Comparison of the use of a vessel-sealing device versus ligatures for occlusion of uterine tissues during ovariohysterectomy or ovariectomy in rabbits (*Oryctolagus cuniculus*)

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Received November 10, 2019.
Accepted January 9, 2020.

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OBJECTIVE
To compare the bursting strength of the uterine horns (UHs) and cervical-vestibule junction (CVJs) of rabbits following sealing with a vessel-sealing device (VSD) or encircling ligatures.

SAMPLE
UHs and CVJs collected from 30 rabbit (*Oryctolagus cuniculus*) cadavers.

PROCEDURES
UHs and CVJs were randomly assigned to sealing with encircling Miller knot ligatures (LIG; n = 10 CVJs and 20 UHs) or a VSD (12 CVJs and 24 UHs). Lumens were infused with saline (0.9% NaCl) solution under pressure until seals burst or to a maximum pressure of 300 mm Hg.

RESULTS
For CVJs, median (range) bursting pressure of the LIG and VSD groups was > 300 mm Hg (224 to > 300 mm Hg) and 35 mm Hg (0 to 60 mm Hg), respectively. Five of 12 CVJs in the VSD group failed at pressures < 33 mm Hg. For UHs, median (range) bursting pressure of the LIG and VSD groups was 255 mm Hg (120 to > 300 mm Hg) and 154 mm Hg (range, 44 to 202 mm Hg), respectively.

CONCLUSIONS AND CLINICAL RELEVANCE
The evaluated VSD was effective in sealing UHs at bursting pressures well in excess of expected physiologic pressures, indicating that the VSD may be useful for ovariectomy procedures in rabbits. However, CVJ seals created with the VSD were ineffective and could potentially burst at low pressures, which could predispose to urine entering the abdomen. Given these results, we do not recommend sealing of the CVJ with a VSD for ovariohysterectomy in rabbits. (*Am J Vet Res* 2020;81:755–759)

The prevalence of endometrial hyperplasia and uterine neoplasia, particularly adenocarcinoma, is greater in rabbits than in other small animals, with up to 79% of rabbits developing uterine abnormalities by 6 years of age.[1–4] Uterine abnormalities can be observed by 2 years of age, so the recommended age for spaying of female rabbits is < 1 year.

Rabbits differ from other small animals in several ways that are important to consider when planning surgery. The uterus of rabbits is formed by separate bicornuate UHs that terminate in 2 cervices, with no uterine body. The cervices are contiguous with a distensible vaginal vestibule that pools urine during and after micturition. This urine pooling differentiates them from other small animals and reinforces the requirement for a reliable seal at the CVJ when performing OHE.[5] The perioperative mortality rate for rabbits anesthetized for routine surgery is up to 5 times that of other small animals.[6] The cause of this increased risk of death is likely multifactorial. In addition, rabbits are a prey species vulnerable to distress during handling and have a small body surface area and low proportional lung volume. They are predisposed to hypothermia, gastrointestinal stasis, and visceral adhesions.[6–10]

Recent investigations of laparoscopic OHE and OVE techniques have suggested lower morbidity rates and faster recovery times than achieved with open surgical approaches.[11–14] Investigations into laparoscopic OVE have demonstrated significant reductions in systemic concentrations of neurohormonal stress and inflammation markers perioperatively. Vessel-sealing devices were developed to seal blood vessels with diameters up to 7 mm. The tissue seal is achieved by denaturation and cross-linking of collagen and elastin fibers in the vessel or tissues.[15,16] These devices have also been used to seal tissues and other hollow luminal organs with varying suc-

ABBREVIATIONS

CVJ	Cervical-vestibular junction
OHE	Ovariohysterectomy
OVE	Ovariectomy
UH	Uterine horn
VSD	Vessel-sealing device

cess rates.[17–26] Additionally, laparoscopic OHE already lends itself to the use of a VSD, notably for hemostasis of the broad ligaments and ovarian pedicle.[27]

The use of a VSD to seal uterine tissues has been evaluated in dogs with promising results.[26] The major determining factor in the efficacy of bipolar VSDs is the collagen-to-elastin ratio of tissues, which is highly variable among species, making the validity of between-species extrapolation questionable.[28,29] An experimental study[30] involving New Zealand White rabbits revealed a mean (SD) maximal intrauterine pressure during parturition of 33.0 mm Hg (15.0 mm Hg). This pressure is well above any physiologic pressures that would be encountered at the CVJ seal in a rabbit after OHE, but it represents the absolute maximal physiologic pressure to which the uterus is exposed. If the UB and CVJ of rabbits can be sealed with a VSD, such a technique could further improve the ease, speed, and morbidity of routine reproductive surgery and lend itself to minimally invasive laparoscopic techniques.

To the authors' knowledge, only 2 case reports[11,14] exist describing the use of laparoscopy and a VSD for OVE or OHE in rabbits. The purpose of the study reported here was to determine whether a VSD would form an effective seal at both the UH and CVJ in rabbits and test the hypothesis that the median bursting pressure in rabbits in which a VSD was used would be lower than that in which encircling ligatures were used instead.

Materials and Methods

Uterine specimens

Reproductive tract tissues were collected via midline celiotomy from 30 rabbit cadavers within 6 hours after euthanasia. The rabbits had been 5 to 6 months old and were euthanized as part of a separate, unrelated study involving evaluation of serologic responses that did not involve any drugs or procedures that might affect the reproductive system or tissues. The uterus was removed from the cadavers by sharp transection of the suspensory, broad, and round ligaments and transection at the most caudally accessible part of the vagina to keep the cervices intact. The right UH was marked with a suture at the ovary so that it could be identified ex vivo. Uterine specimens were placed in individual containers of saline (0.9% NaCl) solution and kept refrigerated at 4°C until testing commenced, which occurred within 12 hours after tissue collection.

The diameters of the CVJs and UHs were measured on the intact uterine tissues with calipers and recorded prior to testing. Specimens were excluded from the study if the diameter of each CVJ or UH was < 10 or < 5 mm, respectively. Seven CVJs < 10 mm in diameter were excluded from the study. One additional uterus was excluded because the cervix was lacerated during collection. Consequently, 22 CVJs were included for bursting pressure testing. Of the 60 UHs collected, 16 were excluded because the diameter was < 5 mm and therefore too small for the equipment used to measure bursting pressures, leaving 44 UHs for bursting pressure testing.

Experimental design

An ex vivo randomized controlled trial was conducted to compare the maximal bursting pressures of tissue seals of the CVJs or UHs created with 2 methods. The UH and CVJ specimens were assigned to undergo sealing with a VSD (VSD group; n = 12 CVJs and 24 UHs) or Miller knot ligation (LIG group; 10 CVJs and 20 UHs) with the aid of a random number generator,[a] whereby the assignment probability was 50% for each specimen. The UHs were treated as paired (left and right) from the same rabbit, with the treatment allocation being randomized within pairs.

Procedures

Each uterus underwent 3 tests: 1 test on each of the UHs and 1 test on the single CVJ. The techniques used were similar to those reported by Barerra and Monnet[26] to evaluate bursting pressures in canine uteri. Testing was completed within 12 hours after euthanasia.

In preparation for testing, the left and right UHs were sharply transected at a level 2 cm proximal to the CVJ. The lumen of each UH was catheterized by inserting a 20F Foley catheter[b] into the UH lumen in a caudal-to-cranial direction until the catheter tip was 2 cm from the level where the VSD or ligature was applied to the UH. The Foley catheter was filled with saline solution within an outer soft plastic shroud (inner diameter, 7 mm) so that the cuff of the Foley catheter and associated uterine wall were held firmly against the inner wall of the shroud. The tissue-catheter combination was secured in this position by placement of an encircling Miller knot of 2-0 nylon.[c] For the UH testing, the ovary, oviduct, and proper ligament were amputated from the uterus. For CVJ testing, this process was repeated with the Foley catheter placed and fixed within the vaginal vestibular lumen distal to the VSD or ligature applied at the CVJ. The inner diameter of the shroud used for CVJs was 14 mm. The Foley catheter was then attached to a 3-way stopcock. A 50-mL syringe containing saline solution in a syringe pump was attached to the middle port, and a pressure manometer was attached to the remaining port. All interlinking tubing[d] was of narrow bore and had locking connectors to prevent leaks.

Uteri were sealed with the assigned method at the predetermined locations: UHs were sealed 10 mm caudal to the insertion of the fallopian tube and CVJs were sealed immediately caudal to the CVJ. Because of the duplex uterine anatomy of rabbits, the site chosen for the CVJ seal was in the cranial vaginal vestibule, which is the location described for ligature placement during OHE in rabbits. Seals were created with an encircling Miller knot of 3-0 glycolide[e] (LIG group) or a VSD[f] (jaw length, 17 mm) attached to an

electrosurgical generator platform[g] (VSD group). The evaluated VSD was a bipolar device that compresses the tissues and delivered high current and low voltage to tissues and continuously adjusted the delivery of energy on the basis of the tissue impedance between the instrument jaws. For the UHs, 1 cycle of the VSD was sufficient to form a seal, whereas for the CVJs, 2 cycles were required to extend across the greater diameter. If a single cycle of the VSD failed to provide a grossly observable seal, the VSD was reapplied until a seal was observed.

Prior to seal testing, the manometer was zeroed and leveled with the evaluated specimen. Saline solution was infused into the lumen at a constant rate of 150 mL/h (UH specimens) or 200 mL/h (CVJ specimens) until leakage occurred or a maximal pressure of 300 mm Hg was reached, whichever was observed first. Leakage was defined as either a sudden decrease in the manometer pressure reading or gross observation of fluid leakage by the investigator. The experimental setup was video recorded to capture the pressure manometer, timer, and uterine specimens in the viewing field. This allowed for review of the recordings in slow motion after testing to determine the time and mode of failure if failure occurred. If leakage occurred immediately, the recorded pressure was recorded as 0 mm Hg, and if a pressure of 300 mm Hg was reached, it was recorded as a nonfailure.

Statistical analysis

Collected data were managed and descriptive statistics were computed with the aid of spreadsheet software.[a] To evaluate the effect of sealing method on tissue bursting pressure, statistical software[h] was used. If bursting of the tissue was not observed at the highest applied pressure, the bursting pressure was assigned a value of > 300 mm Hg. If the system failed at unmeasurably low pressure, the bursting pressure was assigned a value of 0 mm Hg. Because these censored observations may have been validly ranked but did not have a numeric value, inference was conducted by nonparametric methods. To evaluate the effect of sealing method on CVJ bursting pressure, the Wilcoxon rank sum test was used,[31] with the test hypothesis being that the difference between treatments was 0 mm Hg. For UH bursting pressure, whereby paired measures were obtained for each animal, the Wilcoxon signed rank test was used instead. To screen for an effect of horn side (left or right), a second signed rank test was performed. Tied scores were handled by use of a randomly broken tie technique. Exact *P* values were computed, with values < 0.05 considered significant.

Results

Uterine specimens

Mean (SD) diameter of CVJs in the VSD (n = 12) and LIG (10) groups was 17 mm (3.5 mm) and 16.5 mm (4.0 mm), respectively. Mean (SD) diameter of the left UHs was 5.8 mm (0.63 mm) and of the right UHs was 5.7 mm (0.42 mm). Mean (SD) diameter of the UHs in the VSD (n = 24) and LIG (20) groups was 5.8 mm (0.63 mm) and 6.4 mm (0.42 mm), respectively.

All CVJs in the VSD group required 2 cycles of the VSD to span the CVJ diameter and form a gross seal. On the other hand, all UHs were sealed with a single VSD cycle.

Bursting pressure testing

Bursting pressure of the CVJ specimens in the LIG group (median, > 300 mm Hg; range, 224 to > 300 mm Hg) was significantly (Wilcoxon rank sum test, $P < 0.001$) greater than that in the VSD group (median, 35 mm Hg; range 0 to 60 mm Hg; **Figure 1**). Five of the 12 CVJs in the VSD group failed at pressures < 33 mm Hg. Six of 10 CVJs in the LIG group did not burst (ie, bursting pressure > 300 mm Hg). The CVJs that achieved a gross seal failed consistently at the overlap of the first and second cycle of the VSD, even with careful ex vivo alignment.

Bursting pressure of the UH specimens in the LIG group (median, 255 mm Hg; range, 120 to > 300 mm Hg) was also significantly (Wilcoxon signed rank test, $P < 0.001$) greater than that in the VSD group (median, 154 mm Hg; range, 44 to 202 mm Hg; **Figure 2**). The signed rank test indicated low compatibility of these data with a treatment effect of zero ($P < 0.001$). Five of the 20 UHs in the LIG group did not burst. No effect was identified of UH side (left or right) on bursting pressure ($P = 0.83$).

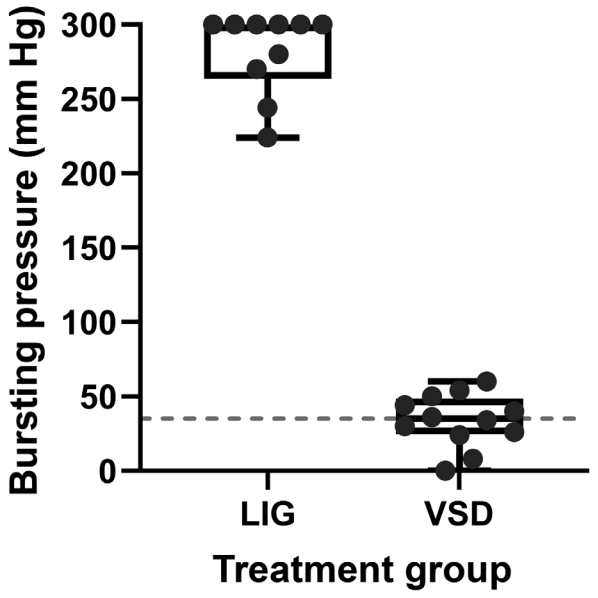

Figure 1—Box-and-whisker plots for bursting pressure in CVJ specimens from rabbit (*Oryctolagus cuniculus*) cadavers in which the CVJ was sealed with a VSD (VSD group; n = 12) or Miller knot ligation (LIG group; 10). The horizontal line within a box represents the median bursting pressure, the bottom and top of each box represent the interquartile (25th to 75th percentile) range, and the whiskers represent the range. Dots represent individual measurements. The dashed line represents the mean maximum physiologic pressure to which a rabbit uterus is subjected during parturition (33 mm Hg).[30] The maximum possible value for bursting pressure was 300 mm Hg.

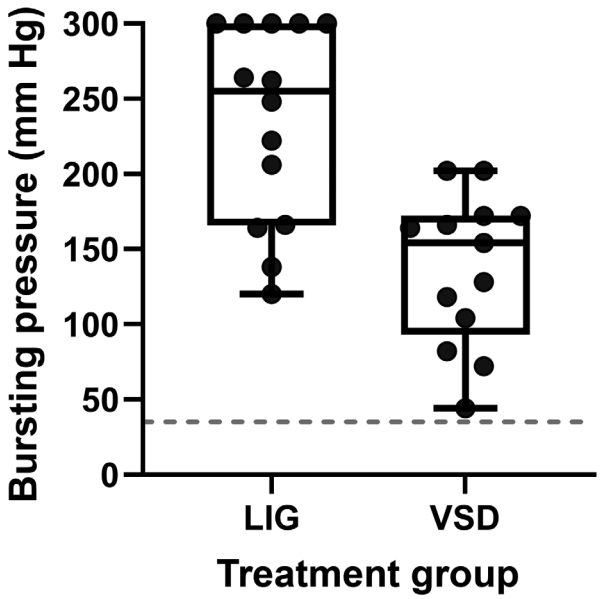

Figure 2—Box-and-whisker plots for bursting pressure in UH specimens from the rabbit cadavers of Figure 1 in which the UH was sealed with a VSD (n = 24) or Miller knot ligation (20). ***See*** Figure 1 for remainder of key.

Discussion

The VSD used in the study reported here failed to achieve a reliable seal in the CVJ of rabbits but did produce a reliable seal in the UHs. The CVJ bursting pressures in the VSD group were consistently lower than those the LIG group, and 5 of the 12 CVJ seals were below the maximum intraluminal pressure to which rabbit uteri can be subjected. Although the UH bursting pressures for the VSD group were lower than those for the LIG group, they exceeded the normal physiologic pressures that UHs would be expected to experience, including those encountered in sexually intact rabbits during parturition.[30]

For 3 CVJs, no seal was grossly observed, which in live rabbits could increase the risk of subsequent urine leakage, infection, and peritonitis from vestibular urine pooling. For the CVJs, 2 cycles were required to span the diameter of the tissue, and the investigator observed that leakage at the CVJ seal occurred invariably at the junction of the first and second cycle of the VSD. We surmise that the seal had been compromised owing to imperfect alignment of the 2 cycles or to variations in the degree of cauterization at the interface of the 2 cycles. The difficulty of achieving a gross, well-aligned seal and evaluating its integrity intraoperatively would likely be compounded by the intrinsic maneuverability problems associated with laparoscopy. Research into the use of a VSD with a longer (50-mm) jaw length than in the present study revealed promising tissue-sealing efficacy during lung lobectomy.[25] This longer jaw length and the mechanism of closing the tip first before compressing tissue within the jaws may mitigate the aforementioned issues associated with using 2 cycles of the VSD in rabbits by spanning the entire CVJ diameter in 1 cycle.

The findings of the study reported here were consistent with the conclusions of Barerra and Monnet,[26] who suggested that VSDs should not be used to seal uterine tissues with a diameter exceeding 9 mm. Our findings also supported the VSD manufacturer statement that the device is efficacious in sealing vessels up to 7 mm in diameter.[11,24] The differences between our results for rabbits regarding use of the VSD to seal uterine lumens and the promising results reported for dogs[26] were likely related to anatomy. Rabbits have a duplex bicornuate uterus, whereas dogs have a bipartite uterus.[32,33] In rabbits undergoing OHE, this difference means that the caudal CVJ seal is placed in cranial vaginal tissue, just caudal to the bifurcation, compared with dogs undergoing OHE, in which the seal is placed in uterine or cervical tissue. The elastin-to-collagen ratios of the tissues at the CVJ and cranial vaginal vestibule are likely to be different from those of the uterine body in rabbits and dogs. Evaluation of the elastin and collagen composition at different levels of the reproductive tract was beyond the scope of the present study. Future research could be directed toward evaluating the efficacy of 2 separate VSD seals across the duplex lagomorphic cervices, immediately cranial to the uterine bifurcation, rather than a single seal placed caudal to the bifurcation as was done in the present study. Such an approach should allow the uterine tissue to be sealed with a single seal, which could potentially address the problems observed with use of 2 seals to span the uterine diameter at the vaginal level. The use of 2 separate VSD seals across the duplex lagomorphic cervices would effectively amputate the UHs and ovaries but would leave some uterine tissue in situ, which might leave the rabbit at risk of developing uterine neoplasia. No long-term outcome studies on the incidence of uterine neoplasia after OVE in rabbits have been reported, to our knowledge.

Surgeons performing laparoscopic OVE in rabbits recommend dissection of the ovary from the fossa to allow extraction of the ovary through the cannula.[14] The larger tissue volume with VSD horn transection or the proposed modified OHE technique may require larger ports or access incisions to remove the tissues.[14] We noted that the creation of seals with the evaluated VSD was subjectively faster and required less tissue handling than ligature placement in the uterine specimens of the present study, which agreed with the observations of other investigators.[26] Another potential advantage of using a VSD is that the device leaves no foreign material behind in the abdomen, unlike suture ligation techniques, which may create a nidus for infection, inflammation, or adhesions. Although the rabbit cadavers included in our study were of similar ages and sizes, substantial variation in the size of the reproductive tract was observed related to degree of maturation, meaning that many specimens were unsuitable for inclusion. Therefore, our

findings should not be generalized to sexually immature rabbits, to rabbits with a CVJ diameter < 10 mm, or to other animal species. It should be noted that the strength of seals was evaluated only ex vivo, providing no information on the progressive healing strength of the tissues. Nevertheless, our findings indicated that the evaluated VSD was effective in sealing the UH of rabbits but not the CVJs. Although the bursting pressures achieved were lower in the VSD group than in the LIG group, they likely exceeded the physiologic pressures expected to occur within the UH. Consequently, although the VSD used in our study did not appear appropriate for sealing of the CVJ during OHE in rabbits, it may be useful for sealing the UHs during OVE.

Acknowledgments

No third-party funding or support was received in connection with this study or the writing or publication of the manuscript. The authors declare that there were no conflicts of interest.

Footnotes

a. Excel, version 16.32, Microsoft Corp, Redmond, Wash.
b. Covidien, Minneapolis, Minn.
c. Ethicon, Johnson and Johnson, New Brunswick, NJ.
d. TUTA, Lidcombe, NSW, Australia.
e. SilverGlide, Castle Hill, NSW, Australia.
f. Covidien Ligasure laparoscopic dolphin-tip sealer/divider, Medtronic, Dublin, Ireland.
g. Covidien ForceTriad generator, Medtronic, Dublin, Ireland.
h. R: A language and environment for statistical computing, version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria.

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