# Evaluation of three ligatures in simulated equine open castration



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**Evaluation of three ligatures in simulated equine open castration**

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**Abstract**

Objective

To compare three surgical knots for preventing leakage from the vascular bundle during ligation in simulated equine open castrations.

Study design

Randomized, case-control, in vitro study.

Sample population

Testes (N = 60) collected from 30 horses.

Methods

Testes were collected from 30 horses and randomly assigned to one of three groups: group G (friction, giant knot), group T (modified transfixing knot), or group S (sliding, strangle knot; n = 20/group). The assigned knot was used to ligate the vascular bundle during open castration. The length of suture material used and the leak pressure of the testicular artery were measured and compared between groups.

Results

Strangle knots consistently leaked at higher pressures (median, 735.5 mm Hg; interquartile range [IQR], 735.5-735.5) compared with giant (median, 441.3 mm Hg; IQR, 367.8-643.6) and transfixing (median, 419.2 mm Hg; IQR, 323.6-643.6; $P < .0001$) knots. Both the strangle (median, 5 cm; IQR, 4.5-5.5) and giant (median, 6 cm; IQR, 5.35-6.075) knots required less suture material compared with the transfixing (median, 9.2 cm; IQR, 8.425-10.38; $P < .0001$) knot.

Conclusion

The three surgical knots tested withstood pressure well above physiological levels in simulated open castrations. The strangle knot withstood higher pressure and required similar (giant) or less (transfixing) suture material than the other two knots.

Clinical significance

This study provides evidence to support the use of a strangle knot to ligate the vascular bundle during simulated open castrations in horses.

## 1 INTRODUCTION

Castration is a common surgical procedure in horses, yet its complications constitute one common cause of malpractice claims against equine veterinarians.1-3 Although there are variations in surgical techniques (open, closed, half-closed) and approaches (scrotal, inguinal, laparoscopic),2, 4, 5 a ligature can be used with or without an emasculator to control bleeding from the vascular bundle before transection.5-7 Despite the possible use of the emasculator and the introduction of newer electrosealing devices, ligatures are still considered the gold standard method to achieve hemostasis in surgery.8 Occlusion of blood vessels requires ligations to include an encircling suture and a knot. The strangle knot is a variation of a two-pass friction knot previously used for hemostasis.8 Friction knots, such as the strangle knot, can cinch down tightly without locking up prematurely. They also resist loosening after they have been placed, allowing time for placement of additional throws to secure the knot.8 In the inguinal castration technique, Kummer et al[5] ligated the vascular bundle with two ligatures, one constating of a modified Miller's knot, and transection with scissors without the aid of an emasculator. The strangle knot has the same configuration as the Miller's knot but is considered easier to tie8, 9 and has been found most effective in securing hemostasis in the hands of inexperienced surgeons.9 No study in which a strangle knot has been tested in a simulated equine castration has been published. We hypothesized that the leak pressure and amount of suture material used in a friction knot (strangle knot) would be comparable to a sliding knot (giant knot) or a modified transfixing knot. The objective of this study was, therefore, to compare these knots in a simulated open castration.

## 2 MATERIALS AND METHODS

Testes and whole spermatic cords were collected from a local abattoir from 30 horses (median age, 24 months [range, 18-28]; median weight 450 kg [range, 420-480]). Vaginal tunic and cremaster muscle were removed before storing the specimens in a 0.9% saline solution for a maximum of 4 hours. We defined the vascular bundle as the testicular artery and vein, nerves, lymphatics, connective tissue, and ductus deferens for the purpose of this study.7

### 2.1 Measurement of the vascular bundle and testicle diameters

An open castration was simulated on all testes by removing the vaginal tunic and cremaster muscle before marking a line on the vascular bundle along the proposed site of transection 3 cm proximal to the epididymis. The testicle was kept hanging from the vascular bundle with graph paper as background, and a digital photograph was taken. This photograph was analyzed in Image J (National Institutes of Health, Bethesda, Maryland), and the diameter of the vascular bundle was measured. The same procedure was used to measure the major and minor axes of the testicle and the diameter of the testicular artery at the proximal aspect of the cord (ie, approximately 9 cm from the epididymis).7 Measurements were taken to ensure uniformity of the sample and also to uncover any possible effects related to the size of the testis, vascular bundle, and artery on the hemostatic ability of the ligatures.7

2.2 Ligation techniques

The testes were randomly (**www.random.org**) assigned to three groups of 20 specimens: group G was ligated with a giant knot,[4, 5, 9] group T was ligated with a modified transfixing knot,[6, 7, 9, 10] and group S was ligated with a strangle knot[8, 9, 11] (Figure 1). For every specimen, one knot was then tied 10 mm proximally to the transection line drawn with the pencil.

In each group, the ligature was completed with a Glicomer 631 USP n°0 (Biosin; Covidien Italia, Milan, Italy) and apposed proximally to the transecting line at a standard length of 10 mm.[5] The transfixing knot was created with a surgeon's knot with four overthrows, for a total of six throws.[12] The giant knot had only one reverse half-hitch on the alternate post.[7] The strangle knot was created as reported previously.[9, 11, 13] Two overthrows were then used to secure the knot.

For all knots, tension was applied and maintained according to the technique of Hazenfield and Smeack.[11] Briefly, a standardized tension of 2 kg (19.61 N) was applied and maintained for 10 seconds by using a hand dynamometer (HCB200K100; Kern & Sohn GmbH, Balingen, Germany) to verify the applied force. A loop on one end of the suture thread, free of the needle, was tied and attached to the dynamometer. The other end was tensioned with the aid of the needle holder.

2.3 Measures of outcome

After completion of the knot, the excess thread was trimmed to a standard length of 3 mm.[14] The total suture material used for each knot was calculated as the difference between the length of the initial thread piece and the trimmed thread pieces.

An orchiectomy was then performed at a standardized distance of 10 mm distal to the ligature with scissors.[5] The detached part of each testis was examined to confirm the presence of the whole epididymis and testis, while the remaining segment of vascular bundle was submitted for additional testing.

The hemostatic effectiveness of ligatures was evaluated with a previously described leak testing protocol.[7] Briefly, the testicular artery was cannulated at the proximal end by first partially advancing (5 mm) a 23-gauge intravenous catheter over the trocar to cover the sharp tip. The catheter with its trocar was advanced about 5 mm proximal to the ligature. The artery was then proximally sealed by using a mosquito forceps with the jaws covered with two latex tubes (Figure 2). The catheter was connected to a 60-mL syringe and an analogue manometer with the aid of three-way inlet tubing, thus forming a closed system. A fluid stain composed of 0.9% saline and methylene blue solution was slowly injected, increasing the intraluminal pressure of the testicular artery. Fluid pressure was measured until leakage from the distal stump through the ligature was visually observed, marking the end of the experiment. The end scale of the manometer was set by the manufacturer at 735.5 mm Hg. If the specimen did not leak at the end scale pressure, a value of

735.5 mm Hg was assigned. To avoid any operator bias, all knots were tied and all orchiectomies were performed by the same experienced surgeon (M.G.).

### 2.4 Data analysis

Power and sample size were calculated on the basis of leaking pressures by using a freely available online sample size calculator (**www.openepi.com**), with an α level of .05 and 80% power according to data in a previous similar report.[7] The normal distribution of data was determined group wise with the Shapiro-Wilk normality test. Normally and nonnormally distributed data were tested as multiple comparisons with an analysis of variance and Kruskal-Wallis test, respectively. Statistical significance was set at $P < .05$. Data were analyzed in Prism 6 (GraphPad Software, La Jolla, California).

## 3 RESULTS

Anatomical features of the testes did not differ between groups (major axis of the testis, $P = .73$; minor axis of the testis, $P = .52$; diameter of the vascular bundle, $P = .20$; diameter of the testicular artery, $P = .16$; Table **1**). The strangle (median, 5 cm; interquartile rage [IQR], 4.5-5.5) and giant (median, 6 cm; IQR, 5.35-6.075) knots required less suture material compared with the transfixing knot (median, 9.2 cm; IQR, 8.425-10.38; $P < .0001$; Table **2**). There was no difference in suture length between group G and group S ($P = .07$).

Specimens ligated with a strangle knot consistently leaked at higher pressures (median 735.5 mm Hg; IQR, 735.5-735.5) compared with those ligated with the giant (median, 441.3 mm Hg; IQR, 367.8-643.6) or transfixing (median, 419.2 mm Hg; IQR, 323.6-643.6; $P < .001$) knots. No difference was detected between group G and group T ($P = .95$).

## 4 DISCUSSION

Ligation of vessels remains the gold standard for achieving hemostasis in veterinary surgery. The three ligatures tested in our study were able to sustain pressures two to three times higher than physiological levels[15]; furthermore, the lowest recorded pressures during the tests were well above physiological values. All tested knot types, therefore, seemed adequate to achieve hemostasis. In addition, the dimensions of vascular bundle/testicular artery did not differ between groups, confirming the homogeneity reported in previous studies[7] and eliminating this variable as a cause for the differences observed in our study. Specimens ligated with a strangle knot sustained higher pressures compared with those ligated with giant or transfixing knots. Such performance was also consistent, with 16 of 20 specimens ligated with a strangle knot reaching the maximum scale value of the manometer. Finally, the giant and strangle knots required less suture material than the transfixing knot.

We speculated that the configuration of the strangle knot, with its two turns around the vessel and overriding turn, was responsible for the knot's ability to achieve the reported results. The overriding turn maintains compression on the underlying turns of the suture, giving the surgeon time to apply additional throws and creating a knot with a high degree of security.[8, 11] Our results are in line with a previous study[7] in which giant and transfixing knots were found effective in equine open and closed castrations when used in combination with an emasculator. The strangle knot has also been found secure at pressures far greater than physiologically relevant arterial pressures.[11] Smeack and Hazenfield[8] and Hazenfield and Smeack[11] also reported that the strangle knot, even before any over-throws are applied, could not be untied when tensioned unless the overriding turn was cut. This characteristic provides time to apply overthrows but requires initial accurate positioning. Indeed, this knot is difficult to untie without scissors, thus creating the potential for iatrogenic damage.

The ideal surgical knot should be strong yet sufficiently small to minimize the amount of foreign body.[16] The length of suture material required for each knot in our study depends on the configurations of knots and the number of overthrows, both influencing knot security.[11] The typical recommendation to secure the knot has been to increase the number of throws, which increases the knot's volume.[17] This strategy increases the amount of material being left in the surgical wound and can prolong healing. In our study, the transfixing knot required more material than the other two knots because of the four overthrows used to maximize its security.[14] Reports in the literature suggest that three half-hitches are required to maximize security in the giant knot, but only one reverse half-hitch on the alternate post is sufficient to secure it.[7, 9, 18] No data have been published regarding the number of overthrows required to secure a strangle knot. On the basis of the authors' clinical experience, a recent publication,[9] and preliminary testing with a tensiometer, applying two or three overthrows does not influence slippage or breaking strength; this provided sufficient evidence to prompt us to select the simplest conformation.

Differences in physical properties between suture materials also influence knot security. Surgeons should, therefore, be aware of how a knot performs with different materials.[19] The selection of monofilament suture material and size 0 USP was derived from published suture recommendations for equine castrations.[6, 7] Monofilament suture material has also been recommended in field conditions for its resistance to contamination.[20-23] Hendrickson[24] also reported that monofilament suture holds knots more securely than braided material. Flat knots created with monofilament suture assumed a sliding conformation when held under unequal tension in a recent study.[25] This configuration change creates stress on the suture material, causing breakage within the knot.[25] Using a friction or sliding knot with monofilament suture avoids such a risk, thereby increasing security.[7, 9]

In a clinical setting, bleeding may not be observed immediately after vessel transection because of generalized hypotension or hypotension-related vascular spasm associated with general anesthesia. However, the spasm typically subsides in the postoperative period, and latent hemorrhage may occur due to insecure ligature placement.[11] Although other causes (insufficient tissue crush, excessive supportive tissue) may contribute to postoperative hemorrhage, ligature failure may reflect a lack of experience in selecting ligature material or technique. The technique varies between surgeons, often on the basis of empirical data or previous training.[6, 11] The use of a monofilament strangle knot was found to be effective even for novice surgeons in a recent study on knot construction by inexperienced surgeons.[9] These findings provide evidence to support the recommendation of Hazenfield and Smeack[11] to teach this knot in veterinary schools. Our study differs from previous publications and recommended techniques by its use of a single rather than a double ligature, a selection made to enhance the ability to differentiate knot types.

The main limitations of our study are inherent to its in vitro nature and justify caution when extrapolating our results to clinical applications. Indeed, our design does not address stresses that a ligature may sustain in vivo, such as postoperative movement and associated tissue oedema or inflammation. In addition, we could not evaluate the effects of vasoconstriction or coagulation on hemostasis. Furthermore, tension exerted on the cord at application of the ligature may impair its effectiveness, although we deliberately avoided placing any tension on the cord when applying the ligatures to reduce this potential confounder.[7] Finally, results may differ with different suture materials and sizes.

In conclusion, a single ligature sufficed to obliterate the testicular artery in this in vitro setting of simulated open castration. Furthermore, all three ligatures tested with monofilament suture withstood pressures well above physiological levels. The strangle knot reached highest leak pressures while requiring less suture material. Our study, therefore, provides evidence to justify clinical evaluations of the strangle knot with monofilament suture material for open castration and encourage its teaching.[9]

## CONFLICT OF INTEREST

The authors have no conflicts of interests to declare.

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**Figure:**

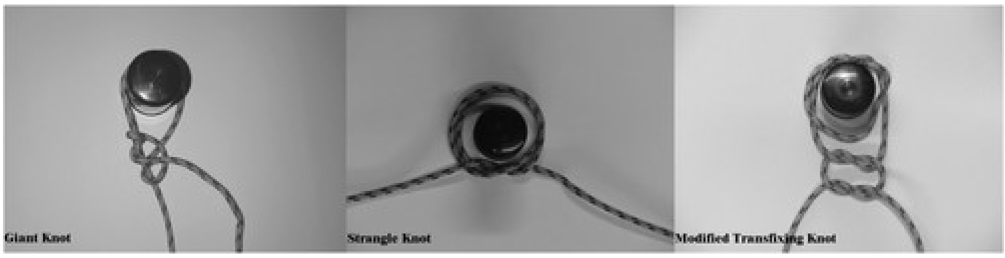


**Figure 1** The knots used in the study

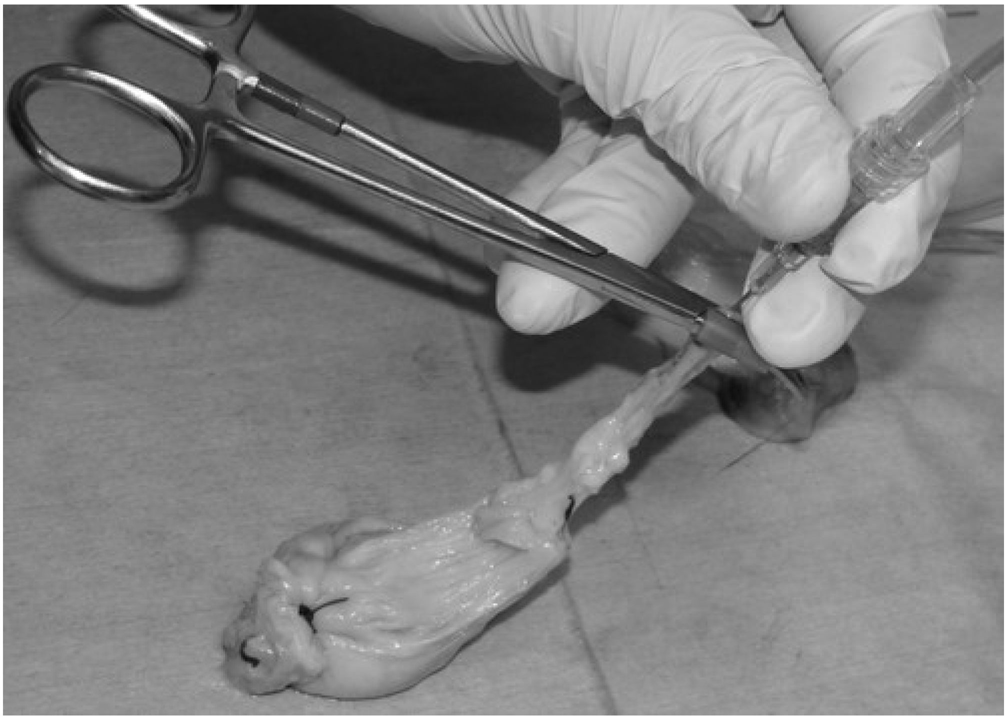

**Figure 2** The catheter is fed into the testicular artery at its proximal end

**Table:**

**Table 1.** Descriptive statistics of the anatomical features of the testes

| Group | Major axis of the testis, mean ± SD, cm | Minor axis of the testis, mean ± SD, cm | Vascular bundle diameter, mean ± SD, mm | Testicular artery diameter, median (IQR), mm |
|---|---|---|---|---|
| G | 9.5 ± 0.98 | 5.67 ± 0.39 | 22.1 ± 1.85 | 4 (3.125-4) |
| T | 9.7 ± 0.88 | 5.6 ± 0.29 | 23.6 ± 2.83 | 3 (3-4) |
| S | 9.6 ± 1.11 | 5.55 ± 0.34 | 24 ± 4.75 | 3.5 (3-4) |

- Abbreviations: G, giant knot; IQR, interquartile range; T, modified transfixing knot; S, strangle knot.

**Table 2.** Descriptive statistics of the features of the ligatures tested

| Group | Leaking pressure, median (IQR), mm Hg | Suture length, median (IQR), cm |
|---|---|---|
| G | 441.3 (367.8-643.6)* | 6 (5.35-6.075)* |
| T | 419.2 (323.6-643.6)* | 9.2 (8.425-10.38)* |
| S | 735.5 (735.5-735.5)* | 5 (4.5-5.5)* |

- Abbreviations: G, giant knot; IQR, interquartile range; T, modified transfixing knot; S, strangle knot.
- * $P < .0001$.