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# Endoscopic Laser Ablation of Endometrial Cysts in Straight Egyptian Arabian Mares: Outcome on Embryo Recovery and Conception Rate

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ENDOMETRIAL cyst is a fluid filled structure originating from the uterine wall of old mares and may complicate the pregnancy diagnosis. This study evaluated the outcome of diode laser ablation of endometrial cysts under the guidance of hysteroscopy. Sixteen infertile Straight Egyptian Arabian mares (9-22 year) with a history of normal cycling and infertility over the last two years and diagnosed with endometrial cysts were enrolled in the study. Trans-rectal ultrasonography, hysteroscopy and laser ablation were performed in all mares. The uterine wall had 1-8 endometrial cysts that were mostly located at the uterine body and at the base of uterine horns. Ultrasonographically, endometrial cysts were visualized as anechoic, mostly rounded, structure with thin echogenic wall of 3-8 cm diameter. Mares were divided into two main groups (n=8 mares) according to breeding management. Group 1 (G1) was intended for embryo transfer while group 2 (G2) was intended for normal pregnancy. Embryo recovery rate in G1 and conception rate in G2 were recorded. The embryo recovery rate was 62.5% in G1 and the conception rate was 75% in G2. In conclusion, laser ablation of endometrial cysts is recommended for infertile mares with endometrial cysts for improvement of fertility. Laser ablation is easy, safe and successful treatment for endometrial cysts in mares.

**Keywords:** Cyst, Embryo transfer, Equine, Hysteroscopy, Laser

## Introduction

Endometrial cyst is a thin-walled single or multiple fluid-filled structures arising from the endometrium [1]. It is a common observation in old broodmares aging 10 years old or older [2, 3]. Approximately 55% of subfertile mares (>10 years old) had endometrial cysts representing an economic impact on the breeding industry [4, 5]. The cysts may present throughout the uterus, but they usually present at the base of the uterine horns near to the body of the uterus [6, 7].

Endometrial cysts have been previously described as originating from uterine glands or from lymphatic lacunae [8, 9]. Accordingly, there are two types of endometrial cysts: glandular and lymphatic cysts. Glandular cysts are small-sized structures (less than 1 cm in diameter) and may be associated with periglandular fibrosis. Glandular cysts are normal finding during pregnancy. Lymphatic cysts are larger in size compared to glandular cysts (1 to 20 cm in diameter) and are usually encountered in multiparous adult mares. Lymphatic cysts may be present in normal uterus or associated with chronic endometritis [10, 11]. The incidence of lymphatic cysts increases with age. These cysts were found in 73.1% of mares over 14 years of age [6, 12].

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The presence of endometrial cyst interferes with embryonic mobility, increases early embryonic death, reduces placentation, and increases abortion rates [13, 14]. Moreover, endometrial cyst may complicate pregnancy diagnosis [3]. The significance of these cysts depends on their size, number and location [11, 15]. Despite these adverse effects, the relationship between presence of uterine cysts and fertility is still unclear and discussed controversially [16]. While certain authors supposed that endometrial cysts do not affect fertility in mares [1], other authors reported 10% lower fertility in mares with uterine cysts [17] or significantly lower pregnancy rates at day 14 and 40 in mares with cysts (77.6% and 71.4%) compared to mares without cysts (91.3% and 88%) [6].

It has been recommended to treat the large and multiple endometrial cysts in mares with history of subfertility and in mares with early embryonic loss in absence of cytological or bacteriological evidence of acute endometritis [3]. Treatment of the endometrial cysts may include; uterine mechanical curettage, hypertonic saline infusions, aspiration, rupture under endoscopic guidance, ablation by endometrial biopsy forceps, electrocoagulation, and laser photoablation [13, 18-20]. Curettage has been largely discredited as ineffective, with the potential to cause excessive scar tissue, hemorrhage and uterine adhesions [3]. Although hysteroscopy guided laser therapy is easy and effective for endometrial cysts resection, this treatment does not improve the underlying endometriosis [19].

The Straight Egyptian Arabian horse of today can trace 100% of its ancestry back to the horses bred by the Bedouin tribes of ancient Arabia [21]. Now, there are less than 6,000 Straight Egyptian Arabians exist worldwide, representing only 3% of all Arabian horses. They are characterized by their extreme rarity and beauty. Therefore, preserving the fertility of these horses is a very important goal for preservation of the bloodlines of these horses [22].

To the authors' knowledge, this is the first study conducted on endometrial cysts in Straight Egyptian Arabians mares. The current study aimed to evaluate the effect of endoscopic laser ablation of endometrial cysts on conception rate and embryo recovery in Straight Egyptian Arabian mares.

## Material and Methods

*Ethical approval*

This study was approved by the Research Committee at Animal Reproduction Research Institute, Agriculture Research Center (ARC), Giza, Egypt (Approval number: 712-1429). All mares were treated in accordance with guidelines established by the international and institutional Animal Care and Use Committees. Animals Sixteen Straight Egyptian Arabian mares with a history of infertility were admitted to Derbala Equine Clinic and Police Heights Equine Hospital, Egypt during October 2019-October 2022. Complete case history, thorough clinical examination and rectal palpation were performed in all mares. Mares aged between 9 to 22 years with a history of normal cycling and infertility over 2 years (2-3 years). Endometrial swabbing was made for culture and sensitivity and positive mares with bacterial endometritis were treated with the suitable antibiotics.

*Ultrasound scanning*

Ultrasound scanning was carried out for all mares using ultrasound device (Sonoscape E1v, linear endorectal probe, China). Trans-rectal ultrasonography, guided by palpation with a 7.5 or 5 MHz linear rectal probe was carried out. Ultrasound scanning aimed to evaluate the reproductive status of the mare, confirm the presence of endometrial cysts and their count, measuring the size and determining their location. Additionally, it was used to determine the onset of ovulation (Day 0) and the proper time of insemination.

*Hysteroscopy and laser ablation of the endometrial cysts*

All mares with confirmed endometrial cysts were included in this study and treated by diode laser ablation under the guidance of endoscope at the diestrus phase. MLT-Diode Laser Premium (MLT-Medizinische Laser Technologie GmbH, Germany) with 980 nm wavelength and adapted with a power up to 18 watts (W) was used. Emitting time was continuous and the laser beam was conducted through a thin and flexible cooled sterilized optical fiber of the endoscope from the diode to the fiber tip (Fig. 1). All persons within the operating room wore eye protecting glasses.

To ensure easy manipulation and comfort of the mares, they were placed in stocks and sedated with 500 µg/kg BW intravenous Xylazine HCl (Xylaject 2%®, ADWIA, Egypt) and 30 µg/ kg BW Butorphanol (Morphasol®, Livisto, Barcelona, Spain). After emptying of the mare's rectum, the perineal region was aseptically prepared with wrapping of the tail. The uterus was inflated with atmospheric air while the cervix was tightly closed during the procedure.

The endoscope was introduced into the uterine horns for thorough inspection of the uterus and

exploration of the endometrial cysts prior to photoablation. The laser fiber was then introduced through the biopsy port of the endoscope till 1-2cm of the fiber was visible. Diode laser was operated at 980 nm and the power was set at 15W in continuous mode.

Each cyst was punctured until complete voiding and collapsing of its wall around the fiber. Multiple punctures at different sites were performed when required for complete voiding. The cystic fluid was then passively drained out into the uterus.

Uterine lavages with 1L of 7% hydrogen peroxide in sterile normal saline solution was performed, followed by 2% Povidone iodine solution in sterile saline solution directly after drainage of cystic fluid. Follow-up uterine lavages with Povidone iodine solution were performed for one or two days until a clear liquid was obtained as previously recommended [19] (Fig. 2). Systemic antibiotic was given intramuscularly twice a day for three days. Oxytocin at a dose of 30 IU/mare was also given intravenously at the end of the procedure. Uterine lavage was also performed during the following estrus to confirm disappearance of endometrial cysts and the absence of intraluminal free fluid or debris [19].

*Breeding management*

At the second estrus cycle following laser ablation procedures, the mares were subjected to trans-rectal uterine ultrasonography to ensure clean uterus and breeding soundness (Fig. 3), and then subjected to natural mating. Mares were either intended for embryo transfer (ET) procedures (Group 1 "G1", n=8) or intended for normal pregnancy (Group 2 "G2", n=8) based on their owner's request. Each mare was kept indoors where sufficient and balanced feed and water were provided. All mares were subjected to breeding management according to the reproductive status of each mare to confirm the best time of insemination.

*Embryo recovery*

In G1, embryo recovery was carried out at 8 days post-ovulation in young donor mares (9-15 years) and at 9 days post-ovulation in old donor mares (16-22 years) [23]. After washing the perineal area with mild soap and Povidone iodine solution, a 36 Foley catheter (Minitüb COP, Germany) was finger-guided through the cervix into the uterine body. Once in, the cuff on the end of the catheter was inflated with 40 mL of Ringer's solution then pulled caudally to ensure a tight seal against the internal os of the cervix. One liter of Lactated Ringer's solution was infused through the catheter into the uterus then drained into a 65 µm EmSafe filter for embryo recovery with integrated Petri dish with a grid (Minitüb COP, Germany). Flushing was continued till the embryos were recovered in the filter and could be seen by the naked eye. Gross evaluation of embryonic shape and microscopic evaluation of the embryo capsule as well as trophoblast cells were carried out. Recovered embryos were transferred to the recipient mares by an insemination catheter in a ready-made holding media (IMV Co., France).

Pregnancy diagnosis in all mares was carried out by ultrasonography on day 15, 30 and 45 [24]. The embryo recovery rate in G1 and the conception rate in G2 were calculated. All recipient mares in G1 and pregnant mares in G2 were followed up till parturition.

## Results

Out of the 16 mares, three had uterine bacterial infections and responded well to antibiotics therapy.

The number of the cysts ranged from one to eight cysts. The cysts appeared as single or multiple anechoic mostly rounded structures with a thin echogenic wall (Fig. 4). Some cysts had ovoid or irregular shape. The cysts appeared as unilocular (Fig. 4a) or multilocular cysts of 3 to 8 cm diameter (Fig. 4c). The most common locations of the endometrial cysts were the uterine body and base of the uterine horns. Some small-sized cysts were also found in the horns. No detectable abnormalities could be visualized within the uterus or the ovaries.

Hysteroscopy-guided photoablation of endometrial cysts using diode laser in the current study was a successfully achieved in all mares. No complications or recurrence were detected.

The embryo recovery rate was 62.5% in G1 (ET group) and the conception rate was 75% in G2 (Normal pregnancy). The overall success in conception was 68.75% in all mares. Increased number of cysts was associated with lower conception rate where mares that conceived had an average of 3 cysts or lower, whereas those that did not had an average of 5 cysts.

In all mares of G1, the recovered embryos showed normal morphology (Fig. 5). Out of 8 mares, three mares were used for two embryo recoveries. All pregnant mares in G2 and the recipient mares showed normal pregnancy and gave birth of normal foals.

## Discussion

Endometrial cysts are one of the challenges in equine practice. These cysts affect fertility and conception in mares, particularly those having numerous and large endometrial cysts [25, 26].

Decreasing the number of endometrial cysts with diode laser under hysteroscopy guidance can improve fertility by reducing the delay for pregnancy confirmation and improving embryo migration between day 6 and 17 [19].

The laser ablation of endometrial cysts improves the uterine environment for receiving a formed embryo and enhances the maternal recognition through increased surface area of the endometrium for embryonic mobility. In the present study laser ablation of the endometrial cysts was successfully conducted and improved the fertility and conception in the studied mares. The improvement in pregnancy outcome and embryo recovery rate may be explained by removal of mechanical hindrance of endometrial cysts for embryo recovery and improvement of maternal recognition mechanism.

Although the effect of endometrial cysts on fertility is controversial, the current results reveal an improvement in the conception rate and embryo recovery rate in 16 infertile mares with multiple endometrial cysts. These results support the adverse effect of endometrial cysts on fertility in mares. In addition, these results are in agreement with the results of earlier authors who observed a history of 10% lower fertility in mares with uterine cysts [17]. Moreover, other authors reported similar results of lowered fertility and low pregnancy rate at day 14 and day 40 in mares with uterine cysts (77.6% and 71.4%) compared to mares without endometrial cysts (91.3% and 88%) [6].

Historically, diagnosis of endometrial cyst was previously based on the postmortem examination [27]. Recently, ultrasonography and hysteroscopy have been used for accurate diagnosis of endometrial cysts in mares [19, 28-30]. In the current study, ultrasonography and hysteroscopy were used for the diagnosis and confirmation of endometrial cysts in mares. Ultrasound examination revealed different numbers, sizes and locations of the cysts. These findings are in agreement with the findings of Pointhier *et al.* [19]. However, hysteroscopy was a helpful tool for thorough inspection of the uterus and detection the exact location of the cysts prior to photoablation. This is in agreement with previous studies [4, 19, 28].

In the current study, MLT-Diode Laser Premium was easily used. The laser beam was introduced through a flexible optical fiber of the endoscope. This facilitated visual monitoring of laser ablation procedures.

Previously Griffin and Bennet described a procedure for photoablation of endometrial cysts with Nd: YAG laser at 18W on mares [28]. Similarly, photoablation of endometrial cysts was performed in the present study by diode laser 980 nm at 18W. In contrast, Blikslager *et al.* previously mentioned a procedure of two stages for photoablation of endometrial cysts using Nd: YAG laser on a small group of mares, both *in vivo* and *in vitro* at 50W in the first stage and 100W in the second stage [31]. This high power was not necessary for cyst ablation and lead to Grade 4 lesions that were obviously undesirable in clinical situation [28, 32, 33]. Moreover, endometrial damage affects the placental and fetal development in mares [34].

In the present study, performing laser ablation of the cysts was carried out in atmospheric environment for good visualization. Similar technique was performed by earlier authors [28]. In contrast, Pointhier *et al.* used liquid environment to avoid smoke emission or foam production inside the uterus during the procedure [19] which could be avoided in the current study by the effect of the fluid leaked from the ablated cysts.

In this study, uterine lavage with 1L of 7% hydrogen peroxide in normal saline was performed directly after laser cyst ablation and followed by 2% Povidone iodine solution in sterile saline solution. Hydrogen peroxide lavage helps to effectively remove tissue debris resulted from the procedure. While Povidone iodine solution acts as an anti-microbial lavage to aid in prevention of possible secondary infection to the procedure as previously recommended [28].

Moreover, uterine lavage was performed during the first estrus cycle after the ablation of endometrial cysts. This was carried out in order to remove any debris or fluids coming from the ablated cysts and to ensure clean uterus.

In group 1, the embryo recovery rate was 62.5% which is quite similar to the result obtained by Griffin and Bennet who reported 66.7% conception rate in mares treated from endometrial cysts by laser photoablation [28].

In group 2, the pregnancy rate reached to75% after laser ablation of endometrial cysts. Similar result was obtained before by Köllmann *et al.* who reported 74% pregnancy outcome in mares treated from endometrial cysts by intrauterine surgery [35].

Continued follow-up of mares, including the live foal rate of those that conceived following laser

cyst ablation, would provide further valuable information regarding the usefulness of this procedure in mares with endometrial cysts.

## Conclusions

Large and multiple endometrial cysts reduce fertility in mares. The fertility of these mares can be improved greatly after laser ablation of endometrial cysts under hysteroscopy guidance. Although this technique is relatively expensive, it is easy, safe and successful treatment for endometrial cysts in mares.

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The authors declare that the present study has no financial issues to disclose.

*Conflict of interest*

None

*Author's contribution*

Both authors contributed to the study's conception and design, data collection, ultrasonography and surgical interferences. Both authors read and approved the final manuscript.

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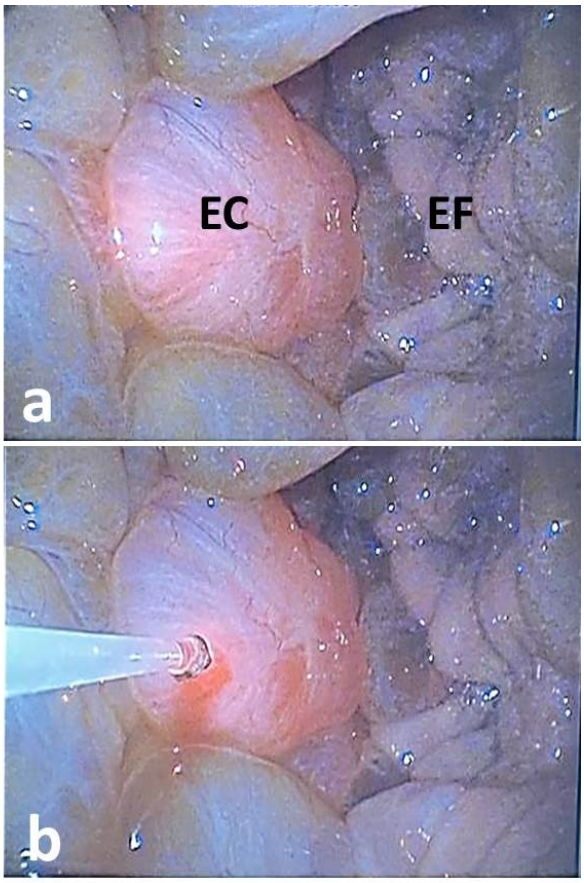


**Fig. 1. (a) Hysteroscopic picture showing an endometrial cyst (EC) located in the body of the uterus. Note the endometrial folds (EF). (b) Hysteroscopic picture showing the diode laser fiber during puncturing and cauterizing the endometrial cyst until collapsing.**

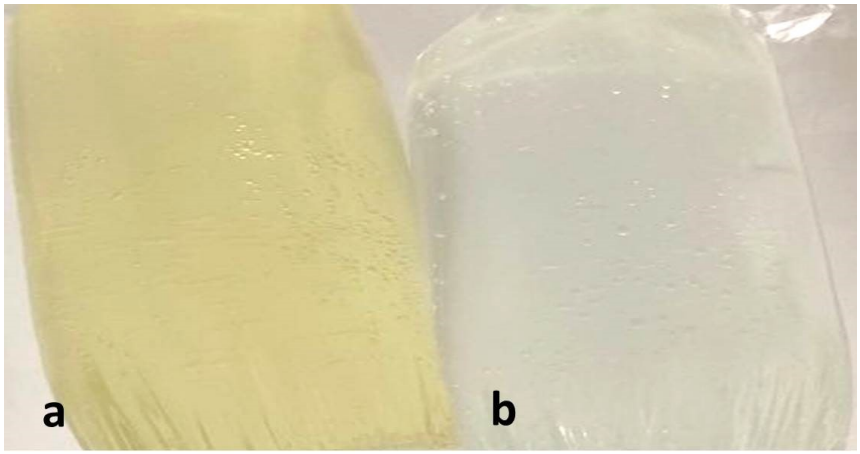


**Fig. 2. (a) The recovered fluid from the uterine lavages directly after laser ablation of the endometrial cysts. (b) Uterine lavage of the same mare after two days of laser ablation of the cysts.**

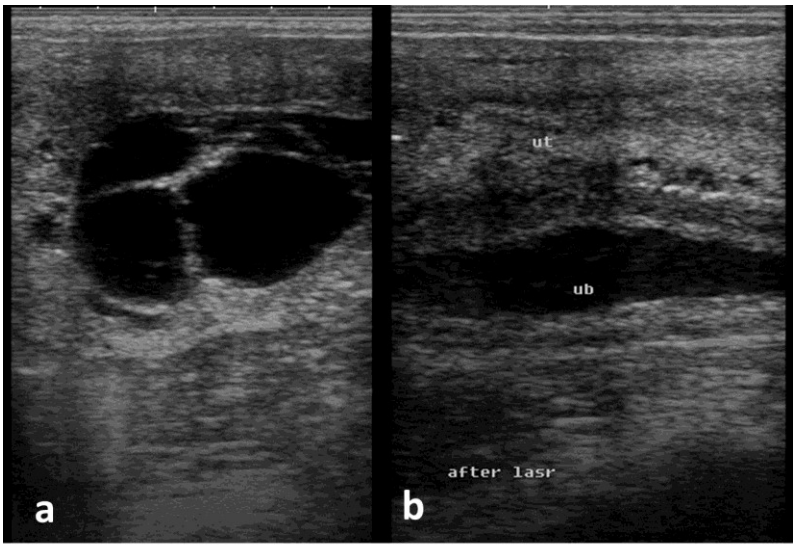


**Fig. 3. Ultrasonography of three endometrial cysts in a 15-year-old Straight Egyptian Arabian mare before (a) and after laser cyst ablation (b).**

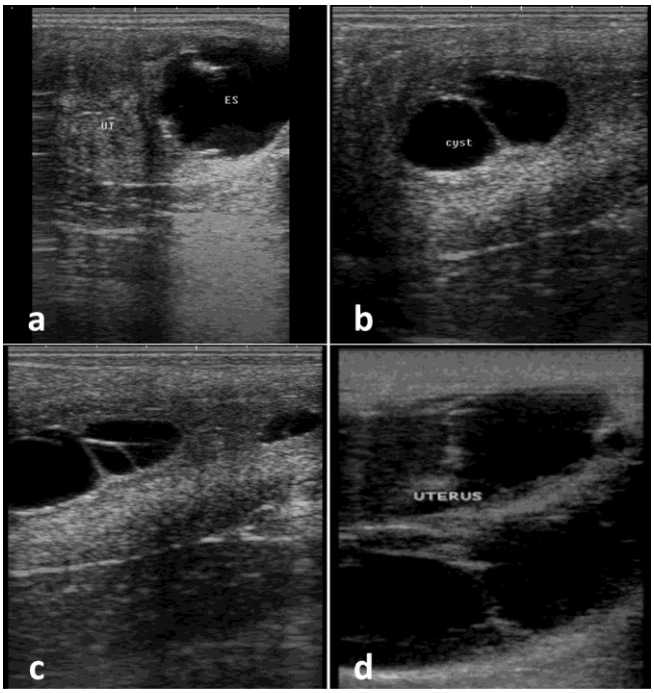


**Fig. 4. (a) Ultrasonography of endometrial cyst showing a large single anechoic cyst in the body of the uterus (close to the cervix) in a 12-year-old Straight Egyptian Arabian mare. (b) Double anechoic endometrial cysts in the body of the uterus of an 18-year-old Straight Egyptian Arabian mare. (c) Triple anechoic endometrial cysts occupying the whole body of the uterus until the base of uterine horns in a 14-year-old Straight Egyptian Arabian mare. (d) Multiple large anechoic endometrial cysts occupying the whole body of the uterus in two parallel rows extending from internal cervical os till the base of uterine horns in a 20-year-old Straight Egyptian Arabian mare.**

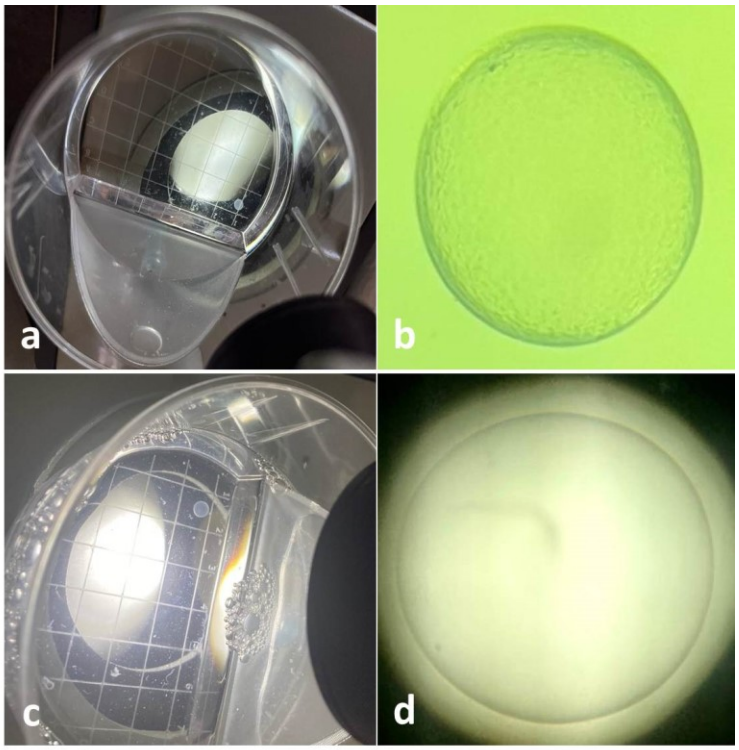


**Fig. 5. (a) The recovered embryo on day 8 post ovulation seen by the naked eye in the Emsafe filter. (b) Stereomicroscopic examination of the same embryo showing the blastocyst stage. (c) The recovered embryo on day 9 post ovulation seen by the naked eye in the Emsafe filter. (d) Stereomicroscopic Examination of the same embryo showing the expanded blastocyst stage.**

# إستئصال التكيسات الرحمية فى الأفراس العربية المصرية بإستخدام التنظير الرحمى و تأثيرة على الحصول على الجنين و نسبة الحمل

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تكيس بطانة الرحم عبارة عن هيكل مملوء بالسوائل ينشأ من جدار الرحم للأفراس خصوصا كبيرة السن وقد يؤدي إلى تعقيد تشخيص الحمل. قيمت هذه الدراسة نتائج استئصال تكيسات بطانة الرحم بالليزر ديود تحت توجية المنظار الرحمى. تم تسجيل ستة عشر من الأفراس العربية المصرية الغير قادرة على الإنجاب (9-22 سنة) مع تاريخ إنتظام دورة الشياع والعقم على مدى العامين الماضيين وتم تشخيص إصابتهم بتكيسات بطانة الرحم في هذه الدراسة. تم إجراء التصوير بالموجات فوق الصوتية عبر المستقيم، والتنظير الرحمى والاستئصال بالليزر في جميع الأفراس. و قد إحتوي جدار الرحم على 1-8 من تكيسات بطانة الرحم و وجد معظمها في جسم الرحم وعند قاعدة قرون الرحم. تم تصوير أكياس بطانة الرحم بالموجات فوق الصوتية على أنها عديمة الصدى، معظمها مستديرة، ذات جدار رقيق يبلغ قطره 3-8 سم. تم تقسيم الأفراس إلى مجموعتين رئيسيتين (8 أفراس فى كل مجموعة) حسب إدارة التخصيب. المجموعة 1 (G1) كانت مخصصة لنقل الأجنة بينما كانت المجموعة 2 (G2) مخصصة للحمل الطبيعي. تم تسجيل معدل الحصول على الأجنة في G1 ومعدل الحمل في G2. كان معدل الحصول على الأجنة 62.5% في المجموعة الأولى ونسبة الحمل 75% في المجموعة الثانية. في الختام، يوصى باستئصال تكيسات بطانة الرحم بالليزر للأفراس المصابة بالعقم والتي تعاني من تكيسات بطانة الرحم لتحسين الخصوبة. الاستئصال بالليزر هو علاج سهل وآمن وناجح لتكيسات بطانة الرحم في الأفراس.

**الكلمات الدالة:** التكيسات، نقل الأجنة ، الخيول، منظار الرحم، الليزر.