In vivo assessment of gastrotomy closure with over-the-scope clips in an experimental model for varicocelectomy (with video)

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Background: Gastrotomy closure remains the major limiting factor for human translation of transgastric surgery; the over-the-scope clip (OTSC) system was proposed as a possibility for this purpose. Transgastric access is good for a pelvic approach, making varicocelectomy a possible indication for natural orifice transluminal endoscopic surgery (NOTES).

Objective: To evaluate the reliability of the OTSC system in vivo after transgastric testicular vessel ligation (varicocelectomy model).

Design: There were 3 experimental groups (5 animals in each): groups 1 and 3, gastrotomy dilation up to 18 mm, surgery was performed with a double-channel endoscope; group 2, gastrotomy dilation up to 13 mm, surgery was performed with a single-channel endoscope.

Setting: Surgical Sciences Research Domain, Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal.

Interventions: Bilateral testicular vessel ligation by transgastric access. Gastrotomy closed with the largest version of OTSC system (12 mm): a single clip in groups 1 and 2, and 2 clips in group 3. Animals were monitored for 2 weeks, killed, and submitted for necropsy.

Main Outcome Measurements: Adequacy of closure and healing after the use of the OTSC system. Statistical analysis.

Results: Vessel ligation was easily achieved in all groups. Although differences in the complication rate did not reach statistical significance ($P = .099$), there was a clear tendency for a better prognosis in groups 2 and 3 than group 1. In fact, only 2 animals from group 1 had complications related to incomplete gastrotomy closure.

Limitations: Small number of animals per group; nonrandomized study.

Conclusions: The OTSC system was shown to be easy and efficient for gastrotomy closure in a survival experimental model of varicocelectomy, when correctly matching the gastrotomy size with the clip size and/or number. (Gastrointest Endosc 2009;70:1137-45.)

Envisioning the potential benefits of natural orifice transluminal endoscopic surgery (NOTES), many investigators tested the feasibility of NOTES in the peritoneal and even in the thoracic cavities.[1,2] From all previous experimental work, the transgastric approach was shown to be particularly applicable for performing surgical procedures in the pelvis.[3,4]

Simultaneously, analyses by leaders in the fields of surgery and endoscopy revealed 3 fundamental challenges and barriers to the safe implementation of NOTES: access creation, prevention of infection, and visceral closure.[5] Gastrotomy creation could be achieved with PEG-like,[6] hybrid or transvisceral combined[7-9] approaches, and the risk of contamination seems a matter of adequate endoluminal disinfection and intravenously administered antibiotics, but the solution to achieving secure closure of the gastric

Abbreviations: NOTES, natural orifice transluminal endoscopic surgery; OTSC, over-the-scope clip.

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defect remains more difficult, and development of endoscopic closing devices continues to be a prime area of research and testing.[10] Indeed, this aspect seems to be the most limiting factor for human translation of transgastric procedures and likely justifies the still-scarce number of reports of successful NOTES in humans.[11,12]

Regarding closure techniques, several methods have been proposed, including conventional endoscopic clips,[1] an over-the-scope clip (OTSC) system,[13] septal occluders,[14] T-tags,[15] and T-bars[16] for tissue opposing, as well as more complex suturing devices such as the Eagle Claw VII (Olympus Optical Co, Ltd, Tokyo, Japan),[17] NDO Plicator (NDO Surgical Inc, Mansfield, Mass),[18] USGI Endosurgical Operating System (San Clemente, Calif),[19] and linear endoscopic staplers.[20] Moreover, special techniques in gastrotomy creation and closure, as described by Sumiyama et al[21] who used submucosal endoscopy with an offset exit gastrotomy or by Sporn et al,[22] who used a PEG technique combined with gastropexy, have been suggested. However, most of these devices and techniques still have limitations that need improving, and most of them are too complex to apply or could not prove their effectiveness in survival studies.

The OTSC system has a simple method for application, and it was already approved for clinical use in cases of bleeding and iatrogenic lesions of the digestive tract.[23] An enlarged version of the OTSC system was evaluated for the use in NOTES with promising results in a nonsurvival study,[24] highlighting the need for such studies.

Aiming to test the efficacy and reliability of the OTSC system in vivo for gastric closure, we used the OTSC system in a survival porcine model after performing a simple pelvic procedure such as bilateral testicular vessel ligation.

MATERIALS AND METHODS

Study design

Male pigs (*Sus scrofus domesticus*) weighing 40 to 45 kg were used to perform a simple pelvic procedure by NOTES, a transgastric bilateral testicular vessel ligation. After an initial learning curve, the results of which results are not reported here, 15 animals were divided in 3 groups: group 1, 5 pigs, all procedures (gastrotomy and surgery) were performed by using a double-channel gastroscope and at the end, a single 12-mm OTSC was applied by using the same endoscope; group 2, 5 pigs, gastrotomy and surgery were performed with a standard single-channel gastroscope and at the end, a single 12-mm OTSC was applied by using a double-channel endoscope; group 3, 5 pigs, all procedures (gastrotomy and surgery) were performed with a double-channel gastroscope and at the end, two 12-mm OTSCs were applied by using the same endoscope. The animals in all groups were monitored with a 15-day survival follow-up. This study was approved by ethics review board of Minho University (Braga, Portugal).

Capsule Summary

What is already known on this topic

- Reliably safe transgastric access could make varicocelectomy a possible indication for natural orifice transluminal endoscopic surgery.

What this study adds to our knowledge

- In a study of bilateral testicular vessel ligation by transgastric access, an over-the-scope system allowed easy and efficient gastrotomy closure in a porcine model when the gastrotomy dimension correlated with the clip size.

Pig preparation

The animals were fed liquids for 3 days and received no food and water for 8 hours before the surgical intervention. The stomach was lavaged with instilled water and aspirated through the gastroscope until free of solid particles. Subsequently, it was decompressed and a cefazolin solution (1 g in 200 mL of saline solution) was instilled. The antibiotic solution was left in the stomach for 10 minutes before also being aspirated. All procedures were performed with the pigs under general anesthesia with endotracheal intubation and mechanical ventilation, as described in previous studies.[8,9]

Surgical technique

Transgastric access. A double-channel endoscope (G28/34; Karl Storz GmbH & Co KG, Tuttlingen, Germany) was advanced into the stomach. After stomach preparation, the preferred gastrotomy site was chosen on the anterior wall by transillumination and external palpation. After gastric wall incision with a needle-knife, puncture dilation was performed with an 18-mm through-the-scope balloon (5837 Microvasive; Boston Scientific Corp, Natick, Mass) for the double-channel gastroscope passage in groups 1 and 3, and with a 13-mm through-the-scope balloon (5836 Microvasive; Boston Scientific Corp) for passage of a regular single-channel gastroscope (G28; Karl Storz GmbH & Co KG) in group 2. Then on balloon semideflection, the gastroscope was pushed forward into the peritoneal cavity and directed to the pelvic cavity.

Testicular vessel ligation. The animal was placed in a slight head-down position (Trendelenburg up to 30 degrees), and the gastroscope was positioned for anteroinferior abdominal wall exploration. Once the internal inguinal ring and the spermatic cord were identified, the gonadal vessels were approached, sparing the vas deferens. In groups 1 and 3, two instruments were allowed: a grasping forceps and a coagulation grasper. By using these instruments, it was possible to grasp and cut the parietal peritoneum overlying the testicular vessels, high above the internal inguinal ring, to create a window to reach and mobilize the vessels. When isolation was completed, the

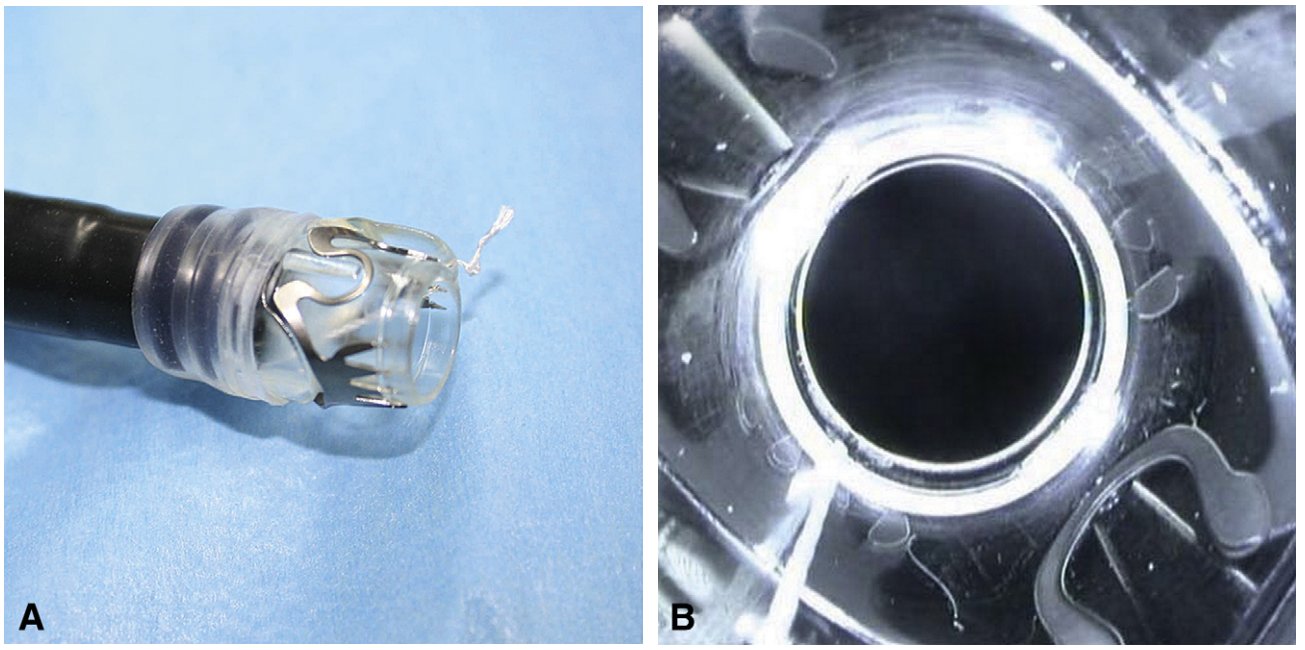

Figure 1. OTSC system mounted on the tip of the gastroscope: **A,** External view. **B,** Restricted image provided by the gastroscope with the OTSC system mounted.

coagulation grasper was used for a soft coagulation appliance in 2 points of the vessel segment and then cut in the middle for section. In group 2, only 1 instrument could be used at a time, and almost all the procedure was done by using the coagulation grasper, although a less careful dissection had been achieved. In all groups, the procedure was repeated on the contralateral side. The CO_2 pneumoperitoneum was controlled and maintained up to a maximum of 12 mm Hg with a Veress needle.

Gastrotomy closure

At the conclusion of the intra-abdominal procedure, the endoscope was withdrawn. The OTSC system (Ovesco Endoscopy GmbH, Tubingen, Germany) was mounted on the tip of the double-channel gastroscope, charged with a 12-mm clip (Fig. 1), and inserted into the stomach. After visualization of the transgastric hole, in groups 1 and 2, a single clip was applied according to the manufacturer's instructions, after centering the clip over the gastrotomy. In group 3, the first clip was slightly deviated for one of the extremities of the gastrotomy, whereas the second was centered on the other extremity of the gastrotomy and involved part of the first clip (Video 1, available online at www.giejournal.org). Once applied in all groups, the clips were inspected and the tightness of the closure was confirmed by means of air inflation and the ability to maintain organ distention.

Postoperative care

At the end of the surgical intervention, all animals received 1.2 g amoxicillin and clavulanic acid intravenously. A liquid diet was resumed 8 hours after surgery and a regular diet 2 days later. The animals were closely monitored for any signs of postoperative complications, distress, behavior changes, anorexia, or weight loss. After the follow-up period, the animals were killed and necropsy was performed to check the healing of the gastric wall incision and signs of intraperitoneal complications.

Statistical analysis

Continuous variables are presented as mean ± standard deviation. In the experimental groups the total operative time of the procedures passed the kolmogorov-smirnov normality test; thus, this parameter was compared with the one-way ANOVA analysis. The postoperative and necropsy findings (rate of postoperative complications related to incomplete gastrotomy closure, gastrotomy position, persistence of clip on site, and presence of omentum mesh) were compared by using χ^2 analysis of contingency because there were more than 2 groups. However, once 20% of the expected values in contingency tables are less than 5, the power of the performed test is below the desired power of 0.8. Statistical significance was set at $P < .05$.

RESULTS

The complete cleansing of the pigs, stomachs was difficult, even with a liquid diet for an easier emptying. The transgastric port creation had no significant complications besides some accidental injuries to the anterior abdominal wall during the needle-knife procedure, but no lesions on adjacent organs occurred. Pelvic assessment, vessel identification, and position adjustments were easily obtained. The Trendelenburg position and a slide left or right rotation favored the displacement of intestinal loops,

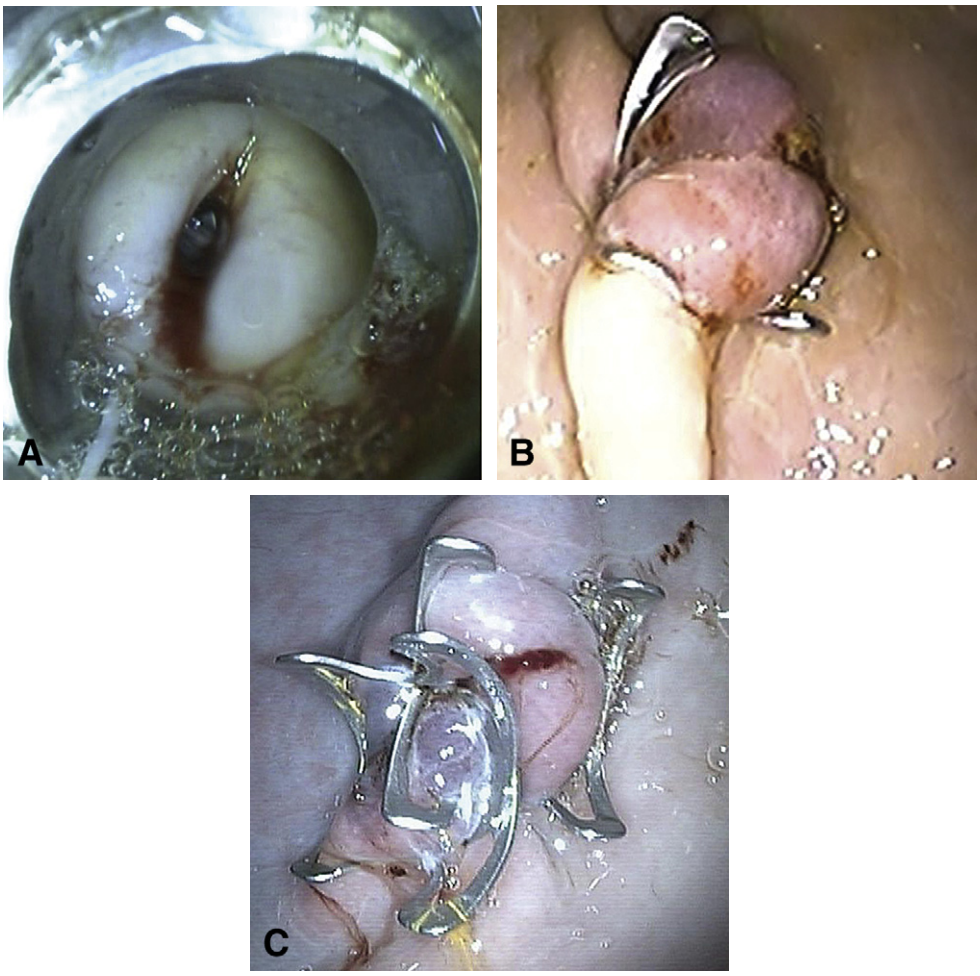

Figure 2. Gastrotomy closure. **A,** OTSC system mounted on the tip of the gastroscope, already in the stomach and having the gastrotomy on the image center. **B,** One clip positioned and the defect closed (groups 1 and 2). **C,** Two clips positioned and the defect closed (group 3).

allowing good anatomical exposure. In groups 1 and 3, the surgical procedure was more elegant than group 2, but we could isolate and safely ligate the vessels easily in all groups. In fact, no adverse events or bleeding complications occurred.

We found the OTSC system easy and intuitive. The most tedious step was the need for gastroscope removal and loading. Before clip release, a mesh of omentum was sometimes pulled into the stomach during gastroscope withdrawn or suction, and its endogastric portion was snared and cut after the clip application (this occurred in 1 pig in group 1, 2 pigs in group 2, and 1 pig in group 3). Immediately after gastrotomy closure, all clips seemed well positioned, and the stomachs were able to maintain distention on air inflation (Fig. 2).

The mean time for the overall procedure, including transgastric port establishment, bilateral testicular vessel ligation, and gastrotomy closure was 64.0 ± 9.4, 53.8 ± 5.5, and 59.2 ± 7.7 minutes for groups 1, 2, and 3, respectively ($P = .18$).

Postoperative follow-up and necropsy results are shown in Table 1. Although comparison of complication rates (related to incomplete gastrotomy closure) among the 3 groups did not reach statistical significance ($P = .099$), there was a clear tendency for a better outcome in groups 2 and 3 than group 1. In fact, in group 1, one animal died within the first 24 hours after the procedure and another experienced anorexia and progressive prostration during recovery and was killed 12 days after surgery (both had evidence of incomplete gastrotomy closure), whereas the remaining animals had an uneventful recovery. In groups 2 and 3, the postoperative recovery and the survival period progressed without adverse events related to surgery. In fact, all the pigs ate heartily and gained weight with no evidence of infection during the 2 weeks after the procedure. All animals that completed the study follow-up period had complete healing of the gastrotomy and no evidence of intra-abdominal abscesses or adhesions (Fig. 3), whereas no significant differences were identified among the groups when comparing gastrotomy position ($P = .74$), persistence of clip on site ($P = .12$), and the presence of omentum mesh on the external side of the stomach ($P = .74$) at necropsy analysis.

TABLE 1. Postoperative follow-up and necropsy data

	Survival follow-up	Necropsy
Group 1 (gastrotomy dilation 18 mm, 1 clip applied)		
1	Dead 24 h later	Leakage Anterior gastrotomy Clip on site; incomplete closure
2	Good recovery	No intra-abdominal complications Posterior gastrotomy No clip; defect healed
3	Progressive clinical deterioration; killed at 12 d	Peritonitis Anterior gastrotomy Clip on site; incomplete closure
4	Good recovery	No intra-abdominal complications Anterior gastrotomy No clip; defect healed Mesh of omentum on external side
5	Good recovery	No intra-abdominal complications Posterior gastrotomy Clip on site; complete closure
Group 2 (gastrotomy dilation 13 mm; 1 clip applied)		
1	Good recovery	No intra-abdominal complications Anterior gastrotomy No clip; defect healed
2	Good recovery	No intra-abdominal complications Anterior gastrotomy No clip; defect healed Mesh of omentum on external side
3	Good recovery	No intra-abdominal complications Posterior gastrotomy No clip; defect healed Mesh of omentum on external side
4	Good recovery	No intra-abdominal complications Posterior gastrotomy Clip on site; complete closure
5	Good recovery	No intra-abdominal complications Anterior gastrotomy Clip on site; complete closure
Group 3 (gastrotomy dilation 18 mm; 2 clips applied)		
1	Good recovery	No intra-abdominal complications Anterior gastrotomy Clips on site; complete closure

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TABLE 1 (*continued*)

	Survival follow-up	Necropsy
2	Good recovery	No intra-abdominal complications Anterior gastrotomy Clips on site; complete closure Mesh of omentum on external side
3	Good recovery	No intra-abdominal complications Anterior gastrotomy Clips on site; complete closure Mesh of omentum on external side
4	Good recovery	No intra-abdominal complications Posterior gastrotomy Clips on site; complete closure
5	Good recovery	No intra-abdominal complications Anterior gastrotomy Clips on site; complete closure

DISCUSSION

We assessed the efficacy and reliability of the OTSC system in vivo for gastric closure in a survival porcine model after bilateral testicular vessel ligation, transgastric varicocelectomy, a simple intra-abdominal surgical procedure that would work as a novel indication for NOTES.

In this study, we used the male pig as an experimental model. This should be taken into account in survival studies such as this one because pig studies are likely the extreme of luminal contamination and peritoneal dissemination risk. In fact, we found it sometimes difficult to obtain a clean stomach in these animals. However, the human stomach is usually clean after some hours of fasting, and some studies suggest that transgastric endoscopic peritoneoscopy does not require stomach decontamination in humans.[25] Other important issues are the safety of gastrotomy creation and the stomach anatomy. Several transgastric access procedures have been described,[1-4,6,9,12] but there is a common step in almost all descriptions, the blind use of a needle-knife with cautery that is highly risky for adjacent organs, and this uncertainty is not acceptable for human application. In our study, we did have some anterior abdominal wall injuries. Regarding the optimal location points for gastrotomy, many authors suggest the anterior gastric wall of the corpus or antrum as the preferred location.[26] This is not always accomplished in pigs because of easy gastric rotation, and in our study, gastrotomy location on the posterior gastric wall was not an uncommon finding.

More crucial and likely the most important step for human translation of NOTES is the method used to achieve safe closure of the gastric defect. Among the current methods,[1,13-22,26,27] the OTSC system seemed to us to be the easiest to apply when a single transgastric port is used for simple procedures. The OTSC system efficacy for NOTES for gastrotomy closure had only been tested in a nonsurvival animal study, and although the gastrotomy lesions could primarily be closed, half of the animals revealed that adaptation of the wound margins was not completely airtight under maximum inflation of the stomach.[24] Believing in the concept of the OTSC system, we designed this protocol with 3 groups in which we varied the gastrotomy size and the number of clips applied to test their in vivo efficacy.

Our experience with OTSC in this study reveals some advantages and disadvantages. We had confirmed its simplicity and intuitive and easy application. However, when mounted over the scope, the cap narrows the field of vision and enlarges the gastroscope tip dimension (up to 18 mm), increasing the risk of esophageal trauma. We think that these aspects were improved with the new transparent and less traumatic cap currently available. Another concern is the risk of adjacent structure involvement during suction. Although bowel loop aspiration never occurred in our protocol, the aspiration of omentum into the stomach through the opening occurred sometimes. We dealt with this by coagulating and cutting it with a snare after the clip application, and there were no related problems. This could even work as an advantage to enhance gastrotomy healing because it is well-known that a patch of omentum could increase the security of bowel perforation repair. Another relevant conclusion drawn from this study is that the stomach's ability to maintain distention with air inflation immediately after closure is not a totally reliable

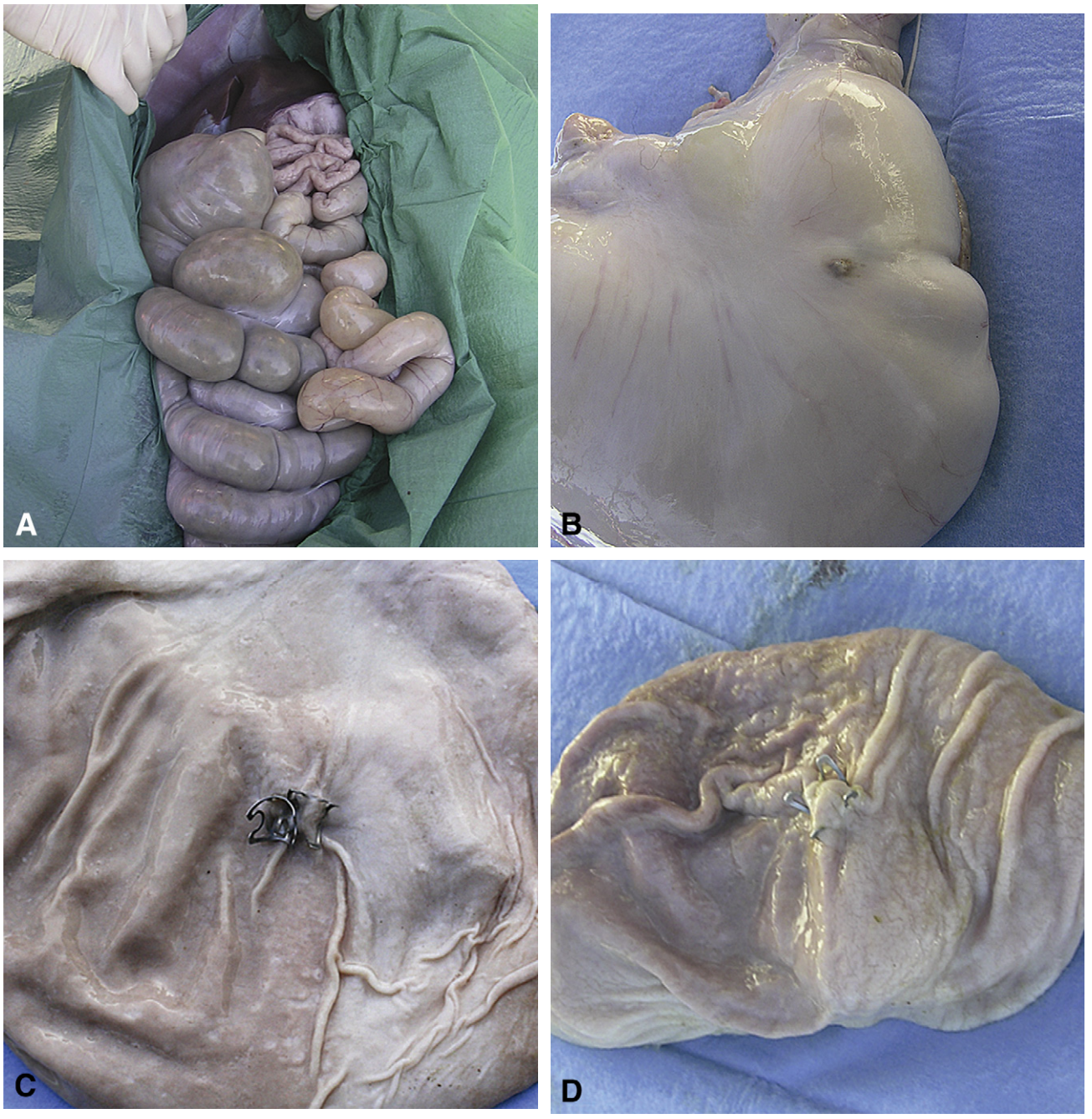

Figure 3. Necropsy findings. **A,** Overall abdominopelvic view. **B,** Gastric external view of the healed gastrotomy. **C,** Internal gastric wall with 2 clips in position. **D,** Internal gastric wall with 1 clip in position. **E,** Internal gastric wall showing the healed defect without clip.

method to confirm good gastrotomy sealing. In fact, although all animals could maintain air distention in the stomach immediately after clip application, in group 1, two animals presented complications related to incomplete gastrotomy closure. Our explanation for this is that acute gastric sealing could be obtained in those animals because of mucosa congestion and/or edema that with time regressed, exposing a leak.

The results of our study validate the potential applicability of the OTSC system in vivo, but they also stress the need to find the perfect match of clip and gastrotomy size. Usually for passing a double-channel gastroscope, we used balloon dilation up to 18 mm; the results for group 1 of 2 incomplete closures in 5 pigs raise some concerns regarding the safety of this clip for this size opening. In group 2, when we dilated just up to 13 mm for single-channel gastroscope passage, the results were 100% successful. Although there are 2 sizes of clips available, each one adapting to the 2 regular diameters of flexible endoscopes, the message here is that it is safer to apply a clip that is larger than the endoscope used

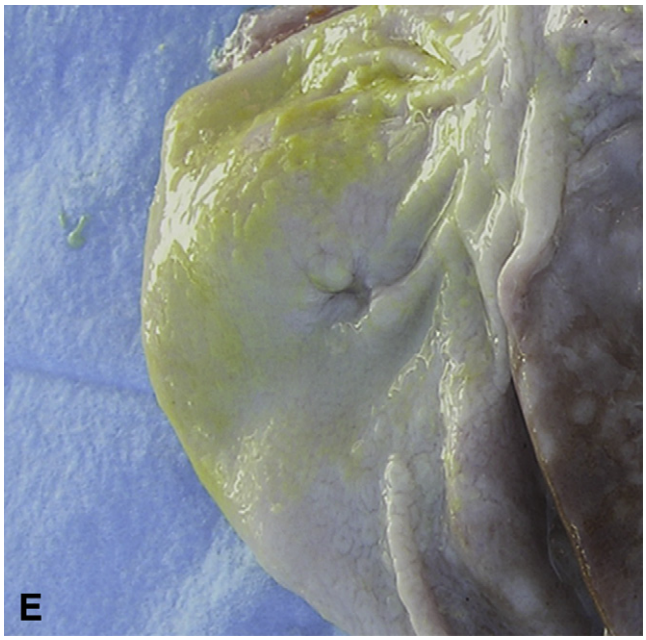

Figure 3 (*continued*)

for transgastric surgery, seeking a better match between the clip and gastrotomy size. Of course a problem still remains: how to close larger openings, mainly when large multitasking platforms are used? Attempting to answer this question, we used a third experimental group with the largest gastrotomy (similar to group 1), in which we applied 2 clips instead of only 1 clip. Surprisingly, we found it easy to apply a second clip even if it is released partially overlapping the first clip. Follow-up of the pigs in group 3 revealed no complications, suggesting that 2 clips could be a reasonable solution for larger gastrotomies. We believe that for simple procedures, as used in our study, thinner endoscopes would be sufficient, and a single-clip adequate to close the gastrotomy. These findings might have implications for other more complex procedures in which a combined hybrid approach might be a smooth transition for pure NOTES while new endoscopes and devices are being developed.

We selected a simple and effective surgical procedure, and the anatomical exposure of the pelvic excavation and anteroinferior abdominal wall for testicular vessel ligation was excellent, with the help of some external adjustments such as use of the Trendelenburg position. Testicular vessel ligation was intentionally chosen because it mimics a current procedure performed in males, varicocelectomy. The term varicocele defines an abnormal tortuosity and dilation of the testicular veins in the pampiniform plexus that has an overall incidence of approximately 10% to 15%, and there is a clear association among varicocele, infertility, and testicular growth arrest. It is also known that varicocelectomy can reverse growth arrest in adolescents with varicocele, and all those aspects support an early intervention in selected cases.[28] Accepting this new era of transforming surgery in an even more minimally invasive field, we tested successfully that varicocelectomy, when using a modified Palomo approach, could became a novel and good indication for NOTES.

In conclusion, the OTSC system was easy to apply and efficient in gastrotomy closure in an experimental survival model of varicocelectomy when correctly matching the gastrotomy size and clip size and/or number. This study encourages further research to make simple pelvic procedures performed by the transgastric approach safe in humans.

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