

Journal of Visualized Experiments

Endoscopy Guided Photoablation of Endometrial Cysts using a 980 nm Laser with a Contact Fiber in Mares --Manuscript Draft--

Article Type:	Invited Methods Collection - JoVE Produced Video
Manuscript Number:	JoVE61569R2
Full Title:	Endoscopy Guided Photoablation of Endometrial Cysts using a 980 nm Laser with a Contact Fiber in Mares
Keywords:	Mare; Fertility; Endometrial cysts; Laser surgery; Hysteroscopy
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Additional Information:	
Question	Response
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Dear Doctor Jewhurst,

I would like to take the opportunity to submit the manuscript entitled: **“Endoscopy guided photoablation of endometrial cyst using laser”** written by Jérôme PONTHER, Geoffroy de la REBIERE, Alexandra SALCICCIA, Stéfan DELEUZE.

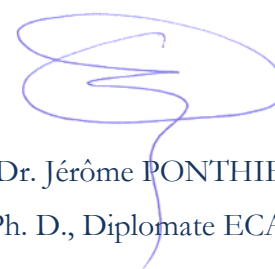
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This widely detailed method is highlighting 3 major points:

- Photoablation of endometrial cysts in mares is easy to perform and does not causes major complication
- After performing this technique, pregnancy diagnosis is easier and is not delayed, improving the mare fertility. Moreover, in some case, cysts photoablation enables better endometrial pregnancy recognition, what could also increase the observed fertility of the mare.
- However, endometrial cysts photoablation does not treat underlying causes of infertility, such as endometrosis or endometritis. This can be disappointing for the breeder if these conditions are not diagnosed or treated before endometrial cysts ablation.

I am looking forward to hearing from you.

Sincerely yours



Dr. Jérôme PONTHER

DVM, M. Sc., Ph. D., Diplomate ECAR



Jérôme Ponthier graduated as DVM in 2003. He did an ECAR residency in Liège University and obtained the ECAR diploma in 2009. He got his PhD degree in 2012 and is an Associate Professor in Equine Reproduction in Liège University.

Geoffroy de la Rebière graduated as DVM in 2004. He got his PhD degree in 2011 and obtained the ECVS diploma in 2018. He is an associate professor in Equine Surgery in Liège University.

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Stefan Deleuze passes the ECAR exam in 2004 and get his PhD degree in 2009. He is now full Professor of Equine Obstetrics and Reproductive Pathologies in Liège University.



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TITLE:

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

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KEYWORDS:

Mare, fertility, endometrial cysts, laser surgery, hysteroscopy

SUMMARY:

The aim of this report is to describe the photoablation of endometrial cysts in the mare using an endoscopic laser device, and to discuss the interest and limitations of this new approach to deal with low fertility issues.

ABSTRACT:

In mares, endometrial cysts are associated with endometriosis and can cause maternal recognition failure or compromise and delay pregnancy diagnoses. Historical treatments were invasive and had adverse effects on the endometrium. Hysteroscopically guided laser therapy is easy and effective for endometrial cysts resection, with no deleterious effects for the endometrium.

A 110 cm long and 1.0 cm wide endoscope is sterilely introduced in the uterus through the open cervix of an estrous mare after vulvar cleaning. The uterus is slowly insufflated with less than 1 L of air and the laser fiber is inserted in the biopsy canal of the endoscope. Cysts are then cauterized with the 980 nm diode laser with a contact fiber set at 20–25 W in continuous mode. Each cyst is punctured and the energy delivered until complete voiding of the cyst and shrinking of the cyst wall around the fiber. Uterine lavages with sterile saline solution are performed directly after the surgery and for one or two days as non-inflammatory fluid can be observed.

This procedure is easy and quickly performed, with no obvious deleterious effects. Cysts resection makes ultrasound pregnancy diagnosis easier and, in some cases, could restore proper embryo migration in the uterine horns between day 6.5 and 17. However, this treatment does

not improve the underlying histological lesions related to endometriosis. These considerations should be clearly expressed to the breeder before this procedure.

INTRODUCTION:

Endometrial cysts in mares were histopathologically described in the seventies by Kenney¹. In the late eighties, the use of hysteroscopy and ultrasonography led to their clinical description and helped to understand their consequences on fertility²⁻⁵.

In the early stages, endometrial cysts have been associated with a degeneration of the endometrium now called endometrosis^{1,3,4,6-8} and it is currently accepted that small (<1 cm) cysts are related to endometrial glands fibrosis when larger cysts are related to lymphatic dilatation⁶. These large cysts are the most likely to disturb equine embryo migration between day 6.5 and day 17, thus decreasing the fertility by impairing maternal recognition^{1,3,4,6,8}. Endometrial cysts may also interfere with early pregnancy diagnosis, especially if they are round and have a size similar to that of an embryonic vesicle^{3,4,6,9} (see **Figure 1**). Thus, they can potentially increase the interval between two inseminations by delaying first embryo ultrasonography and twin gestation assessment (see **Figure 2**). Endometrial cysts may also interfere mechanically with fluid collection during uterine lavage performed to treat endometritis or to collect the embryo. Consequently, endometrial cysts have effects on an observed mare's fertility, with potential economic consequences in breeding farms.

Besides attempts to treat the underlying endometrosis¹⁰, endoscopically-guided focal treatments of cysts have been proposed. Electrosurgical cauterization has been described, but heat exposure could injure the endometrium around the cyst¹¹. Aspiration of the cyst is only efficient if the secreting structure is no more active at the time of the puncture¹². Laser irradiation with a 980 nm diode laser with a contact fiber seems to show smaller subsequent endometrial scars^{6,13}. Improvement of laser devices and procedures⁶ now allows us to propose this safe and efficient technique in equine clinical practice. However, mare owners must be informed about the limits of this option. Photoablation of the cysts will increase embryo recognition in some mares, will advance the pregnancy diagnosis in some cases and could limit the negative mechanical effect of endometrial cysts on uterine lavage performed to treat endometritis or to collect embryos. However, it will never treat the concomitant underlying endometriosis or endometritis.

This report aims to describe an efficient procedure to perform hysteroscopy-guided diode laser cyst ablation in mares. Preparation and procedures will be practically described for a clinical application.

PROTOCOL:

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

NOTE: Perform endometrial cysts photoablation before anestrus: heats observed in late July or August thus seem most reliable. Do not perform this procedure too late in the breeding season as it can limit the number of estruses available to treat a potential endometritis.

89
90 **1. During the preceding estrus**
91

92 1.1. Perform a complete breeding soundness examination of the mare, including an
93 endometrial biopsy¹ (not described here) to ensure that the mare can potentially get pregnant
94 after the procedure.
95

96 1.2. In case of poor endometrial biopsy staging according to Kenney's classification¹, give the
97 embryo transfer or intra cytoplasmic sperm injection (ICSI) program preference over endometrial
98 cysts photoablation.
99

100 **2. During the preceding diestrus**
101

102 2.1. Once the rectum is empty, count, measure and map endometrial cysts of the mare in the
103 uterus using trans-rectal ultrasonography guided by rectal palpation with a 7.5 or 5 MHz linear
104 rectal probe and calipers on the screen (**Figure 3**) in a contention stock.
105

106 2.2. Administer 250 µg of cloprostenol intra-muscularly to induce estrus within 2 or 3 days at
107 day 6–7 post-ovulation.
108

109 2.3. On a daily basis, perform routine daily ultrasonography and cervix palpation to observe
110 estrus signs, including significant endometrial edema, antral follicle of 35 mm and softened and
111 opened cervix.
112

113 **3. Preparation when the mare is in heat**
114

115 3.1. Restrict access to the operation room to people wearing eye protection glasses.
116

117 3.2. After emptying of the rectum, wrap the tail and clean the vulva aseptically using iodine-
118 povidone.
119

120 3.3. To ensure easy manipulation and comfort of the mare, administer detomidine (10 µg/kg)
121 and butorphanol (0.1 mg/kg) intravenously in the jugular vein with a 21 G needle.
122

123 3.4. Per vagina, install the embryo collection catheter through the cervix and insufflate the
124 balloon with 40 mL of air.
125

126 3.5. Infuse the uterus with 1.5 L of sterile saline solution (0.9% NaCl) through a sterile to inflate
127 the uterus.
128

129 3.6. Deflate the balloon and remove the catheter.
130

131 3.7. Pass the endoscope aseptically through the cervix within a sterile sleeve.
132

4. Technique

4.1. Connect the 600 μ m quartz fiber to a diode laser operating at 980 nm. Set the power at 20–25 W in continuous mode.

4.2. Pass the flexible quartz fiber via the biopsy channel of a 10 mm diameter video-endoscope until 3–4 cm of free fiber can be identified on the screen. A minimum of 10 mm from the end of the fiber should be visible.

4.3. Activate the laser to puncture the cyst ideally at its apex: a dedicated pedal is available on the device to keep hands free. In some cases, multiple punctures at various locations are necessary to achieve complete voiding of the cyst. The cystic fluid is passively drained out into the uterus

4.4. After the cyst lining has collapsed around the fiber, deploy the laser until the membrane shrinks.

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

5. Post-operative treatments

5.1. Administer general antibiotic treatment: 22,000 UI/kg penicillin-procaine suspension, intramuscularly, twice a day, for 3 days.

5.2. Just after intervention, perform uterine lavage as described above (see steps 3.4-3.6).

5.2.1. Collect the liquid infused in the uterus before the endoscopic procedure through a sterile cuffed catheter.

5.2.2. Flush the uterus with successive instillations of 1 L sterile isotonic solution (0.9% NaCl) until a clear liquid is collected.

5.2.3. At the end of the procedure, administer 20 IU of oxytocin intra-muscularly.

5.2.4. Perform transrectal ultrasonography of the mare uterus the day after: intraluminal free liquid is frequently observed (see step 2.1).

5.3. As long as free intra-luminal fluid is observed by transrectal ultrasonography and until the end of the heat, perform uterine lavages daily with the procedure described above (steps 3.4-3.6). Sometimes, serum-like, limpid and yellow stained liquid is collected for several days.

NOTE: Giving repeated intra-muscular injections of oxytocin (20 IU) every 4 h or less when the mare is in estrus can avoid fluid accumulation and reduce the risk of bacterial contamination in the uterus.

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

NOTE: If free intraluminal fluid is observed, prostaglandin injection is recommended to induce a new estrus, to diagnose an eventual subsequent endometritis and treat it.

REPRESENTATIVE RESULTS:

Surgeries were performed on mares having cysts with diameters over 15 mm. However, if other smaller cysts were also present, photoablation of the other cysts was attempted. Generally, these large cysts were at the bifurcation or the basis of the horns. However, some of them were present in the body of the uterus and were equally treated.

In our experience, intraluminal cysts are very easy to localize with the endoscope. Moreover, performing this procedure in saline solution increases the visibility inside the uterus: it avoids emission of vapors, foam production due to endometrial mucus and lymphatic liquid mixing. Manual closure of the cervix while maintaining and guiding the endoscope helps to keep the liquid inside. Trans-rectal manipulation of the endoscope in the uterus sometimes helps to guide the endoscope into the desired horn. Thereafter, laser intervention is quite easy and quick, with a maximum treatment time of 5 min per cyst, depending on the size and on presence of inner walls or loculations that may require multiple laser activations for the same structure. The direct contact decreased the overall temperature in the uterus. Parietal cysts were more difficult to identify, because of their localization and their smaller size (see **Figure 4**). In some cases, it was impossible to visualize (with the endoscope) the parietal endometrial cysts previously identified by transrectal ultrasonography. However, 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, because of their size and localization. No direct surgical complications have been observed after the performed procedures.

Liquid accumulation was frequently observed by ultrasonography for several days after surgery. However, the collected liquid was clear and yellow and no inflammatory cells were observed on endometrial swabs. We attributed this feature to lymphatic fluid accumulation in the uterus after the opening of the cysts. Routine lavage procedures and repeated oxytocin boluses were sufficient to treat this condition before the end of estrus.

Delay between breeding and pregnancy diagnosis was shortened by decreasing the number of large size cysts. In a mare, the diagnosis was made possible again at day 14, whereas before treatment, it was only possible after day 24 or day 32 when cardiac activity or embryo migration could be respectively assessed. While laser treatment of endometrial cysts has obvious advantages, concluding about its direct effect on fertility would be dangerous, because of the

many factors impacting fertility and even the birth of a foal the following season would not allow a statistically valid conclusion.

FIGURE LEGENDS:

Figure 1: Day 14 embryonic vesicle (~14 mm diameter). Normal appearance of an embryo observed on early pregnancy diagnosis

Figure 2: Day 14 embryonic vesicle and endometrial cyst. The embryo (above) is attached to the endometrial cyst (below).

Figure 3: Endometrial cysts in diestrus. Multiple cysts in the bifurcation of the horns of a non-pregnant mare (measure scale on the right side is in centimeters).

Figure 4: Small parietal endometrial cyst. The mare is non-pregnant and in diestrus (scale bar is on the left side is in centimeters).

DISCUSSION:

Hysteroscopy and laser photoablation of endometrial cysts is an easy procedure to reduce large intra-luminal cysts in the mare, as previously suggested¹⁴. Technically, this method is improved when performed on a mare in estrus. Inflating the uterus with sterile saline solution improves our visibility when compared to air filling and continuous aspiration while performing the procedure. Small intra-parietal cysts (<10 mm) are more difficult to reach and to treat. Moreover, treating these small cysts could lead to piercing of the endometrium and myometrium if laser treatment time is prolonged. However, they seldom interfere with the embryonic vesicle's mobility and the early pregnancy diagnosis, due their localization and their size⁶.

Reducing numbers of intra-luminal large cysts under hysteroscopic control with the laser⁶ improves the mare's annual fertility by reducing the interval between breeding and pregnancy confirmation. On day 14 post-ovulation, ultrasonographic presence of a ~14 mm diameter anechogenic embryo is used to confirm gestation, or, if it is not observed, leads to use the next heat to inseminate or breed again^{9,10}. However, large and/or multiple endometrial cysts can delay this decision, because embryos may be hidden by endometrial cysts or confused with them^{6,9,10}, thus limiting opportunities to get the mare pregnant, due to unexploited heats happening between two ultrasonographic controls. Hysteroscopy and laser ablation of cysts makes the day-14 pregnancy diagnosis possible again in mares that had large endometrial cysts, and thus maximizes the number of attempts, as recommended to manage difficult mares¹⁰. It also decreases the risks of an inadequate twin pregnancy diagnosis or to miss such a pregnancy.

Assessing a statistical effect of hysteroscopy and laser resection of endometrial cysts on observed fertility is very hazardous: several management factors can vary before and after surgery that comparison seems to be impossible. However, embryo migration between day 6.5 and day 16-17 could be mechanically impeded by large endometrial cysts^{1,4}, and this incomplete uterine pregnancy recognition can lead to premature luteolysis and early pregnancy loss¹⁵⁻¹⁷. In this case

of mechanical obstruction, photoablation of cysts can effectively decrease embryonic losses and improve the fertility of the mare.

However, laser resection of cysts cannot resolve the underlying causes of infertility such as endometritis or endometriosis. Response to the treatment can be disappointing when these conditions are present as they are more likely to be the cause of the poor fertility than the cysts themselves. Complete breeding soundness examination, including endometrial biopsy^{1,10}, should be performed prior to considering cyst ablation by laser in order to exclude the presence of endometrial pathologies and to define their prognosis. Any other condition compromising the mare's fertility should be first treated, and alternatives such as embryo transfer or ICSI program should be proposed when applicable. Hysteroscopically guided endometrial cysts resection with laser is solely of interest when the endometrial cysts delay the pregnancy diagnosis or interferes with embryo migration and recognition.

ACKNOWLEDGMENTS:

Authors would like to thank Mr. Pascal Lejeune for his technical support during all procedures.

DISCLOSURES:

All authors are fully employed by Equine Clinic of Liège University and have no conflicts of interest with any company trading one of the products mentioned above.

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322
323

Figure 1

[Click here to access/download;Figure;JOVE-Fig1-Embryo.jpg](#)



Figure 2

[Click here to access/download;Figure;JOVE-Fig2-Embryo&cysts.jpg](#)



Figure 3



Figure 4

[Click here to access/download;Figure;JOVE-Fig4-Parietal cysts.jpg](#)



Name of Material/Equipment	Company	Catalog Number	Comments/Description
Butorphanol	Animedica	Morphasol 10 mg/mL	
Detomidine	Dechra	Domidine 10 mg/mL	
Endoscope	Olympus	Vet 170-10 SN2012729	
Endoscope Vidéo Tower	Olympus	CLE-E & CV-E	
	Wuhan Giga		
Laser device	Optronics	Velas II-60B GA19-V3186	
NaCl 0.9%	Baxter		
Pénicilline-procaïne	Kela	PENI-Kel 300.00 UI/ml	
		Aloka 500 & 5mHz	
Ultrasonography	Aloka	transrectal probe	

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Changes have been made in the protocol section.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We tried to highlight these technical aspects in the new protocol description.

• Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the animal care guidelines of your institution

Added on line 73.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

Sorry for that mistake, the word application is sometimes difficult to deal with when uploading files.

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1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

4) Notes cannot be filmed and should be excluded from highlighting.

Our protocol description was 2.5 pages long and we tried to reduce it to 2 pages to only keep the actions of the procedure.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

The reviewer modifications and remarks improved the modifications and limitations of the technique parts of the discussion. The limitation of the technique haven't been changed because we really think that they are a key point before to perform such procedures.

• **Figures:** Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

We added description of the figures after the title.

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We deleted the only commercial reference of the text.

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These are personal and unpublished figures.

Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

The manuscript describes how endoscopic guided photo ablation of endometrial cysts in the mare is performed however presents no data as to the derivation of opinions by conducting the procedures on how many mares.

Major Concerns:

How photo ablation can be different from simple aspiration of cysts as most endometrial cysts are lymphatic have not been mentioned.

Thank you for that recommendation, we tried to highlight and to improve this aspect in the introduction part (lines 57,58, 59).

The outcome of the procedure is speculated and not based on actual number of observations which have not been described anywhere in the manuscript. The location of cysts, their size and the clinical outcome need to be described somewhere.

We added some extra-details about the procedures (lines 139-140-141-142-147-148-149-157). However, we didn't aim to make a study about the outcomes of the cysts photoablation by laser. The aim of this paper is to describe the method in a video about this treatment used in the case of infertility in mares.

No endoscopic pictures have been included in a text only manuscript. No video of performing the procedures have been included.

As the aim of this article is to perform a video of the procedure with professionals, we don't know if it's really necessary. I don't know what's the recommendation of the Editor, but professional picture will be better than our home-made files.

Minor Concerns:

The authors mention that historical treatments but do not mention even one reference such as that of De Luca et al., 2009 which described the use of snare for removal of endometrial cysts. The language has many errors that need to be improved

Could you be more specific about this reference? It was impossible to find it on Pubmed with these keywords.

Reviewer #2:

Manuscript Summary:

Missing relevant material, see "Major Concerns"

Major Concerns:

The title needs to be modified from "Endoscopic guided photoablation of endometrial cyst using laser" to "Endoscopic guided photoablation of endometrial cyst in (number) mares using a 980nm laser with a contact fiber. The reason is reference # 13 in 1993 originally describes the procedure using a Nd:YAG laser or high powered diode laser (810nm or 980nm) in non-contact application.

This has been modified.

A good portion of this manuscripts described technique is a reiteration of previously published/presented material. The uniqueness is that although contact application has been presented at veterinary meetings/proceedings as an alternative to non-contact there are no refereed publications describing this technique and outcomes using contact application. In regard to this what is missing in this manuscript is the number of mares treated, presenting complaint, number of cyst in each mare, endometriosis presence or not, the outcome, how evaluated, and when evaluated. Without these facts this manuscript is hypothetical as to success of the technique and justification of its application.

We are sorry for the request for this manuscript was to describe a technique used to deal with infertility. The aim was to describe a clinical approach in order to produce a video to explain how it can be done. At this point, we never had the ambition to make a comparative study between the different procedures to reduce endometrial cysts in mares.

This manuscript is potentially unique in that it advocates using an aqueous medium within the uterus and a 10mm active section of the fiber (cladding removed). The benefits of this needs to be discussed as well as potential complications. One complication as an example, is the possibility of piercing the endometrium when cyst are less than 10mm in diameter. Whereas an advantage is contact irradiation produces less intrauterine heat than non-contact application.

It has been amended in different parts of the results and discussion (lines 143-144-145-146-174-175-176-177-178).

The laser production of smoke is easily dealt with by activating the endoscope's air flow and evacuation and does not justify the addition of saline to laser through. The author needs to discuss advantages of aqueous insufflation versus air inflation, and how it is maintained? What is the possibility of the same outcome without it? The discussion needs to be expanded to address these points.

We added dedicated comments in the results and in the discussion (lines 143-144-145-146-174-175-176-177-178).

Other rational for ablating endometrial cyst are that they may interfere with embryo collection. Cyst have been also observed to enlarge along with the embryo competing for space and potentially causing abortion. These observations/possibilities also need to be discussed or add to your justification for laser ablation.

We added some points about it (lines 52-53-54-64-65-67).

In the author introduction electrosurgical removal was cited. This produces a significant amount of coagulum and endometrial damage, more than laser. The first form of treatment was manual "popping" followed by endoscopic snare removal. Both frequently resulted in incomplete removal and or reoccurrence. This also supports the need for this manuscript it provide outcome information. It is perceivable that draining a cyst without destroying the secretory membrane could lead to recurrence. In that you do not have or expected to provide histologic description of damage produced by this contact technique supports the need for the manuscript to provide outcome information to validate this form of laser application.

Following your suggestion and the comment of reviewer #1, we added some data about it in the introduction (lines 58-59-60). However, it is very difficult to get histologic information about the treated region as it is difficult to reach them with a conventional biopsy forceps if the cyst is in a horn and it is difficult to identify the treated region with the endoscope.

I like but not required authors give credit to the first laser treatment of a disease or application in a condition which would be the 1993 publication. Lastly the discussion should also include the possible use of a diode laser with maximum output of 15 watts which is a common obtainable machine. I have also provided a few editorial remarks by line of the manuscript to direct the author are in "Minor Concerns".

In our hands, using low power (15W) did increase the time of photoablation or was ineffective.

Minor Concerns:

Line 17- be specific a 980nm diode laser in with a contact fiber: *it has been modified*

Line 22-possible place to add interference with embryo collection: *it has been added*

Line 35- replace resection here and other locations with ablation: *it has been modified*

Line 38- use owners rather than breeder here and other locations: *it has been modified*

Line 60- removal of cyst ma assist in treatment of endometritis: *it has been amended*

Line 62- add diode laser: *it has been modified*

Reviewer #3:

Manuscript Summary:

Your article titled "endoscopy guided photoablation of endometrial cyst using laser" is a clinical study on the treatment of a common fertility problem in mares. Your article has the potential to be published after some minor corrections.

Major Concerns:

It should be stated in the article title that the study is in mares (lines 2-41-47-62)

Minor Concerns:

Different treatment procedures can be mentioned briefly in the introduction.

In the protocol, the subtitles of item 3 continue with 2. *It has been modified (line 79)*

it would be better to write the result sentence. Some changes have been made in the beginning of the results part to improve it (139>146).

Dear Dr. DSouza,

Thank you for your careful revision of the manuscript. The corrections you suggested have been made in this new manuscript version. We also added information in the Table of Material file. They are highlighted in red.

Line 15,16,17: email addresses of co-authors have been added.

Line 82: thank you for your wise modifications, we appreciated.

Line 86: we simplified the sentence with a more scientific expression.

Line 93: reference has been added.

Line 101-103: we tried to be more specific and we added the material in the xls file.

Line 109: we changed the sentence to specify that these are routine examination, that doesn't require anesthesia.

Line 113: we tried to be more specific.

Line 115-116: we tried to be more specific

Line 118-119: we added the required information.

Line 121-122: we described the procedure.

Line 124-132: we re-write this part of the protocol method to improve the comprehension of the different steps.

Line 136: description of the quartz fiber is more detailed in point 4.1. The fiber description has been added in the xls file.

Line 156: it has been added in the xls file.

Line 159: we changed this part of place to improve comprehension of post-operative treatments and we added the previous description of this procedure.

Line 170: as it has been stated previously in the manuscript, we didn't change this part.

Line 173-174: we added recall to the previously described procedure.

Line 194-202: We mixed 2 paragraphs of the previous version to make an only paragraph that describe temporally the procedure and how it is performed.

Line 220: we changer the sentence, in an easier way.

Once again, thank you for your careful revision and your advices!

Best,

Dr. J. Ponthier