat, but they do not interfere with the embryonic vesicle's movements and are not easily confused with day-14 embryos [1].
 - 5.2.1. LAB MEDIA: Figure 4.

Conclusion

6. Conclusion Interview Statements

6.1. **Jérôme Ponthier**: Never forget the associated underlying causes of infertility: perform uterine swabs and biopsies, then treat and prevent these fertility dealing pathologies with vulvar surgeries or uterine lavages.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

6.2. **Stéfan Deleuze**: Multicentric studies about fertility observed before and after laser cysts ablation should be performed. However, semen quality, insemination's timing and other external factors may induce extrinsic causes of variation.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

