Ex vivo comparison of the giant and transfixing knot in equine open and closed castration

Francesco Comino, Gessica Giusto, Vittorio Caramello, Eleonora Pagliara, Claudio Bellino, Marco Gandini

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Summary

Reasons for performing this study: In equine castration, the application of a ligature on the spermatic cord can prevent evisceration in the closed technique and haemorrhage in the open but not in the closed technique. We hypothesized that this may be caused by the characteristics of some ligatures commonly used in equine castration.

Objectives: To compare the modified transfixing knot with a slip knot (Giant knot) in open or closed equine castration techniques.

Study design: Experimental, randomized, case-control in vitro study.

Methods: Seventy-two testes were assigned to two groups (open or closed castration). Both groups were further divided into three subgroups: E (emasculator only), G (emasculator plus Giant knot), T (emasculator plus modified transfixing knot). The leaking pressure of the testicular artery was measured by dye injection. In closed techniques the tensile strength of the parietal tunic was measured with a tensiometer.

Results: In both techniques, the leaking pressure was higher (although not significantly so) when using a Giant knot. In the closed technique, minimum leaking pressure values for the T and E subgroups were close to physiological arterial pressure. Tensile strength of the parietal tunic was higher (although not significantly so) when ligatures were applied.

Conclusions: The fact that some of the samples in group T were able to withstand very low leaking pressures may explain failure of the modified transfixing knot in providing haemostasis in the closed technique, as previously reported. In the closed technique, a Giant knot can provide higher arterial leaking pressure as well as higher parietal tunic tensile strength, thus possibly reducing complications in equine castration.

Introduction

Orchiectomy is one of the commonest procedures performed on equines. Despite being considered routine practice, a number of complications may arise which, not unfrequently, lead to veterinary malpractice claims being filed [1]. Most postoperative complications are mild in nature and only require minimal treatment. Others, such as peritonitis, haemorrhage and evisceration, are severe and even potentially fatal to the animal [2, 3, 4].

In equine orchiectomy, ligation of the spermatic cord is recommended, in order to reduce the risk of postoperative complications [3, 5]. The procedure is reported to prevent evisceration in closed castration techniques, but does not reduce the risk of haemorrhage and even increases the risk of infection due to suture material being left *in situ* [1, 2 3].

A number of different ligation techniques are disclosed in literature. Rijkhenuizen et al. assessed the efficacy of two haemostatic ligatures without application of the emasculator [6]. They did not mention, however, whether either technique could be preferred over those available when used in combination with the haemostatic action exerted by the emasculator, with regard to both haemostasis and the prevention of evisceration in closed castration.

The aim of this study is to evaluate, for each castration technique (i.e., open or closed technique): (1) whether or not a particular type of ligature can improve the haemostatic capability of the emasculator when used alone; (2) whether a slip knot (Giant knot) configuration is equal to, or better than, a transfixing ligature; and (3) whether or not adding a ligature in closed castration can increase parietal tunic tensile strength.

Materials and methods

Testes and whole spermatic cords were acquired from 36 horses (mean age: 24 months, range: 18-24 months, mean weight: 450 kg, range 420-480 kg) sourced from a local abattoir and maintained in 0.9% saline solution for up to 4 hours. The 72 testes were randomly assigned to two different groups (36

samples each). An orchiectomy procedure was then performed, with either open (Group A) or closed (Group B) technique, as previously described [5]. The two groups were further divided into three subgroups, each comprising 12 testes.

In subgroup E, the procedure was carried out using the Serra emasculator (Kruuse)[a] alone. In subgroup G, the procedure was carried out using the emasculator plus a Giant knot [7, 8] and in subgroup T, the procedure was carried out using the emasculator and a transfixing modified knot [6, 9]. In all three groups, Glycomer 631 USP (United States Pharmacopeia) size 0 (Byosin®)[b] was used as suture material and ligatures were placed proximal to the application site of the emasculator. The transfixing knot involved a surgeon's knot and four additional half-hitches [10], whereas the Giant knot only required one half-hitch. After application of the ligature, excess thread was cut at a 3-mm standard end length [11]. The thread used for each ligature was measured by subtracting the remaining thread from the initial thread length, including waste. All procedures were performed by the same surgeon (MG), so as to avoid any operator influence on the results.

Prior to surgery, the major (cm) and minor (cm) axes of each testis were measured. Next, a line was marked on the spermatic cord, along the proposed application site of the emasculator. The spermatic cord was then kept hanging and photographed on graph paper. Using a dedicated software (Image J)[c], the diameters of the spermatic cord (closed technique) and of the vascular bundle (open technique) were measured. The same procedure was used to measure the diameter of the testicular artery.

In the closed technique group (Group C) the testis, still encapsulated by the parietal tunic, was stripped of the remaining part of the dartos and spermatic fascia. The cremaster muscle was transected and emasculated separately before proceeding with the tunica vaginalis and spermatic cord [1, 2 , 5] .

After having separated the testis, the emasculator was placed proximal to the operator's fingers and left in place for 2 minutes before being removed [2, 5]. In the open technique group (Group A), an incision was made on the distal face of the parietal tunic to prolapse the testis and the ligament of the tail of the

epididymis was severed. Next, the testis, the epididymis and the distal part of the spermatic cord were excised using the emasculator. Again, the emasculator was left in place for 2 minutes before being removed [2, 5].

Once the emasculator removed, the detached part of each testis presented all parts of a testis with epididymis. An intravenous, 23-G catheter (TERUMO)[d] was slid partially along its inner trocar so as to protect tissues from its sharp tip (Fig.1 , 2) The testicular artery was cannulated with the catheter and its spindle about 5 mm proximal to the ligature or to the emasculation site. The catheter was then attached and the femoral artery proximally sealed using a mosquito with two pieces of latex tube around its jaws (Fig.). Finally, the catheter was connected to a 50-mL syringe (TERUMO)[d] and to an analogue manometer (mmHg) with the aid of a three-way inlet tubing, thus forming a closed system [6, 12, 13]. A fluid stained with methylene blue was slowly inoculated, increasing the intraluminal pressure of the testicular artery. Fluid pressure was measured until leakage from the distal stump through to the ligatures and the application site of the emasculator. Fluid leakage marked the end of the experiment and the value thus obtained was identified as the leaking pressure [6, 13].

Additionally, in Group C a tension test was carried out on both the internal spermatic fascia and the spermatic cord in order to assess whether knot placement could increase parietal tunic tensile strength. Two mosquitos were attached, one to the tunica vaginalis proximal to the emasculation site and the other to the spermatic cord. The latter was then connected to a digital dynamometer (Kern&Sohn)[e] and tension was applied in an incremental manner until excision of the tunica itself. Rupture of the tunica vaginalis marked the end of the experiment and the maximum value obtained identified the maximum parietal tunic tensile strength.

Statistical Analysis

Data were tested for normality using the Kolmogorov-Smirnov test. Those found to be not normally

distributed were analysed using nonparametric tests. The largest testis diameter, the diameter of either the spermatic cord or the vascular bundle, and that of the testicular artery were compared using either the Kruskal-Wallis test (for all three subgroups) or the Mann-Whitney test (Groups A and C). The smallest testis diameter was compared using either ANOVA (all three subgroups) or a Welch corrected, unpaired *t*-test (Groups A and C). The length of suture thread for all subgroups involving ligation (namely, -G and –T) was compared using the Mann-Whitney test. Leaking pressures were compared using either the Kruskal-Wallis test (across subgroups) or the Mann-Whitney test (across groups). For each group, the correlation between leaking pressure and the respective sizes of the testis, the spermatic cord and the artery was assessed using a Spearman Rank Correlation. Finally, tensile load was analysed with ANOVA.

All statistical analyses were carried out using a commercially available statistical software (Graphpad-InStat®)[f]. The level of significance was set at 5%.

Results

Part of the results are summarised in Table 1.

Largest testis diameter

The median (min-max) largest testis diameter was 9 cm (7-13) for subgroup AE, 9.5cm (5-12) for subgroup AG and 10.5 cm (8-14) for subgroup AT. The difference across all three subgroups was not statistically significant (p=0.3).

The median (min-max) largest testis diameter was 10 cm (8-16) for subgroup CE, 10.75 cm (7.5-13.5) for subgroup CG and 10.25 cm (7.5-13.5) for subgroup CT. Again, the difference across all three subgroups was not statistically significant (p=0.9).

The median (min-max) largest testis diameter was 10 cm (5-14) for Group A and 10.5 (7.5-16) for

Group C. The difference was not statistically significant (p=0.1).

Smallest testis diameter

The mean (±SD) smallest testis diameter was 5.80±1.53 cm in subgroup AE, 5.60±1.87 cm for subgroup AG and 6.69±1.86 cm for subgroup AT. The difference across all three subgroups was not statistically significant (p=0.3).

The mean (±SD) smallest testis diameter was 6.34±1.96 cm in subgroup CE, 6.25±0.96 cm un subgroup CG and 6.31±1.13 cm for subgroup CT. Again, the difference across all three subgroups was not statistically significant (p=0.9).

The mean (±SD) smallest testis diameter was 6.02±1.78 cm in Group A and 6.30±1.40 cm in Group C. The difference was not statistically significant (p=0.4).

Spermatic cord/vascular bundle diameter

The median (min-max) vascular bundle diameter was 20.3 mm (16.3-22.8) for subgroup AE, 19.75 mm (16.5-22.9) for subgroup AG and 20.5 mm (16.25-22.9) for subgroup AT. The difference across all three subgroups was not statistically significant (p=0.8).

The median (min-max) spermatic cord diameter was 28.1 mm (25.1-30.25) for subgroup CE, 28.15 mm (24.1-30.5) for subgroup CG and 27.82 mm (24.9-30.4) for subgroup CT. Again, the difference across all three subgroups was not statistically significant (p>0.9).

The median (min-max) was 20.4 mm (16.5-22.9) for Group A and 28.1 mm (24.1-30.4) for Group C. The difference was statistically significant (p=0.0001).

Testicular artery diameter

The median (min-max) testicular artery diameter was 4 mm (2-6) for subgroup AE, 5 mm (2-6) for subgroup AG, and 5 mm (2-6) for subgroup AT. The difference across all three subgroups was not statistically significant (p=0.5).

The median (min-max) testicular artery diameter was 4 mm (2-6) for subgroup CE, 5 mm (2-6) for subgroup CG, and 4 mm (2-6) for subgroup CT. Again, the difference across all three subgroups was not statistically significant (p=0.4).

The median (min-max) was 4 mm (2-6) for Group A and 5 mm (2-6) for Group C. The difference was not statistically significant (p>0.9).

Thread length

Median (min-max) length of the suture material used in the open technique was 7.5 (2.5-10) cm for Group A and 11 (6.5-13.5) cm for Group A-T. The difference was highly statistically significant (p=0.0008). For Group C-G, median (min-max) length of the suture material used was 8.8 (4-13), whereas for Group C-T it was 13.2 (8-19). Again, the difference was highly statistically significant (p=0.004).

Leaking pressure across subgroups

In open castration, median (min-max) leaking pressure was 294.20 (220.65-713.44) mmHg when using the Serra emasculator alone (Group A-E), 544.28 (382.47- 706.09) mmHg when using the emasculator plus a Giant knot (Group A-G), and 514.86 (220.65- 720.80) mmHg when using the emasculator and a transfixing knot (Group A-T). Differences were not considered statistically significant (p=0.06).

In closed castration, median (min-max) leaking pressure was 301.56 (147.10- 720.80) mmHg when using the Serra emasculator alone (Group C-E), 356.72 (205.94- 720.80) mmHg when using the emasculator plus a Giant knot (Group C-G), and 257.43 (147.10- 588.41) mmHg when using the

emasculator plus a transfixing knot (Group C-T). Again, differences were not considered statistically significant (p=0.7) .

Leaking pressure across groups

Median pressure was 514.86 mmHg (220.65-720.8) in Group A and 308.91 mmHg (147.10-735.51) in Group C. The difference was considered statistically significant (p=0.006).

Correlation between leaking pressure and respective sizes of testis, spermatic chord and artery

In Group C, no correlation was evidenced between leaking pressure and testis size (p=0.7), spermatic cord diameter (p=0.2) and artery diameter (p=0.5).

The same was true for Group A where, again, no correlation was observed between leaking pressure and testis size (p=0.7), spermatic cord diameter (p=0.6) and artery diameter (p=0.07).

Tensile load

In closed castration, tensile load (mean±SD) was 1.56±0.926 kg when using the Serra emasculator alone (Group C-E), 2.611±0.957 kg when using the emasculator and a Giant knot (Group C-G) and, finally, 2.611±0.995 kg when using the emasculator and a transfixing knot (Group C-T). Differences were not regarded as statistically significant (p=0.2).

Discussion

Although no statistically significant differences were evidenced in the results, a positive trend is found in relation to ligature placement, for both intra-arterial pressure and parietal tunic tensile strength (when present).

Our study showed that the Giant knot is as efficient as (and even more efficient than) a modified transfixing ligature in achieving haemostasis in open castration, although it should be noted that the latter is superfluous when a Serra emasculator is applied. In closed castration, a ligature with Giant knot can provide valuable support in achieving haemostasis but does not significantly increase the tensile strength of the tunica vaginalis. What is more, the reduced amount of suture material left *in situ* might also reduce the risk of possible complications related to the ligature itself.

Across all subgroups, no statistically significant differences were found in relation to the respective sizes of the testis, the spermatic cord and the artery, thus ruling out a possible confusing factor related to the sizes of the latter two elements. *Par contra*, a statistically significant difference was found across groups in relation to the size of the spermatic cord and to leaking pressure. This suggests that a larger tissue area correlates with a reduced haemostatic capacity of the emasculator (when used alone or in combination with a given ligature).

As it uses only one surgeon's knot for wound closure, a transfixing ligature needs at least four half-hitches amounting to 6 throws in total. This results in more suture material being used and then left *in situ* [10]. Although in literature the addition of 3 half-hitches is recommended to ensure optimal security of Giant knots [14], we decided to use one half-hitch only taking into account preliminary results. The tensile strength thus obtained was way different from physiological pressure. In addition, during the investigation none of the knots slipped out before the dye material started to leak from the distal stump. We can therefore conclude that a Giant knot allows for fewer half-hitches to ensure adequate tensile strength, resulting in a reduced amount of suture material left *in situ*.

In open castration, our results indicate that tensile strength is equivalent irrespective of the technique being used. In closed castration, the application of the Serra emasculator either alone or in combination with a transfixing ligature, was able to withstand very low intra-arterial pressures approximating physiological values [17]. This may provide an explanation for the occurrence of post-operative

haemorrhage when using this technique. In closed castration, parietal tunic tensile strength was also higher when using a ligature as compared to the emasculator alone. However, no statistically significant differences were observed when comparing values, which may suggest that the application of ligatures may provide added safety but is by no means mandatory for the purpose of reducing the risks of postoperative evisceration. As noted earlier, the Giant knot also allows for a reduced quantity of suture material being left in the animal's body, thus minimising the risk of complications related to the presence of ligatures [3, 4, 2].

Rijkhenuizen et al. [6] recommend using a monofilament material and a transfixing knot as the ligation method of choice in castration. However, recent studies on monofilament sutures have demonstrated that flat knots assume a sliding conformation when held under tension [18]. This in turn generates stress on the suture, which breaks precisely at the point in which the configuration of the knot changes [19, 20] . For these reasons, starting off using a slip knot is considered far more beneficial given that tensile strength in this case has proved equal or even superior to that of a transfixing ligature closed with a surgeon's knot.

As regards parietal tunic tensile strength, our findings do not appear to corroborate previous results reported in the literature [21, 22]. Until recently, it was thought that using the emasculator alone would not guarantee adequate tensile strength to prevent possible evisceration and therefore a ligature was required. Regarding the haemostatic capacity of the emasculator used alone, the application of a ligature to increase haemostasis should be considered an effective measure in closed castration. Again, this contrasts with previous evidence [1, 2] probably due to the fact that cutting a larger tissue area reduces the haemostatic capacity of the emasculator.

Knot security ultimately depends not only on the chosen technique but also on the type and size of suture material and on the number of half-hitches [23]. A slip knot requires less tension to be applied on the suture strands to ensure a satisfactory haemostatic effect. This allows for smaller gauge,

monofilament sutures to be used, thus reducing knot volume and minimising any risks of infection related to the material itself [24].

In our study, using monofilament Glycomer 631 (USP 0) ensured adequate haemostasis and parietal tunic tensile strength, with no lacerated tissues and an optimal knot strength [6, 24]. A further advantage of slip knots is that they can be applied over the emasculator [22] once the latter has been applied on the spermatic cord, resulting in an improved operative comfort for the surgeon especially during field castration.

This study is not free of limitations. A first area of possible concern lies in its in-vitro configuration, which made it clearly impossible to account for any tensions applied on the sutures and on the emasculation site by the actual movement of the animal in the post-operative period, as well as any oedemas or tissue inflammation that might reduce the haemostatic effect of the chosen technique. In open castration, using the emasculator alone may be sufficient to achieve haemostasis. A ligature may provide additional security to the surgeon but is in no way essential for optimal results. In closed castration however, using a ligature in combination with the emasculator is to be considered mandatory for the purpose of inducing haemostasis, probably because the larger tissue area between the jaws of the emasculator limits its efficacy.

When selecting the type of ligature, a Giant knot offers more advantages in terms of tensile strength and also helps reduce the quantity of suture material required, thus keeping to a minimum the risk of complications related to the presence of ligatures. For these reasons, in closed castration procedures we recommend applying a ligature with Giant knot using monofilament suture material so as to reduce the risk of complications.

Manufactures' addresses

[a] Kruuse, Langeskov , Denmark

[b] Covidien , Brunn Am Gebirge, Austria

[c] ImageJ , NIH , Bethesda , Usa

[d] Terumo , Tokyo , Japan

[e] Kern&Sohn, Balingen, Germany

[f] Graphpad , La Jolla, CA

Table 1. Summary of results. For normally distributed data we report the mean±SD. For non-normally distributed data we report the median (min-max). When like superscript characters appear, a statistically significant difference is indicated.

Reference

1. Kilcoyne I (2013) **Equine castration: A review of techniques, complications and their management**. *Equine vet. Educ.* **25** (9) , 476-482

2. Kilcoyne I , Watson JL, Kass PH, Spier SJ (2013) **Incidence, management, and outcome of complications of castration in equids: 324 cases (1998–2008).** *J Am Vet Med Assoc* **242 ,** 820-825

3. Schumacher J. (1996) **Complication of castration**. *Equine vet. Educ.* **8***(5) ,* 254-259

4. Moll HD, Pelzer KD , Pleasant RS , Modransky PD , May KA (1995) **A Survey of equine castration complications**. *J Equine Vet Sci* **15** , 522-526

5. Schumacher J (2012) Testis . In *Equine Surgery* , 4nd edn., Ed: Auer JA and Stick JA. Elsevier Saunders, St. Luis , pp 804-839

6. Rijkhenuizen A.B , Sommerauer S. , Fasching M. , Velde K , Peham C (2013) **How securely is the testicular artery occluded by using a ligature ?** . *Equine Vet J* **45** , 649-652

7. Fleega BA , Sokkar SH (1999) **The Giant Knot** : **A new One-Way Self-Locking Secured Arthroscopic Slip Knot** . *Arthroscopy* **15** , 451–452

8. Banieghbal B (2009) **Extracorporeal " Giant" Locking Sliding Knot Pediatric and Neonatal Minimally Invasive Surgery : Indications , Techniques , and Outcomes**. *Journal Laparoendosc Adv Surg Tech A* **19** , 831-834

9. Botet X , Surinyach S , Molinas J (2005) **A New Knot Technique for Vessel Ligatures**. *World J. Surg***. 29** , 1356-1358.

10. Muffly TM, Kow N, Iqbal I , Barber MD (2011) **Minimum Number of Throws Needed for Knot Security** *J Surg Edu* **68** , 130-3

11. Muffly TM , Cook C , Distasio J, Bonham AJ , Blandon RE (2009) **Suture End Length as a Function of knot Integrity** *J Surg Edu* **66** , 276-80

12. Masked for review**.**

13. Rumbaugh ML, Burba DJ, Natalini C, Hosgood G and Moore RM (2003) **Evaluation of a Vessel-Sealing Device for Small Intestinal Resection and Anastomosis in Normal Horses** *Vet Surg* **32**,574-579.

14. Kim SH , Yoo JC , Wang JH , Choi KW , Bae TS , Lee CY (2005) **Arthroscopic Sliding Knot: How Many Additional Half-Hitches Are Really Needed?** . *Arthroscopy* **21**, 405-411

15. Harold KL, Pollinger H, Matthews BD , Kercher KW , Sing RF , Heniford BT (2003) **Comparison of ultrasonic energy , bipolar thermal energy, and vascular clips for the haemostasis of small-, medium -, and large-sized arteries .** *Surg Endosc* **17,** 1228-1230

16. Rajbabu k , Barber NJ , Choi W , Muir GH (2007) **To knot or not to knot ? Sutureless haemostasis compared to the surgeon's knot.** *Ann R Coll Surg Engl* **89 ,** 359-362

17. Bright Mcintosh J , Marr CM. (2010) Introduction to cardiac anatomy and physiology. In :*Cardiology of the Horse ,* 2nd edn. *,* Ed: Marr CM and Bower MI. , Elsevier , China.
pp 14

18. Davis DA, Pellowski DM, Rawdon EJ (2013) **All Monofilament Knots Assume Sliding Conformation In Vivo.** *Dermatol Surg* **39** , 729-733

19. Aanning HL, Hass T, Jorgensen DR (2007) **Square Not a Running Knot.** *J. Am. Coll. Surg.* , **204** , 422-425

20. Aanning HL, Van Osdol A, Allamargot C, Becker BE, Howard TC, Likness ML, Merkwan CE, Tarver DD (2012) **Running sutures anchored with square knots are unrieable** . *Am J Surg* , **204** , 384-388

21. Shoemaker R , Bailey J , Janzen E , Wilson DG (2004) **Routine castration in 568 draught colts: incidence of evisceration and omental herniation**. *Equine vet. J.* **36** , 336-340

22. Carmalt JL , Shoemaker RW , Wilson DG (2008) **Evaluation of common vaginal tunic ligation during field castration in draught colts**. *Equine vet. J.* **40** , 597-598

23. Ivy JJ , Unger JB , Hurt J , Mukherjee D (2004) **The effect of number of throws on knot security with nonidentical sliding knots.** *Am. J. Obstet. Gynecol.* , **191** ,1618-1620

24. Kummerle JM (2012) Suture materials and pattern. In: *Equine Surgery*, 4nd edn., Ed: Auer JA and Stick JA. ,Elsevier Saunders, St. Luis . pp 182-189.

Supplementary information items

Fig. 1 : The intravenous catheter partially fed along the internal trocar and clamped with the mosquito forceps- note free flowing of fluid through the clamped catheter and trocar.

Fig. 2 : The system inserted into the ligated testicular artery