

Use of a Vessel Sealing Device During Elective Orchiectomy in Client-Owned Pet Rabbits: A Clinical Comparison with Conventional Ligation

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Abstract

Background

Vessel sealing devices provide rapid hemostasis and may reduce tissue trauma. Although they have been evaluated in rabbit ovariectomy and ovariohysterectomy, their use during orchiectomy in pet rabbits has not been investigated, and postoperative pain has not previously been assessed using the Rabbit Pain Behavior Scale (RPBS). To compare the effects of a vessel sealing device (Ligasure) and conventional suture ligation (Suture) on surgical duration, physiological variables, recovery time, and postoperative pain in client-owned pet rabbits undergoing elective orchiectomy.

Methods

Twelve healthy intact male pet rabbits were allocated to either Suture or Ligasure groups. Heart rate, respiratory rate, and rectal temperature were recorded before anesthesia and after surgery. Surgical and recovery times were measured. Postoperative pain was independently assessed by two blinded observers using the RPBS. Physiological variables and pain scores were analyzed using linear mixed-effects models, whereas surgical and recovery times were compared using Welch's two-sample *t*-tests. Interobserver agreement was evaluated using the intraclass correlation coefficient (ICC).

Results

Surgical duration was significantly shorter in the LigaSure than in the Suture ($p < 0.001$), whereas recovery time did not differ. Physiological variables changed significantly over time but were unaffected by surgical technique. Interobserver agreement for RPBS scoring was excellent ($ICC = 0.93$). Rabbits treated with LigaSure had significantly lower postoperative pain scores throughout the observation period ($P < 0.001$).

Conclusions

Vessel sealing devices significantly reduced surgical duration and postoperative pain without adversely affecting physiological stability or anesthetic recovery, supporting their use as an effective alternative to conventional ligation for elective rabbit orchiectomy.

Background

Pet rabbits have become increasingly popular companion animals worldwide, resulting in increasing expectations for high standards of veterinary surgical care and postoperative welfare (1–3). Elective orchiectomy is one of the most frequently performed surgical procedures in male pet rabbits for population control, prevention of reproductive disorders, reduction of hormonally mediated behaviors,

and facilitation of group housing (4–6). As with any surgical intervention, minimizing tissue trauma, achieving meticulous hemostasis, reducing operative time, and providing effective pain control are essential components of successful operative management (7–9). Improving postoperative welfare has become a major focus of contemporary rabbit surgery, as inadequate pain management may adversely affect feeding behavior, gastrointestinal function, and overall recovery (7, 10, 11).

Reproductive surgeries are commonly performed in rabbits. Depending on the patient, the owner's request, and whether the surgery is elective or therapeutic, different surgical techniques can be used for the same procedure (8). Conventional rabbit orchiectomy is typically performed using suture ligation of the spermatic cord before transection (2, 5, 8). Although this technique is well established and generally safe, it requires meticulous dissection and ligation to achieve adequate hemostasis. Consequently, surgical duration may be prolonged, potentially increasing anesthetic exposure and tissue manipulation. Longer procedures may also increase surgical stress and delay recovery, emphasizing the importance of techniques that improve surgical efficiency while maintaining patient safety (12–14).

Advanced bipolar vessel sealing devices, such as LigaSure™, simultaneously seal and divide blood vessels using a controlled combination of pressure and bipolar energy (15–17). These devices have been widely adopted in both human and veterinary surgery because they provide rapid and reliable hemostasis during surgery (12, 18). Previous veterinary studies in dogs, cats, pigs, and rabbits have consistently demonstrated reductions in surgical time when vessel sealing technology is used for a variety of soft tissue procedures (7, 9, 19, 20). As prey species, rabbits are particularly susceptible to perioperative stress and anesthetic complications (1, 10, 11); reducing surgical manipulation and operative time may be especially beneficial in this species.

Only a limited number of studies have evaluated vessel sealing devices during rabbit ovariectomy or ovariohysterectomy (7, 21–23), and their findings have been conflicting. Although reductions in surgical duration have been reported, improvements in postoperative pain have not been consistently demonstrated. To the authors' knowledge, no clinical study has evaluated the use of a vessel sealing device during elective orchiectomy in client-owned pet rabbits. Current evidence in rabbits is limited almost exclusively to ovariectomy and ovariohysterectomy, procedures that differ substantially from orchiectomy in surgical approach, tissue handling, operative field, and duration. Consequently, the potential benefits of vessel sealing technology for rabbit orchiectomy remain unknown.

Moreover, most previous investigations assessed pain using the Rabbit Grimace Scale (RbtGS)(24), which relies primarily on facial expressions and may not fully capture the multidimensional behavioral indicators of pain in rabbits. Behavioral scales designed to assess pain stand out for providing evidence of validity, reliability, and sensitivity (25). The utilization of composite pain assessment scales in rabbits is useful for evaluating pain through the analysis of facial expressions, physiological parameters, and behavioral signs (25–27). These scales therefore provide valuable guidance for recognizing and managing postoperative pain in clinical practice. The recently developed and validated Rabbit Pain Behavior Scale (RPBS)(25), provides a more comprehensive behavioral assessment by incorporating

posture, activity, interaction and appetite, facial expression, attention to the affected area, and other pain-related behaviors. Nevertheless, to the authors' knowledge, no previous clinical study has investigated the RPBS to compare postoperative pain following different orchiectomy techniques in rabbits.

Therefore, the present study was designed to address these two important knowledge gaps by providing the first clinical comparison of a vessel sealing device and conventional ligation during elective orchiectomy in client-owned pet rabbits, while simultaneously providing the first clinical evaluation of postoperative pain using the RPBS in this surgical model. We hypothesized that the use of a vessel sealing device would reduce surgical duration and postoperative pain without adversely affecting recovery time or physiological variables.

Methods

Animals and study design

Thirteen client-owned intact male pet rabbits of various ages, weights, and breeds presented for elective orchiectomy were prospectively enrolled in the study. Only clinically healthy rabbits displaying clearly visible testes within two scrotal sacs without a history of previous surgery or evidence of cryptorchidism were included. Before surgery, all rabbits underwent a complete physical examination and routine hematological evaluation to confirm their suitability for general anesthesia. Rabbits were admitted to the hospital a day before the procedures to minimize the stress associated with transportation to allow environmental acclimatization and perioperative management. Because rabbits should not be fasted before anesthesia, no food or water restrictions were imposed before surgery (8).

No formal sample size calculation was performed because this was a prospective clinical study involving client-owned rabbits presented for elective orchiectomy during the study period. All rabbits meeting the inclusion criteria and whose owners provided informed consent were enrolled consecutively. Treatment allocation was not randomized; Treatment allocation was based on owner preference after informed discussion of both surgical techniques and their associated costs. Allocation therefore depended on owner preference and willingness to pay the additional cost associated with the vessel sealing device. Thirteen rabbits were initially enrolled. One rabbit allocated to the Suture group developed postoperative inguinal herniation requiring additional surgery and was excluded from postoperative analyses. Consequently, data from twelve rabbits (six per group) were included in the final statistical analyses. All surgical procedures were performed by the same experienced veterinary surgeon (H.S.), and all anesthetic procedures were administered by the same anesthetist (H.G.) between 5:00 pm and 7:00 pm.

Anesthetic protocol

Anesthetic protocol

The rabbits were anaesthetized with a combination of ketamine, xylazine and midazolam. First, sedation was achieved by intramuscular (IM) administration of 2 mg/kg xylazine (Rooyan Darou®, 20 mg/ml, Semnan, Iran) combined with 1 mg/kg midazolam (Exir® 50 mg/mL, Boroojerd, Iran). Ten minutes later, general anesthesia was induced using IM injection of 40 mg/kg ketamine (Exir® 50 mg/mL, Boroojerd, Iran)(11). Following induction, a 26-gauge intravenous catheter was placed in the marginal ear vein. Because endotracheal intubation in rabbits is technically demanding and the expected duration of surgery was short, anesthesia was maintained using intermittent intravenous (IV) anesthesia, and oxygen supplementation was provided via face mask throughout the procedure. Additional intravenous doses of ketamine (10 mg/kg) and midazolam (0.5 mg/kg) were administered IV as required according to anesthetic depth, based on clinical monitoring and assessment of reflex responses.

Surgical procedures

Following induction of anesthesia, rabbits were positioned in dorsal recumbency on a heating pad and connected to a multiparameter monitoring system (Guangzhou Leyte Medical Equipment, Guangzhou, China) to monitor vital parameters via electrocardiography (ECG) and pulse oximetry. The hair surrounding the testicle was clipped, and the surgical site was aseptically prepared with chlorhexidine solution. A standard scrotal approach orchiectomy was performed using closed technique in all rabbits. A 1.0–1.5 cm longitudinal skin incision was made over each scrotal sac. Following exteriorization of the testis, the spermatic cord was isolated and occluded according to the assigned treatment group. In the conventional ligation (Suture) group, the spermatic cord was ligated using a single circumferential ligature of 3 – 0 Vicryl and transected approximately 5 mm distal to the ligature. In the vessel sealing device (Ligasure) group, the spermatic cord was sealed and transected using a 5-mm Maryland, 23-cm handpiece LigaSure™ vessel sealing system (Covidien®, Colorado, USA) using the ForceTriad generator. Tissue sealing was achieved by a single activation lasting approximately 2–4 seconds for each spermatic cord, after which transection was completed using the integrated cutting mechanism.

Postoperative management

Following surgery, rabbits recovered from anesthesia, during which they were closely monitored. The rabbits were then transferred back to the filming pen for post-surgery video recording. All rabbits received 0.9% sodium chloride solution (10 mL/kg/h, IV) during surgery and anesthetic recovery. 1 mg/kg Meloxicam (Razak® 20 mg/mL, Tehran, Iran) was administered subcutaneously (SC) before surgery as pre-emptive analgesia and continued every 12 hours for three days postoperatively. 10 mg/kg Marbofloxacin (Rooyan Darou®, 20 mg/ml, Semnan, Iran) was administered SC before surgery as postoperative antimicrobial prophylaxis and continued every 24 hours for five days postoperatively (28). The rabbits were discharged the day after surgery.

Outcome measurements

Heart rate, respiratory rate, and temperature were measured by the same investigator immediately before anesthetic induction and again after completion of surgery through thoracic auscultation, observation of chest movements, and a rectal thermometer, respectively. Surgical time was defined as the interval between the initial skin incision and completion of the final surgical step. Recovery time was defined as the interval from completion of surgery until the rabbit achieved sternal recumbency and was able to maintain its head in an upright position without assistance. The primary outcome measures were surgical duration and postoperative pain scores. Secondary outcomes included physiological variables and recovery time.

Postoperative pain assessment

Following anesthetic recovery, rabbits were housed individually in identical cages within a quiet room free from human activity and external disturbances. Each cage contained commercial rabbit feed, fresh water, a piece of carrot, and a pine cone as environmental enrichment. The cage floor was covered with a soft absorbent pad. Video recordings were obtained remotely in the absence of an observer using a fixed camera positioned approximately 1 m in front of each cage. Rabbits were recorded continuously for 20 minutes at 1, 2, 4, 6, 8, 10, and 12 hours after recovery.

Pain was assessed from randomized video recordings using the Rabbit Pain Behavior Scale (RPBS)(25), which evaluates six behavioral domains, including posture, activity, interaction/appetite, facial expression, attention to the affected area, and miscellaneous pain-related behaviors. Two observers (H.G. & M.A.) independently scored all videos while blinded to treatment allocation and postoperative time. Videos were presented in a randomized order and could be reviewed as many times as required before assigning scores. Interobserver agreement for RPBS total scores was assessed using the intraclass correlation coefficient (ICC).

Statistical analysis

Statistical analyses were performed using R version 4.6.0 (R Foundation for Statistical Computing, Vienna, Austria) within the RStudio integrated development environment.

Linear mixed-effects models were fitted using the lme4 and lmerTest packages. Physiological variables were analyzed with surgical technique (Ligasure vs. Suture), time point (preoperative vs. postoperative), and their interaction as fixed effects. At the same time, rabbit identity was included as a random effect to account for repeated measurements. Type III analysis of variance with Satterthwaite's approximation was used to evaluate fixed effects.

Surgical time and recovery time were compared between groups using Welch's two-sample t-tests because the data satisfied the assumption of normality.

RPBS total scores and individual behavioral components were analyzed using linear mixed-effects models with surgical technique, postoperative time, and their interaction as fixed effects. Rabbit identity and observer were included as random effects to account for repeated measurements and interobserver

variability. RPBS scores from both blinded observers were included in the mixed-effects models, with observer incorporated as a random effect to account for interobserver variability.

Estimated marginal means (EMMs) and their 95% confidence intervals (CL) were obtained using the emmeans package, and pairwise comparisons between groups at each postoperative time point were adjusted using Tukey's method. Interobserver agreement between the two observers was assessed using the two-way random-effects intraclass correlation coefficient (ICC) psych package.

Model assumptions were evaluated by visual inspection of residual plots and quantile–quantile plots. Statistical significance was set at $P < 0.05$.

Results

Orchiectomy was completed in all rabbits in both treatment groups. No intraoperative hemorrhage requiring extra-ligation and/or postoperative bloody scrotal discharge occurred in any studied rabbit. One rabbit in the Suture group developed postoperative inguinal herniation several hours after surgery while undergoing behavioral monitoring for postoperative pain assessment. The rabbit required additional surgical treatment and was excluded from subsequent analyses. No additional intraoperative or postoperative complications were observed in either group.

Physiological Variables

Linear mixed-effects analysis revealed no significant effect of surgical technique on heart rate, respiratory rate, or rectal temperature (all $p > 0.05$). However, a significant effect of time point was observed for all three variables. Heart rate decreased significantly after surgery compared with pre-induction values ($F = 29.45$, $p < 0.001$), irrespective of surgical technique. Similarly, respiratory rate showed a significant postoperative reduction ($F = 24.27$, $p < 0.001$), and rectal temperature also decreased significantly following surgery ($F = 38.81$, $p < 0.001$). No significant Group $\times$ Time interactions were detected for any physiological parameter (all $p > 0.05$), indicating similar physiological responses to surgery in both groups (Table 1).

Table 1

Physiological variables in rabbits undergoing orchiectomy using Ligasure or Suture techniques.

Variable	Heart rate (bpm)	Respiratory rate (breaths/min)	Temperature (°C)
Ligasure - Before induction	196.0 ± 30.9	65 ± 20.8	38.8 ± 0.32
Ligasure - After surgery	172.0 ± 24.2	55.5 ± 13.4	37.6 ± 0.94
Suture - Before induction	198.0 ± 30.6	66 ± 25.8	38.6 ± 0.60
Suture - After surgery	178.0 ± 26.1	47.5 ± 21.2	36.8 ± 1.25
p Group	0.779	0.770	0.242
p Time	< 0.001	< 0.001	< 0.001
p Interaction	0.761	0.144	0.246

Values are presented as mean ± SD. Statistical analyses were performed using linear mixed-effects models with surgical technique (Ligasure vs. Suture), time point (preoperative vs. postoperative), and their interaction as fixed effects and rabbit identity as a random effect. P-values are derived from Type III analysis of variance (ANOVA) using Satterthwaite's approximation.

Surgical and Recovery Times

Values are presented as mean ± standard deviation (SD). Welch's two-sample *t*-test analysis demonstrated a significantly shorter surgical duration in the Ligasure group compared with the Suture group (97 ± 18.5 vs. 163 ± 16.5 sec, *t* = -6.556, *df* = 9.867, *p* < 0.001). The use of the vessel sealing device reduced surgical duration approximately 40% compared with the conventional ligation technique. In contrast, recovery time following anesthesia did not differ significantly between groups (63.5 ± 22.7 vs. 69.0 ± 18.6 min, *p* = 0.699) (Fig. 1).

Rabbit Pain Behavior Scale (RPBS)

Excellent interobserver agreement was observed for RPBS total scores (ICC = 0.93; 95% CI: 0.86–0.97), indicating high consistency between the two observers. This excellent agreement supports the reliability and reproducibility of the behavioral pain assessments. Therefore, both observers were retained in the subsequent analyses using linear mixed-effects models that accounted for interobserver variability.

Linear mixed-effects analysis demonstrated a significant effect of surgical technique on RPBS total scores (*F* = 58.88, *P* < 0.001). Rabbits in the Ligasure group consistently exhibited lower postoperative pain scores than rabbits in the Suture group. Postoperative time also had a significant effect on pain scores (*F* = 236.05, *P* < 0.001), reflecting the expected progressive reduction in pain during recovery. A significant Group × Time interaction was detected (*F* = 3.25, *P* = 0.005), indicating that the magnitude of the difference between surgical techniques varied slightly throughout the postoperative observation

period. Post hoc comparisons demonstrated significantly lower RPBS scores in the Ligasure group at every postoperative assessment (1, 2, 4, 6, 8, 10 and 12 h; all $P < 0.001$). Estimated marginal means (EMMs) RPBS scores progressively decreased over time in both groups, with consistently lower values observed in the Ligasure group throughout the postoperative period (Fig. 2, Table 2).

Analysis of individual RPBS components demonstrated significant effects of surgical technique on posture, activity, interaction/appetite and miscellaneous behaviors scores. Posture and interaction/appetite additionally showed significant Group $\times$ Time interactions, indicating differences in their temporal recovery patterns between surgical techniques. Although no overall effect of surgical technique was observed for facial expression, a significant Group $\times$ Time interaction suggested modest temporal differences between treatment groups. Attention to the affected area did not differ between groups (Table 3).

Table 2
RPBS total scores following orchiectomy using Ligasure or Suture techniques in rabbits.

Time (h)	Ligasure (EMM $\pm$ SE)	Suture (EMM $\pm$ SE)	t ratio	p value
1	6.17 $\pm$ 0.30	8.25 $\pm$ 0.30	-6.201	< 0.0001
2	4.08 $\pm$ 0.30	6.83 $\pm$ 0.30	-8.185	< 0.0001
4	3.50 $\pm$ 0.30	5.08 $\pm$ 0.30	-4.713	< 0.0001
6	2.75 $\pm$ 0.30	4.25 $\pm$ 0.30	-4.465	< 0.0001
8	2.00 $\pm$ 0.30	3.58 $\pm$ 0.30	-4.713	< 0.0001
10	1.50 $\pm$ 0.30	3.08 $\pm$ 0.30	-4.713	< 0.0001
12	0.75 $\pm$ 0.30	2.75 $\pm$ 0.30	-5.953	< 0.0001

EMMs $\pm$ Standard error (SE) Values obtained from linear mixed-effects models including surgical technique, postoperative time, and their interaction as fixed effects with rabbit identity and observer as a random effect. Pairwise comparisons between treatment groups at each time point were adjusted using Tukey's method.

Table 3
RPBS components following orchiectomy using Ligasure or Suture techniques.

RPBS component	Group	Time	Group × Time
Posture	F = 12.62, p = 0.005	F = 80.53, p < 0.001	F = 7.50, p < 0.001
Activity	F = 16.13, p = 0.002	F = 36.90, p < 0.001	F = 0.72, p = 0.631
Interaction/Appetite	F = 13.44, p = 0.004	F = 66.43, p < 0.001	F = 8.62, p < 0.001
Facial Expression	F = 4.00, p = 0.073	F = 5.65, p < 0.001	F = 5.65, p < 0.001
Attention to the Affected Area	F = 0.38, p = 0.549	F = 6.44, p < 0.001	F = 0.89, p = 0.506
Miscellaneous Behaviors	F = 13.43, p = 0.004	F = 25.00, p < 0.001	F = 1.46, p = 0.195

Results of linear mixed-effects models evaluating the effects of surgical technique (Ligasure vs. Suture), postoperative time, and their interaction on the individual RPBS behavioral components. Rabbit identity and observer were included as random effects to account for repeated measurements and interobserver variability. P-values were obtained from Type III analysis of variance using Satterthwaite's approximation. Statistically significant effects are shown in **bold**.

Discussion

The present study evaluated the effects of a vessel sealing device compared with conventional ligatures on physiological variables, surgical duration, anesthetic recovery, and postoperative pain in pet rabbits undergoing orchiectomy. The principal findings were that the use of the vessel sealing device significantly reduced surgical time and resulted in lower postoperative pain scores throughout the observation period, while no differences were observed in recovery time or physiological parameters between groups. To our knowledge, this is the first clinical study to evaluate a vessel sealing device during elective orchiectomy in client-owned pet rabbits.

One of the most notable findings of the present study was the significant reduction in surgical duration associated with the use of the vessel sealing device. Rabbits undergoing orchiectomy with LigaSure required approximately 40% shorter surgical time than those treated with conventional ligation. This finding is consistent with previous studies evaluating vessel sealing devices in rabbits undergoing ovariohysterectomy or ovariectomy (7, 21–23), as well as in dogs, cats, and pigs undergoing a variety of reproductive procedures, all of which have demonstrated shorter operative times compared with conventional ligation techniques (9, 12, 19, 20, 29). Although the magnitude of time reduction varied according to the complexity and type of surgery, these studies collectively indicate that vessel sealing technology consistently improves surgical efficiency across different species and surgical procedures. The shorter operative time observed in the present study most likely reflects the ability of the device to simultaneously seal the spermatic cord structures—including the testicular vessels, vas deferens, and surrounding connective tissue—without the need for meticulous placement of multiple suture ligatures (12, 13, 30).

The extent to which this technical advantage translates into a reduction in overall surgical duration appears to depend on the characteristics of the surgical procedure. In one rabbit ovariohysterectomy study, although vessel sealing significantly reduced the time required for ovarian pedicle sealing, no reduction in total surgical time was detected because the benefit was offset by longer abdominal incisions and additional time required for laparotomy closure (21). In contrast, orchiectomy is a relatively simple extra-abdominal procedure that requires neither abdominal entry nor multilayer closure (2, 5, 31); therefore, the time saved during vascular sealing contributes directly to the overall operative time. This likely explains why the reduction in total surgical duration was particularly evident in the present study. The benefit of vessel sealing devices is expected to be greatest in procedures in which vascular control represents a substantial proportion of the total operative time. From a clinical perspective, shorter procedures may reduce tissue manipulation, improve operating room efficiency, decrease anesthetic and consumable costs, and enhance workflow in busy veterinary practices (7, 32). Moreover, previous studies have suggested that vessel sealing devices are technically straightforward to use, allowing surgeons with moderate experience to perform the procedure efficiently [7].

Despite the shorter surgical duration observed with the vessel sealing device, recovery time following anesthesia did not differ significantly between techniques in the present study. In contrast to our study, the shorter anesthesia time was reported in previous study between the vessel sealing device compared with conventional ligation after ovariohysterectomy (7). This discrepancy may be explained by several factors: First, the magnitude and clinical implications of the time savings may vary with the complexity of the surgical procedure. Ovariohysterectomy is an intra-abdominal operation involving multiple surgical steps, including abdominal entry and multilayer closure, whereas orchiectomy is a less invasive procedure with fewer operative stages. Consequently, the reduction in operative time observed during orchiectomy is more directly reflected in the overall duration of the procedure because fewer additional surgical steps are required. Second, recovery from general anesthesia is a multifactorial process that depends not only on the duration of anesthesia but also on anesthetic protocol, drug pharmacokinetics, individual variability, metabolic status, thermoregulation and perioperative management (11, 33), which may explain the absence of detectable differences between groups. Finally, the shorter anesthesia duration (minutes from the beginning of isoflurane delivery to its discontinuation) reported during ovariohysterectomy is consistent with the reduction in surgical time. In contrast, the present study demonstrated no difference in postoperative recovery time (minutes from the end of surgery until the animal wakes up). Differences in study design may also account for the variation between studies, because anesthesia duration and recovery time represent different clinical outcomes influenced by different physiological factors; direct comparison between these findings should be made with caution.

Although the vessel sealing device significantly reduced surgical duration, the magnitude of this reduction was relatively modest. It was therefore unlikely to influence perioperative physiological variables under the standardized anesthetic protocol used in the present study. No significant differences were detected between the two surgical techniques before and after surgery with respect to heart rate, respiratory rate, and rectal temperature. These findings suggest that the use of a vessel sealing device does not adversely affect physiological stability during routine rabbit orchiectomy. Similar

observations have been reported in rabbits undergoing ovariectomy, where no significant differences in heart rate, respiratory rate, rectal temperature, food consumption, fecal output, body weight, or blood glucose concentration were detected between laparoscopic and open surgical approaches (22). Collectively, these findings suggest that, when appropriate anesthetic and perioperative management are provided, the choice of surgical technique may have only a limited influence on short-term physiological variables in healthy rabbits (10, 33). Similar observations have also been reported in other species, where postoperative changes in body temperature were more closely associated with anesthetic duration and perioperative management than with the surgical technique itself (11, 19). The postoperative decreases observed in these variables were expected and likely reflected the combined effects of anesthesia, surgical manipulation, and postoperative recovery.

The most clinically relevant finding of the present study was the significant reduction in postoperative pain observed in rabbits undergoing orchiectomy with the vessel sealing device. Although pain scores progressively decreased in both groups throughout the postoperative period, rabbits treated with Ligasure consistently exhibited lower Rabbit Pain Behavior Scale scores than those treated with conventional ligatures at every assessment time point. These findings suggest that, in addition to improving surgical efficiency, the use of a vessel sealing device may also enhance postoperative welfare by reducing pain following rabbit orchiectomy. Although a significant Group $\times$ Time interaction was identified, pain scores declined progressively in both groups throughout the postoperative period. This finding indicates that postoperative recovery followed a similar overall pattern in both groups. At the same time, rabbits treated with the vessel sealing device consistently experienced lower pain scores, with the magnitude of this difference varying modestly over time. These results differ from those of previous studies, in which vessel sealing or minimally invasive surgical techniques did not result in significantly lower postoperative pain scores compared with conventional techniques (7, 22). Several factors may explain this discrepancy. First, d'Ovidio and Adami (2024) proposed that although vessel sealing devices provide rapid and effective hemostasis, the thermal energy generated during tissue sealing may induce transient thermal nociception that partially offsets the reduction in tissue trauma. Similarly, Kabakchiev et al. (2021) suggested that the true differences in postoperative pain between surgical techniques may be relatively small and therefore difficult to detect, particularly in rabbits where pain-related behavioral changes can be subtle.

Another important explanation may be the method used to assess pain. Both previous studies evaluated postoperative pain using the Rabbit Grimace Scale (RbtGS)(24), which is based primarily on facial action units. The authors acknowledged that facial expressions in rabbits may be subtle and that the limited sensitivity of the RbtGS, particularly in lop-eared rabbits, together with relatively small sample sizes, could reduce the ability to detect clinically meaningful differences between surgical techniques (7, 22). In contrast, the present study employed the Rabbit Pain Behavior Scale (RPBS)(25), a multidimensional validated pain assessment tool that evaluates posture, activity, interaction and appetite, facial expression, attention to the affected area, and miscellaneous pain-related behaviors. Consistent with this approach, the observed differences between treatment groups were mainly attributable to posture, activity, interaction/appetite, and miscellaneous behaviors. Although facial expression showed no overall

effect of surgical technique, a significant Group $\times$ Time interaction indicated modest temporal differences between treatments. Attention to the affected area did not differ between groups. These findings suggest that multidimensional behavioral assessment may be more sensitive than facial-expression-based scoring alone for detecting differences in postoperative pain following rabbit orchiectomy. The excellent interobserver agreement observed in the present study further supports the reliability of the RPBS for assessing postoperative pain in clinical rabbit patients.

Nevertheless, this interpretation should be made with caution because postoperative pain was assessed exclusively using the RPBS in the present study. Direct comparisons between studies employing different pain assessment tools remain difficult, and future investigations simultaneously applying both the RPBS and the Rabbit Grimace Scale would help determine their relative sensitivity for detecting postoperative pain in rabbits. Another possible explanation is that reduced tissue manipulation and the absence of foreign suture material around the spermatic cord may have contributed to less postoperative inflammation and discomfort in rabbits treated with the vessel sealing device. Although this mechanism was not directly evaluated in the present study, it represents a plausible explanation for the consistently lower behavioral pain scores observed throughout the postoperative period.

An important clinical implication of the present study is that improvements in postoperative welfare may be achieved without fundamentally altering the surgical procedure itself. Rather than introducing a new surgical approach, replacing conventional ligation with a vessel sealing device provided effective hemostasis while significantly reducing postoperative pain, without adversely affecting physiological variables or recovery time. This finding supports the concept that refinement of surgical technique, even through relatively small procedural modifications, can positively influence animal welfare. Such refinements are particularly valuable in rabbits, in which pain is often difficult to recognize and may adversely affect appetite, gastrointestinal function, and postoperative recovery.

Several limitations should be considered when interpreting these findings. The relatively small sample size may have reduced the statistical power to detect subtle differences in some behavioral variables. In addition, treatment allocation was based on owner preference rather than randomization, which may have introduced selection bias. Moreover, pain assessment was limited to the first 12 hours after recovery; therefore, potential long-term differences in postoperative recovery could not be evaluated. Future studies involving larger populations, longer follow-up periods, and objective indicators of inflammation or stress would help clarify the mechanisms underlying the apparent analgesic benefit of vessel sealing devices in rabbits.

An additional observation of the present study was the occurrence of postoperative inguinal herniation in one rabbit from the conventional ligation group. The herniation was attributed to breakdown of the tunica vaginalis, a recognized complication of rabbit orchiectomy when vaginal tunic is opened; Still, adequate closure of the inguinal canal is not achieved (8). Previous reports have emphasized the importance of performing rabbit orchiectomy using a closed or open-to-closed technique to minimize the risk of abdominal organ herniation through the patent inguinal canal (2, 5, 31). Although no comparable

complication occurred in the vessel sealing group, the present study was not designed or sufficiently powered to compare complication rates between surgical techniques. Nevertheless, the ability of the vessel sealing device to achieve simultaneous tissue sealing may represent a potential technical advantage that deserves further investigation in larger clinical studies.

Collectively, the available evidence suggests that the principal and consistently demonstrated benefit of vessel sealing technology is improved surgical efficiency across different species and procedures. The present study extends this evidence by demonstrating that, in pet rabbits undergoing elective orchiectomy, improved surgical efficiency is accompanied by significantly reduced postoperative pain without compromising physiological stability or anesthetic recovery. Although the study population was relatively small, the inclusion of client-owned rabbits managed under routine clinical conditions may enhance the clinical applicability of the findings.

Conclusion

The use of a vessel sealing device during elective orchiectomy in pet rabbits significantly reduced surgical duration and postoperative pain compared with conventional ligation, while recovery time and physiological variables remained comparable between techniques. These findings indicate that vessel sealing technology can improve surgical efficiency and postoperative welfare without compromising perioperative safety.

Given the challenges of pain recognition and the importance of minimizing surgical stress in rabbits, refinement of hemostatic technique may provide meaningful clinical benefits without altering the overall surgical approach. Further prospective randomized clinical studies involving larger populations, longer follow-up periods, and objective biomarkers of inflammation and tissue injury are warranted to confirm these findings and further clarify the mechanisms underlying the observed reduction in postoperative pain.

Abbreviations

ANOVA Analysis of Variance

CL Confidence intervals

ECG Electrocardiography

EMMs Estimated marginal means

ICC Intraclass Correlation Coefficient

IM Intramuscular

IV Intravenous

IQR Interquartile Range

mg/kg Milligram per Kilogram

RbtGS Rabbit Grimace Scale

RPBS Rabbit Pain Behavior Scale

SC Subcutaneously

SD Standard Deviation

SE Standard Error

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Shahid Bahonar University of Kerman Biomedical Research Ethics Committee (IR.UK.VETMED.REC 2025-027) before commencing the study. Written informed consent was obtained from the owners of all rabbits before enrollment. All procedures were performed at the Veterinary Hospital of Shahid Bahonar University of Kerman, Iran between, November 2025 and May 2026.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

Conceptualization, M.A., H.S., and H.G.; methodology, M.A., H.S., and H.G.; software, M.A. and H.G.; validation, M.A., H.S., and H.G.; formal analysis, M.A., H.S., and H.G.; investigation, M.A., H.S., and H.G.; resources, M.A., H.S., and H.G.; data curation, M.A., H.S., and H.G.; writing—original draft preparation,

H.G.; writing—review and editing, M.A., H.S., and H.G.; visualization, M.A., H.S., and H.G.; supervision, H.S., and H.G.; project administration, H.S., and H.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

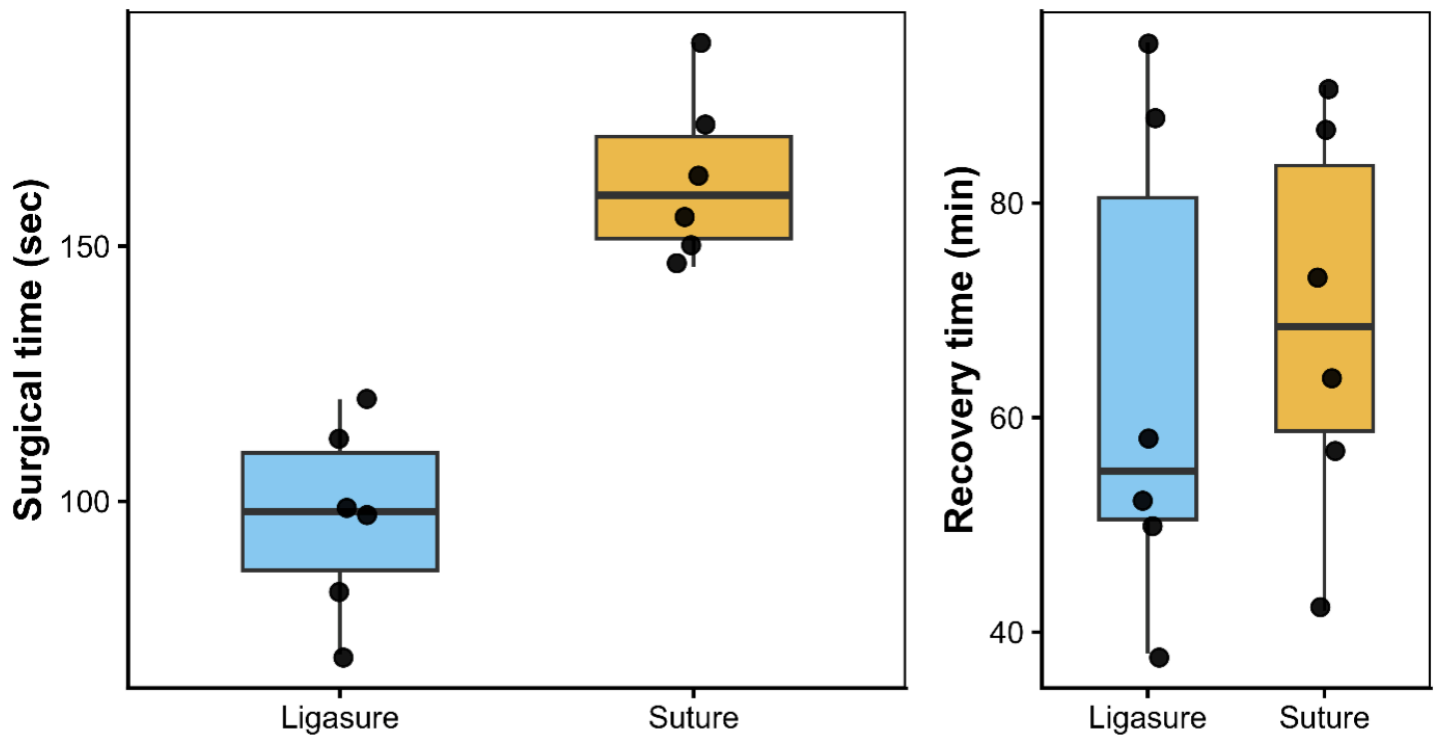


Figure 1

Surgical time and Recovery time in rabbits undergoing orchiectomy using Ligasure or Suture techniques. Panel A: Surgical time (seconds), and Panel B: Recovery time (minutes). Boxes represent the interquartile range (IQR), horizontal lines indicate the median, whiskers represent the range, and points represent individual rabbits. Surgical time was significantly shorter in the Ligasure group than in the Suture group, whereas recovery time did not differ significantly between groups (Welch's two-sample *t*-test, $P < 0.001$ and $P = 0.699$, respectively).

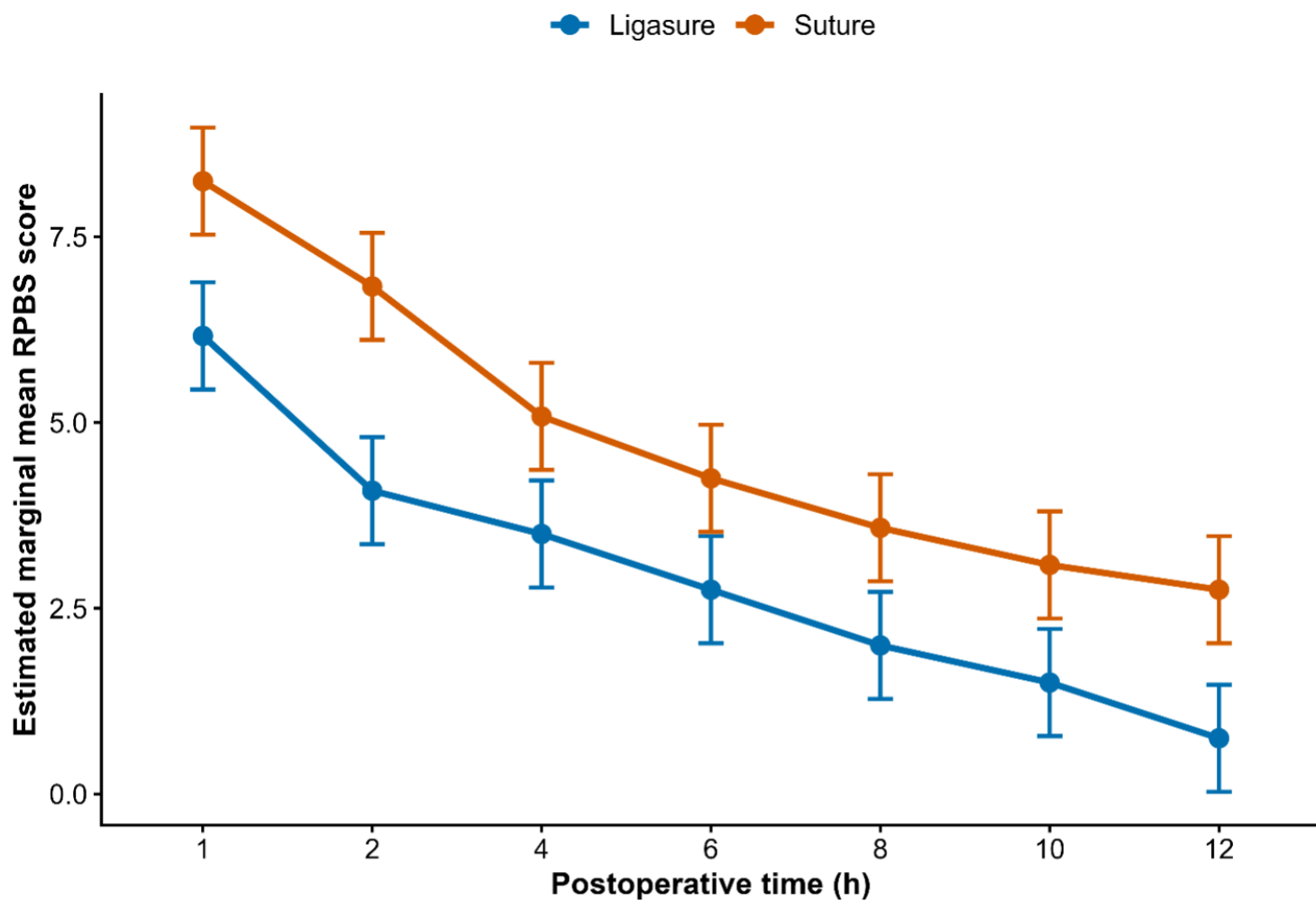


Figure 2

RPBS total scores following elective orchiectomy in rabbits treated using the Ligasure or Suture technique. EMMs ($\pm 95\%$ CL) were obtained from a linear mixed-effects model including surgical technique, postoperative time, and their interaction as fixed effects, with rabbit identity and observer included as random effects.