



Coelioscopic-assisted prefemoral ovariosalpingectomy in a d'Orbigny slider (*Trachemys dorbignii*) using a digital otoscope

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ABSTRACT. The purpose of this study was to assess the unilateral prefemoral coelioscopic-assisted approach for ovariosalpingectomy in a d'Orbigny slider (*Trachemys dorbignii*) using a digital otoscope. Twenty healthy turtles were randomly assigned to one of two groups, for right (GR; n=10) or left (GL; n=10) prefemoral access, for coelioscopic-assisted ovariosalpingectomy. Anesthesia and surgery times, body weight, and ovary/oviduct weight data were recorded. Anesthesia and surgery times did not differ significantly between the groups. Wound closure was the most time-consuming surgical step. Ovary and body weights significantly affected the exposure time of the ipsilateral and contralateral ovaries, respectively. Two intraoperative complications were reported. All the animals recovered uneventfully. The digital otoscope can be safely and effectively used for coelioscopic-assisted single-access, unilateral prefemoral ovariosalpingectomy in d'Orbigny slider.

KEY WORDS: chelonians, coelioscopy, soft tissue surgery, *Trachemys dorbignii*, wildlife

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Interactions between mankind and wildlife are becoming closer as urban complexes expand toward the native environment. Such proximity increases the incidence of accidents involving the surrounding fauna [3]. Illegal trade and irrational introduction of captive species in wildlife milieus may severely alter the local environmental equilibrium and affect predation, transmission of parasites, and extinction of naturally occurring species [3, 21]. Chelonians may live for over 40 years, with puberty occurring at approximately 4 years; females can generate over 30 newborn turtles per oviposition and nearly 2,000 offspring in a lifetime [13].

Disorders of the reproductive tract are some of the main concerns observed in the clinical routine of captive breeding chelonians [20]. Surgery is the treatment of choice in cases of complicated dystocia [1, 7]. Plastronotomy or prefemoral access is the main surgical approach used in such cases [1]. Surgical sterilization may be considered for population control and prophylactic management of reproductive disorders in turtles. The coelioscopic-assisted approach has been reported as a minimally-invasive alternative for this purpose [20]. The coelioscopic approach enables detailed inspection of intracoelomic organs, especially the reproductive tract [7].

Through a single prefemoral approach, the view of and access to the contralateral ovary without bladder emptying can be challenging [20]. A bilateral prefemoral approach for ovariosalpingectomy without endoscopic assistance has been described [15]. Other available techniques either require expensive endoscopic equipment [10] or are time-consuming or highly invasive [10, 14]. Therefore, the purpose of this study was to assess the unilateral prefemoral coelioscopic-assisted approach for ovariosalpingectomy in a d'Orbigny slider (*Trachemys dorbignii*) using a digital otoscope.

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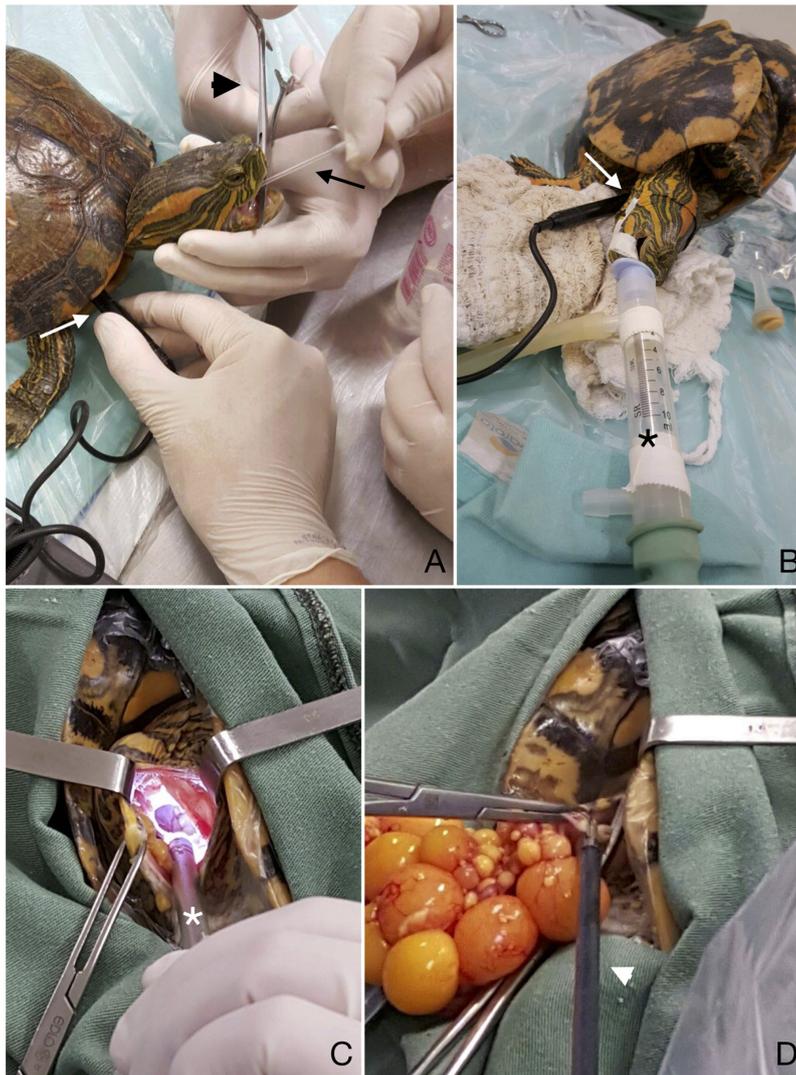


Fig. 1. Prefemoral coelioscopic-assisted ovariosalpingectomy using a portable digital otoscope in a d'Orbigny slider (*Trachemys dorbignii*). (A) Tracheal intubation and positioning of the vascular Doppler probe (white arrow). Intubation was performed using a 14-G vascular catheter (dark arrow) as tracheal tube and forceps as mouth-opener (dark arrowhead). (B) Turtle positioned in dorsal recumbency, with a 45° reverse Trendelenburg position. A 10-ml syringe (dark *) was used as a modified Baraka circuit. (C) Endoscopic view of the coelomic cavity using a 7 mm × 17.5 cm digital otoscope (white *). (D) Exposure of the ovary ipsilateral to the surgical access side for prophylactic hemostasis and transection of the vascular plexus using 5 mm × 33 cm bipolar hemostatic/cutting laparoscopic forceps (white arrowhead).

MATERIALS AND METHODS

This study was approved by the Committee for Ethics in Animal Use of the Passo Fundo University (CEUA-UPF-036/2016). Twenty healthy adult female captive d'Orbigny sliders (*Trachemys dorbignii*) from the Gramado City's Zoo (Rio Grande do Sul State, Brazil) were used in this study. The animals had previously been selected to undergo surgical spay as part of a population control program at the Zoo.

The d'Orbigny sliders were assessed clinically. Exclusion criteria were based on clinical and morphological disorders. The animals were conditioned in climatized individual plastic boxes at a temperature of 25–30°C and minimum humidity of 60%. The animals were fed with commercial food for the d'Orbigny slider and water *ad libitum*.

The turtles were starved of dry food only for 72 hr. They received prophylactic enrofloxacin (2.5 mg/kg) and morphine sulfate (1.5 mg/kg) intramuscularly (IM), 60 and 30 min, respectively, prior to anesthesia induction. General anesthesia was induced with intravenous propofol (10 mg/kg), via the supraoccipital sinus. Endotracheal intubation was performed using a 14-G catheter sleeve (Fig. 1A) coupled to the anesthesia machine. Isoflurane was delivered in 100% oxygen through a nonrebreathing gas circuit (Fig. 1B) for anesthesia maintenance. An assisted ventilation regimen was established and adjusted to 3–4 breaths per min. Additionally, epidural block was performed. Thus, lidocaine chloride (0.2 ml/kg) was injected through the coccygeal intervertebral space, as cranially as possible, using a 1-ml syringe with a 13 × 4.5 mm needle. The carotid artery pulse was monitored (Fig. 1A and 1B) using a vascular Doppler probe (DV 610B; Medmega, Franca, Brazil).

The turtles were randomly grouped into one of two groups of 10 animals each, according to the side of endoscopic single-access prefemoral approach used for the coelomic cavity: the right (GR) and left (GL) groups. The animals were placed in an oblique 45° dorsal recumbent position to the contralateral side of the surgical access (Fig. 1B). Aseptic preparation of the prefemoral fossa was performed using chlorhexidine–alcohol-based surgical skin preparation, according to the groups (GR or GL).

All surgeries were performed by a nonproficient surgeon, who was undergoing training for endosurgery procedures for exotic

animal practice. A 3-cm skin incision was performed, followed by blunt dissection of the underlining muscular layer. Subsequently, the peritoneum was incised ensuring access to the peritoneal cavity. A 7 × 161 mm digital otoscope (Y002; Supereyes Tech Co., Ltd., Shenzhen, China), connected to a laptop to display the image, was inserted through the prefemoral single access (Fig. 1C).

The coelomic cavity was inspected. Regardless of the group, the ovary ipsilateral to the surgical access side (Ova 1) was gently grasped and withdrawn from the coelomic cavity using 5 mm × 36 cm laparoscopic Kelly forceps (Kelly forceps; Edlo/Exatech, Canoas, Brazil). Hemostasis and transection of the mesovarial vessels were performed using 5 mm × 33 cm laparoscopic bipolar hemostatic/cutting forceps (Lina Tripol Powerblade; LiNA medical ApS, Glostrup, Dinamarca) (Fig. 1D). Subsequently, the oviduct ipsilateral to the surgical access side (Ovi 1) was properly exposed and resected after bipolar coagulation. The otoscope was reinserted through the surgical access site, after which the contralateral ovary and oviduct (Ova 2 and Ovi 2, respectively) were exposed and resected as reported for Ova 1 and Ovi 1. Muscle layers and subcutaneous tissue were closed with 3-0 USP polyglecaprone 25 cruciate mattress and simple continuous sutures, respectively. The skin was closed with 4-0 USP polyglecaprone 25 intradermal suture.

During early postoperative period, the animals were managed in a temperature-controlled dry environment (25–30°C) until complete recovery from anesthesia. All the ovaries and oviducts were scaled. Total anesthesia duration (from induction to extubation), total surgery time, and time taken to perform surgical steps were recorded. The following surgical steps were considered: (1) exposure of Ova 1; (2) hemostasis of Ovi 1; (3) exposure of Ova 2; (4) hemostasis of Ovi 2; and (5) wound closure. Exposure of Ova 1 was defined as the time taken from performing skin incision to full exposure of Ova 1. Hemostasis of Ovi 1 was defined as the time lapse between exposure of Ova 1 and final transection of Ovi 1. Exposure of Ova 2 was considered as the end of transection of Ovi 1, reinsertion of the otoscope, and complete exposure of Ova 2. Hemostasis of Ovi 2 involved end of exposure of Ova 2 and final transection of Ovi 2. Wound closure was defined as the time lapse between the final transection of Ovi 2 and the last intradermal knot tying.

Postoperative care included tramadol chloride administration (2 mg/kg, IM, SID, for 3 days) for pain management and wound dressing using normal saline for 14 days. The animals were closely monitored for possible postoperative complications. For patients with iatrogenic perforation of the bladder, enrofloxacin (10 mg/kg, IM, SID, for 10 days) was administered. Wound healing, gait, food/water intake, and social interactions were assessed on a daily basis for 15 days.

Statistical analysis

Data distributions were tested using the D'Agostino-Pearson's normality test, with descriptive analysis. Body weight; ovary/oviduct weight; and time required for exposure of Ova 1, hemostasis of Ovi 1, and wound closure were compared between the groups using Mann-Whitney test. Furthermore, total surgery time, total anesthesia duration, time required for exposure of Ova 2 and for hemostasis of Ovi 2 was compared between the groups using unpaired *t* test, with bicaudal *P*-values. The correlation between the animals' weight and time required for exposure of Ova 2 was assessed using Spearman's test. Correlations between ovary weight and the other surgery times were assessed using Pearson's test. For all the tests, significance levels were set at *P*<0.05.

RESULTS

The mean weights (± SD and range) of animals, ovaries, and oviducts are presented in Table 1. There was no significant between-group difference in body weight [mean body weight, GR: 2.17 ± 0.25 kg (range 1.90–2.65 kg); GL: 2.21 ± 0.36 kg (range 1.96–2.93 kg); *P*=0.7054] (Table 1). Furthermore, there were no significant intra (Ova 1 vs Ova 2)- and between-group differences in ovary weight (*P*=0.1046). Likewise, there were no significant intra (Ovi 1 vs Ovi 2)- and between-group differences in oviduct weight (*P*=0.1414).

Data on anesthesia duration, total surgery time, and time required for the surgical steps are displayed in Table 2. The mean anesthesia duration was 68.2 ± 12.8 min in GR and 70.8 ± 27.4 min in GL (*P*=0.7890); anesthesia recovery was uneventful in all patients. The mean surgery time was 31.7 ± 7.8 min (range 22–45 min) in GR and 42.8 ± 20.0 min (range 11–77 min) in GL (*P*=0.1201). Coelioscopic-assisted ovariosalpingectomy was feasible in all the animals included in this study. There was no significant between-group difference in the time required for each surgical step (*P*>0.05). Wound closure was the significantly most time-consuming step in both groups (*P*<0.0001). Time required for the other surgical steps was similar within the groups, in both groups (*P*>0.05).

There was a significant positive correlation between ovary weight and the time required for exposure of Ova 1 (*P*=0.011; *r*=0.558). Likewise, body weight was significantly positively correlated with the time required for exposure of Ova 2 (*P*=0.011; *r*=0.554). However, there were no significant correlations of ovary weight with body weight (*P*=0.222) and time required for exposure of Ova 2 (*P*=0.447).

One of the turtles from GR had two oviducts ipsilateral to the surgical access side, which were equally resected after bipolar coagulation and transection. Of the 20 operated turtles, two (10%) experienced intraoperative complications. In one animal from GL, an ovum ruptured inside the coelomic cavity during exposure of Ova 2. The ruptured ovum was retrieved using Kelly forceps, and the coelomic cavity was thoroughly rinsed using warmed normal saline (1 l/kg) and suctioned. There was one case of iatrogenic bladder perforation while establishing access to the coelomic cavity. The bladder was immediately sutured with 4-0 polyglactin in a simple continuous pattern, after which the coelomic cavity was rinsed with sterile saline and ovariectomy regularly performed. All the animals recovered uneventfully from both anesthesia and surgery. Wound healing was complete in all the turtles by the 14th postoperative day.

Table 1. Body, ovaries, and oviducts weight of d'Orbigny slider (*Trachemys dorbignyi*) undergoing prefemoral coelioscopic-assisted ovariosalpingectomy, using a portable digital otoscope

Animals	Weight					
	Body ^{a)} (kg)	Right ovary ^{b)} (g)	Right oviduct ^{c)} (g)	Left ovary ^{b)} (g)	Left oviduct ^{c)} (g)	
GR	1	2.24	19	8	26	7
	2	1.94	6	5	8	80
	3	2.26	33	13	33	13
	4	1.96	17	7	10	11
	5	2.65	33	14	70	14
	6	1.93	20	8	20	6
	7	1.9	13	11	12	11
	8	2.38	18	11	20	9
	9	2.36	13	13	11	12
	10	2.06	17	12	26	12
Mean/SD	2.17 ± 0.25	18.9 ± 8.45	10.2 ± 3.01	23.6 ± 18.26	17.5 ± 22.11	
GL	1	2.11	78	18	72	20
	2	2.33	72	14	62	40
	3	2.1	65	30	65	30
	4	1.99	45	13	21	50
	5	1.97	6	9	7	9
	6	2.77	7	10	8	10
	7	2.93	16	14	16	94
	8	1.98	42	16	62	15
	9	1.95	13	6	9	4
	10	1.96	70	14	33	14
Mean/SD	2.21 ± 0.36	41.4 ± 28.97	14.4 ± 6.50	35.5 ± 26.82	28.6 ± 27.23	

GR=Right prefemoral approach group, GL=Left prefemoral approach group, SD=Standard Deviation. Mean/SD were no significant difference between both groups (a as $P=0.7054$, b as $P=0.1046$ and c as $P=0.1414$).

Table 2. Total anesthesia and surgical time, and time elapsed for each surgical step of prefemoral coelioscopic-assisted ovariosalpingectomy using a portable digital otoscope, in d'Orbigny slider (*Trachemys dorbignyi*)

Animals	Time (min)							
	Surgery ^{a)}	Anesthesia ^{b)}	Exposure of Ova 1 ^{c)}	Hemostasis of Ovi 1 ^{c)}	Exposure of Ova 2 ^{c)}	Hemostasis of Ovi 1 ^{c)}	Wound Closure ^{c)}	
GR	1	45	100	6	7	8	5	19
	2	32	65	3	7	1	6	15
	3	35	63	6	3	3	3	20
	4	31	80	4	4	3	7	13
	5	25	55	7	3	1	2	12
	6	42	66	4	8	3	13	14
	7	22	65	5	2	1	4	10
	8	35	60	2	3	7	6	17
	9	28	62	4	2	5	7	10
	10	22	66	3	2	4	3	10
Mean/SD	31.7 ± 7.8	68.2 ± 12.8	4.4 ± 1.6	4.1 ± 2.3	3.6 ± 2.5	5.6 ± 3.1	14 ± 3.7	
GL	1	77	97	18	5	4	6	44
	2	65	120	11	8	5	5	26
	3	35	55	10	2	4	8	11
	4	50	96	3	6	3	1	37
	5	54	77	2	22	7	2	21
	6	47	50	4	5	4	4	30
	7	38	53	13	2	7	2	16
	8	20	40	1	4	2	7	7
	9	11	40	2	1	3	2	3
	10	31	80	4	3	1	2	12
Mean/SD	42.8 ± 20.0	70.8 ± 27.4	6.8 ± 5.8	5.8 ± 6.1	4.0 ± 1.9	3.9 ± 2.4	20.7 ± 13.4	

GR=Right prefemoral approach group, GL=Left prefemoral approach group, SD=Standard Deviation, Ova 1=Ovary ipsilateral to the surgical access, Ovi 1=Oviduct ipsilateral to the surgical access, Ova 2=Ovary contralateral to the surgical access, Ovi 2=Oviduct contralateral to the surgical access. Mean/SD were no significant difference between both groups (a as $P=0.1201$, b as $P=0.7890$ and c as $P>0.05$).

DISCUSSION

Reproductive tract disorders are frequently seen in chelonian's clinical routine, with confined animals being at high risk [20]. Spaying prevent follicular stasis, dystocia, and neoplasm development in the reproductive tract of confined turtles [7]. Exposure of the ovaries is one of the most challenging steps of ovariosalpingectomy in turtles. However, the coelioscopic approach is both safe and effective in all steps of gonadectomy [17]. The coelioscopic approach is also indicated for biopsy sampling and gender determination in chelonians [6, 9].

To the authors' knowledge, the use of a standard digital otoscope for coelioscopic procedures in chelonians has not yet been reported. Coelioscopic-assisted ovariosalpingectomy using a digital otoscope was successfully performed in all the animals in this study. Secondary access or wound enlargement were not required in any animal, regardless of complications. Pneumoperitoneum was not required to enhance visualization, despite the limited working space of the coelomic cavity.

In procedures that require some form of insufflation, CO₂ delivered via a dedicated endoflator is preferred; however, air delivered via a syringe or a small aquarium air pump can be used [6]. Moreover, it can quickly dry out the organs and mucosa [4]. The use of sterile saline infusions, as described in the literature [4, 6], could be helpful. In the authors' opinion, pneumoperitoneum could be potentially harmful and would not improve intracoelomic visualization substantially.

In small chelonians, the use of pure intracorporeal techniques is challenging. Poor port triangulation and limited working space increase the risk of bowel perforation or thermal injury [14]. Although bladder perforation occurred in one of the animals while establishing surgical access, the coelioscopic-assisted approach itself was safe and provided an optimal field of view. Thus, both complications were readily identified and managed without need for conversion to plastron osteotomy.

To avoid perforation, the bladder should be drained before surgery via catheterization [18, 20] or ultrasound-guided cystocentesis [12, 18]. Depending on the surgical technique, bladder perforation can be avoided regardless of whether drainage is performed [18]. Conversely, a distended bladder may impair intracoelomic visualization, requiring drainage [14, 17]. In our study, bladder emptying was not performed in all the turtles, and iatrogenic perforation occurred in one case. This perforation occurred while establishing prefemoral access. We believe that bladder rupture occurred when coelomic membrane incision was performed. This surgical maneuver demands care, due to the often-voluminous and closely-associated bladder [18], considering that iatrogenic bladder perforation while using the celiotomy approach has been discussed in previous studies [5, 12, 17]. Furthermore, the membranous wall may be mistaken for the peritoneal membrane and incised, especially when viewed through a small surgical incision [12].

There was a significant positive correlation between the time required for exposure of the ovary located on the contralateral side of the surgical access and body weight. Exposure was significantly poorer in animals with high body weights. Additional cautious handling and traction was required to expose Ova 2 in those animals. Furthermore, the study revealed a significant positive correlation between the time required for exposure of Ova 1 and Ova 1 weight. Thus, increased surgery time may be expected in animals with massive ovaries.

The approach to the coelomic cavity should be based on anatomic considerations and diagnostic imaging findings [8]. A bilateral prefemoral approach is recommended when a single-side access provides poor view or exposure of the contralateral ovary [17]. However, there was no need for the bilateral approach in this study. The reproductive tract was completely exposed in all the cases. Moreover, there was no significant difference between the durations of surgeries performed using the right and left approaches.

The total surgery time in this study was comparable to that reported in other video-assisted studies using 2.7 mm [11] and 10 mm [2] rigid endoscopes for spaying of chelonians via unilateral prefemoral access. In the previous study using a 2.7 mm rigid endoscope, the mean surgery time was 36 ± 6 min [11], whereas in that using a 10 mm rigid endoscope for spaying of 20 red-eared sliders (*Trachemys scripta elegans*), the mean surgery time was 50.2 ± 14.6 min with left access and 48.0 ± 11.8 min with right access, with there being no difference in surgery times between the access sides. Moreover, similar durations of surgery were reported in bilateral approaches for video-assisted spay in Galapagos tortoises (*Geochelone nigra*) [14].

Both conventional and coelioscopic-assisted prefemoral approaches provide proper access to intracoelomic organs and lead to short surgery time in chelonians; in contrast, plastron osteotomy access is associated with a longer surgical duration [14, 16], longer convalescence, and higher risk of complications such as plastron flap sequestrum [12] and osteomyelitis [2]. In a trial, the prefemoral approach was found to be less time-consuming for both anesthesia and surgery compared with the conventional access through the plastron [16].

Everted suture patterns are usually indicated for wound closure in chelonians [5]. In this study, only appositional sutures were performed. Wounds healed uneventfully in all the animals within 14 days. Antibiotics were administered only to the animal with iatrogenic perforation of the bladder. Time taken for complete wound healing in this study was comparable to that reported in a study involving prefemoral celiotomy in red-eared sliders (*Trachemys scripta elegans*) [16]. In other chelonian species, wounds may heal in 4–8 weeks post-operatively [11]. In the authors' opinion, the absence of wound-related complications was due to the minimally-invasive approach used in the study.

Although we have been successful with electrocoagulation hemostasis using the video-assisted technique, accurate hemostasis becomes increasingly critical for endosurgery. Radiosurgical and laser devices have become available to veterinarians, facilitating the ability to incise and debride internally without significant hemorrhage [6].

The use of the portable digital otoscope is the main highlight of this study. The portable system is relatively inexpensive, easy to assembly and use, safe, and easy to transport. It can be set for use in a smartphone, tablet, or small monitor. Moreover, the handpiece can be disinfected by soaking it in a disinfectant (4% glutaraldehyde solution). The quality of view obtained with the

portable digital otoscope used in the present study is questionable compared with that obtained with conventional endosurgery or endoscopy systems. However, in the authors' opinion, the view was adequate. The use of conventional endoscopy systems is difficult in on-field conditions. Furthermore, most recently developed high-definition portable endoscopy systems are still expensive, and thus, cost-prohibitive for most exotic animal field practitioners [19]. Further studies are required to compare the cost-effectiveness of the portable digital otoscope used in this study and conventional endoscopy systems.

In conclusion, the use of a digital otoscope ensures safe and effective video-assisted prefemoral ovariosalpingectomy in a d'Orbigny slider (*Trachemys dorbignyi*). Either left or right unilateral prefemoral approach should be considered for coelioscopic-assisted ovariosalpingectomy. Therefore, for a d'Orbigny slider, the prefemoral coelioscopic-assisted approach should be considered as a minimally-invasive alternative for surgical sterilization.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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