Closed Inguinal Castration technique in horses compared with Field Castrations using post-operative Serum Amyloid A analysis.

Dirk Riemersma[1], Gerold Fahlbusch[1], and Astrid Rijkenhuizen[2]

[1]Pferdepraxis Den Heyberg
[2]Equine surgery consultancy

July 26, 2023

Abstract

Introduction: A closed inguinal castration technique was compared with field castrations based on the post-operative (p.o.) inflammatory marker Serum Amyloid A (SAA). Third day p.o. SAA, which has been related to trauma, has been reported after field castrations in four different studies in literature between 500 mg/l and 700 mg/l. These values were compared with SAA values measured on day three after routine castration using a closed inguinal approach applied under general anaesthesia under strict aseptic conditions. Materials and Methods: 51 Male horses were presented for routine castration. Serum was collected pre-operatively and on day 3 post operatively. Castration was performed using a closed inguinal approach in dorsal recumbence under general anaesthesia. The vaginal process was retrieved by blunt dissection over an inguinal skin incision. The closed proximal vaginal process was crushed by a Sand emasculator and ligated at this site. Vaginal Process with content was cut off 10 mm distal to the ligated site. Skin incisions were closed intra cutaneously. Antibiotics or anti-inflammatory drugs were not applied. Results: 48 horses tested negative (SAA< 3mg/l) pre-operatively. Two ponies and one standardbred showed pre-operative 5mg/l, 7mg/l and 75mg/l respectively. Mean SAA value of all 51 horses, three days post operatively, measured 94mg/l. 27 Horses showed no elevation in SAA (<3mg/l). Only 8 horses showed SAA values >100mg/l, of which 4 horses > 500mg/l, (514mg/l, 747mg/l, 1110 mg/l and 1160 mg/l) possibly related to additional trauma. Excluding these 4 cases results in a mean SAA value of 27mg/l. Conclusion: Castrations, using a closed inguinal approach, in which proper surgical standards can be maintained, proved to be far less traumatic than field castrations.

Summary

The closed inguinal approach in equine castration is a technique in which optimal surgical standards can be maintained. The inflammatory response to this method of castration was quantified by Serum Amyloid A (SAA) measurements to be 94 mg/l. on day three after surgery. The SAA value after field castrations, using a scrotal approach, was reported in literature by several authors to be between 480 - 708 mg/l on day three post-operatively (p.o.). The difference in SAA levels between these different approaches proved to be highly significant, quantifying a highly significant lower inflammatory response of the closed inguinal technique over regular field castrations using a scrotal approach.

Introduction

Castration of the male horse is the most frequent routine equine surgical procedure, traditionally performed in practice, referred to as field castrations. Reported complication rates for field castrations range from 10% to 48% (Moll et al.1995, May and Moll 2002, Mezerova et al. 2004, Mason et al. 2005, Kilcoyne et al. 2013a). The wide spread in reported complication rates may depend on variable field and stall conditions, differences in surgical experience and in applicable equipment and medication, differences in p.o. care and patient observation and differences in definition and recognition of complications. The majority of these complications, however, result from contamination (i.e. acute and chronic infections) and/or insufficient

ligation (i.e. p.o. haemorrhage and eventrations). The contamination risk in field castrations requires intra- and p.o. antimicrobial treatment, and inflammatory responses are usually anticipated by anti-inflammatory medication. Castrations performed under acceptable surgical conditions show significant less complications than castrations performed under variable sub optimal field conditions (Mason, 2005). Castrations in this study were performed under optimal surgical conditions using a closed inguinal approach. The complication rate using this technique has been reported previously (Riemersma, 2005) to be 2% (11 out of 554 cases), without the routine use of antimicrobial or anti-inflammatory medication. The complication rate may be indicative for the quality of the surgical procedure, but the wide range of complications reported in literature makes this parameter less suitable for quantification of the quality of a surgical procedure, which is determined by its intended outcome and its p.o. inflammatory response caused either by trauma or by infection.

SAA proved to be the most sensitive acute phase reactant associated with inflammation after field castrations using a scrotal approach in either standing (Jacobsen et al.2005, Busk et al.2010), or recumbent horses (Haucke et al.2017, Bergstrom et al. 2021). Each of these authors compared 2 groups of horses: horses with or without post- operative fever (Jacobsen et al.2005), horses with or without peri-operative administration of Penicillin (Busk et al.2010), horses receiving a single dose of penicillin or a triple dose on three consecutive days (Haucke et al.2007) and horses receiving either Ceftiofur or Procaine penicillin intra-operatively (Bergstrom et al. 2021). Infection may not always be clinically evident until the fourth day after castration (Kilcoyne 2013b) and it has accordingly been stated by Jacobsen et al. (2005) and Haucke et al. (2007) that the SAA levels up to the first three days after surgery would rather be indicative for surgical trauma, unlike SAA levels recorded several days up to several weeks postoperatively which would be related to infection. Average SAA levels recorded at day three after field castration, have been reported to be 480-570 mg/l (Jacobsen et al. 2005), 543-708 mg/l (Busk et al. 2011), 698 mg/l (Haucke et al. 2017) and 570-608 mg/l (Bergstrom et al. 2021). These SAA levels, although recorded after different studies in different countries and even different continents, show remarkable resemblance in magnitude.

Hypothesis.

It is hypothesized that the third day p.o. value of the inflammatory marker SAA using a closed inguinal approach will be significantly lower than the values which have been presented in literature recorded at day three after field castrations.

Materials and Methods.

4.1 Surgical Technique

The closed inguinal approach is performed under general anaesthesia and strict aseptic conditions (disinfection, draping, using gloves and gowns etc.). A 5-10 cm skin incision is made over the palpable outer inguinal portal. The underlying vaginal process, containing Testis, Epididymis, Ductus deferens, testicular artery and vein forming the Plexis pampiniformis distally and their mesenteries, is approached by strict blunt dissection using Mayo scissors in spreading fashion and digital separation of tissue. The vaginal process is hooked by a finger and retrieved by gently pulling and some pushing on the scrotum. The remaining attachment to the scrotum by remnants of the Gubernaculum is separated by disruption. A Sand emasculator is applied to the closed proximal process at the level of the skin incision and the crushed site (Fig.1) is ligated with a double strand nr.4 metric Polyglactin 910, which both, ligates vascularity and closes the vaginal cavity permanently. The distal part of the vaginal process including content is cut by scissors 10 mm distal to the ligature and the combined weight of the left and right removed tissue was measured. The proximal vaginal process including pedicles is checked for haemorrhage and released. The skin wound is closed by Nr.3 metric Polyglactin 910 or Nr. 3 metric Poliglecaprone 25 in a double layer sub- and intra-cutaneous continuous suture and sealed by a sterile wound spray (CE 0124, Beiersdorf).

4.2 Surgical patients

The closed inguinal castration procedure was performed on 51 colts and stallions (34 Warm bloods, 8 Ponies, 6 Standardbreds, 2 Quarter horses and 1 Thoroughbred) admitted as routine patients to be castrated with

full consent of the owners. Mean age of the horses was 3.6 years, ranging from 0.8 years to 19.7 years (Fig.2).

4.3 Serum Amyloid A measurements

Serum was taken from the patients prior to surgery for estimating their basic SAA level. A second probe was taken on the third day after surgery. The SAA levels were measured photometrically by a certified commercial veterinary laboratory (SYNLAB, Leverkusen, GE). Detection level of the method was 3 mg/l. All values <3 mg/l were considered to be negative but were given the value 2 mg/l for calculation reasons.

4.4 Post-operative management:

All horses were checked during three days p.o. for clinical signs of surgical complications such as fever, heart and respiratory rate, oedema of scrotum or prepuce, wound swelling, disruption or -drainage and judgement of post-operative posture and behaviour indicating possible discomfort or pain.

*4.5 Statistical analysis*Data were tested for normal distribution using Kolmogorov-Smirnov Test.

Possible correlations between SAA, age and weight of removed tissue was tested either using the variance analysis for normal distributed data, or using the non-parametric Jonckheere-Terpstra-test for not normal distributed data. Possible cross correlations were tested using Spearman Rho test. Significance of differences between the resulting mean SAA values from this study and the mean SAA values derived from literature for field castrations was tested using a paired T-test. The effect size statistic Cohen's d was calculated as a measure of relevance of the tested differences in third day SAA between the closed inguinal castration and the field castrations reported in the literature. Where applicable, significance was tested two-sided at significance level P<=0.05.

Results.

48 Horses tested negative (SAA< 3mg/l) pre-operatively. Two ponies and one Standardbred showed a pre-operative SAA level of 5mg/l, 7mg/l and 75mg/l respectively. 50 Patients recovered from surgery without showing clinical signs of surgical complications. One horse showed a unilateral incisional infection three days after surgery.

P.o. SAA values ranged from 0 (<3) mg/l to 1150 mg/l (Fig 3). The mean value of p.o. SAA is 94 mg/l. The results are separated in three groups of horses based on the value of SAA (Table 1),**Negative** : SAA 0-2mg/l (26 horses, 51%), **Moderate** : SAA 3-100 mg/l (17 horses, 33%) and **High** : SAA >100mg/l (8 horses,16%). Four horses in the High-group (8%) showed a SAA value over 500mg/l (Table 2), which strongly influence the calculated mean, being 27 mg/l without these values.

The data for age (Fig.2) and p.o. SAA (Fig.3) did not fulfil the criteria for a normal distribution. The weight of removed tissue (Fig.4) showed an acceptable normal distribution. Comparison between p.o. SAA and age and removed tissue weight (Fig.5, Fig.6 and Fig.7) showed a moderate but significantly (P=0,015) higher p.o. SAA in older horses. No statistical significance was shown between p.o. SAA and weight of removed tissue (P= 0,531). A not very surprising significant (P=<0,002) correlation was found between age and weight of removed tissue (larger reproductive organs in mature horses). All other possible correlations did not prove statistically significance.

The third day p.o. SAA data using the closed inguinal approach was tested highly significant lower (P<0,001) than the data recorded in the four previously referred different field castration studies (Table 3). This is true for each of eight groups of horses described in the field castration studies (2 groups in each study). Calculated **Cohen's *d*** ranged from 1.11 to 2.75 (Table 3)

Conclusion.

The results support the hypothesis that the third day p.o. value of SAA after castration using the closed inguinal approach is significantly and relevantly (Cohen's d >>0.8) lower than the third day p.o. values of SAA after field castrations, indicating that the closed inguinal castration is a significant and relevant less traumatic surgical procedure than a field castration.

Discussion.

The inflammatory response to a surgical procedure is caused by trauma and infection and determines the quality and duration of the p.o. healing process. For this reason, modern equine surgery relies on minimal invasive techniques whenever possible. Since Valdez et al. (1979) presented a minimal invasive inguinal approach for cryptorchidectomy (Turner and McIlwraight 1989, Adams and Fessler 2000) it was a logic step to develop an inguinal approach for normal castrations over unilateral cryptorchids (Sedrish and Leonard 2001). Although the advantages of an inguinal approach have been presented (Keller et al. 1996, Sedrish and Leonard 2001, Riemersma 2005, Kummer et al. 2009) a scrotal approach is still the standard in field castrations. The closed inguinal castration is a minimal invasive surgical procedure. Despite the renounce of anti-inflammatory medication, the inflammatory response of the closed inguinal castration, applied in this study, quantifies to be 5 to 7.5 times less than in field castrations; 51% showing no elevation in SAA at all (Table 1). The four horses showing extreme values, comparable with average field castrations (>500 mg/l) in this study may have suffered additional trauma (Table 2). There may be arguments to exclude these patients from the study, but the high impact on the mean value could be considered to bias the objective results. It is also possible that comparable patients may have been included in the reported field studies.

Apart from a significant lower contamination under acceptable surgical conditions, the inguinal approach also provides, in comparison with the scrotal approach, a more secure ligation on the proximal vaginal process, which is thinner on this site than the more distal parts, where the developing of the Plexus pampiniformis increases vascular complexity and tissue diameter (Fig.1).

The closed inguinal approach by its technique therefore eliminates the two main sources of complications in field castrations being contamination and insecure ligation. Apart from this advantage, there is also a strong indication that the closed inguinal approach is less traumatic. Although it remains to be proven whether the third day p.o. SAA level solely depends on non-septic inflammatory causes, it is feasible that at least a considerable influence of traumatic origin will contribute to these SAA values. Love et al. (2009) showed that field castrations in ponies resulted in altered behaviour indicating p.o. pain first day after surgery. This indicates an inflammatory response to trauma, since most infections become usually clinically significant some days after surgery (Kilcoyne 2013b), especially when p.o. treated with antimicrobial and anti-inflammatory medication. Whereas p.o. oedema of Scrotum and Prepuce is common after field castrations (Kilcoyne 2013b), it is extremely rare (<1%) after the castration by a closed inguinal approach (Riemersma, 2005). Less trauma may have its origin in the better surgical conditions under general anaesthesia, but may additionally depend on the difference in technical approach being either inguinal or scrotal and the difference between a true closed procedure or a procedure in which the vaginal tunic is opened, entering the peritoneal cavity. The results of this study show no correlation between the SAA and the mass of removed tissue, representing the size of the reproductive organs. This implies that retrieval of the complete vaginal process does not contribute to the SAA level regardless of the size of the organs and will therefore be minimal invasive. It has been stated that some haemorrhage (a possible source of inflammation) is normal in field castrations (Kilcoyne 2013b), but it is extremely rare in the inguinal approach (Fig.1). This is explained by not disturbing significant vascular supply while the vaginal process is surrounded by the loose connective tissue of the Tunica dartos. Even disruption of the vascular supply of the Gubernaculum remnant does not cause haemorrhage in the fast majority of cases. The only remaining trauma would be from the small skin incision over the outer inguinal area and the blunt separation of the fat containing loose connective tissue to reach the vaginal process. The closed inguinal approach additionally represents a true closed procedure and prevents any blood spilling or other contamination into the peritoneal cavity, which is known to be a source of peritoneal inflammation (Schumacher et al. 1988). The absence of a potentially irritating intraperitoneal ligature may be another advantage of the closed inguinal approach.

Although the scrotal incision provides an easy access to the Testis, the site of ligation of the more proximal located vaginal process may require some more dissection to be reached, and may therefore result in increased tissue trauma. This could be truer in colts and stallions with larger Testes, which may explain a correlation between Testis size and complication rate for scrotal castrations (Birrell et al. 2020). Each additional

handling of tissues may also increase the risk of intra-operative contamination, which is the actual reason for omitting a wound closure in field castrations. The larger diameter of the distal vaginal process may hamper the proper application of an emasculator in closed fashion in many occasions (Rutgers and Merkens 1983, Kilcoyne 2013b), especially in combination with an intra-testicular anaesthesia, which results in enlargement and stiffening of the distal tissues. Therefore, using a scrotal approach often requires opening the vaginal process and pulling the internal organs (Testis, Epididymis, Plexus pampiniformis) and mesenteries distally prior to intra- or extra-vaginal ligation of testicular vascularisation (Rutgers and Merkens 1983). The vaginal process may be closed consecutively (Rutgers and Merkens 1983, Kilcoyne 2013b) but this will not prevent contamination of the peritoneal cavity with blood or tissue or the irritation of an intra-peritoneal ligature, initiation a non-septic peritonitis (Schumacher et al. 1988). These additional inflammatory responses may explain an increase in SAA value in field castrations.

The closed inguinal approach has the advantage over a field castration to be a minimal invasive castration technique without opening the peritoneal cavity. The routine intra- or p.o. medication can be omitted in the closed inguinal approach which may show the public the responsible professionalism of veterinarians in terms of preventing antibiotic resistance (Bowen, 2013). Return time to full training after a closed inguinal castration is less than 7 days, instead of several weeks after field castrations, which is highly appreciated by professional trainers. The closed inguinal approach is a fast procedure, requiring about 45 minutes from induction to recovery and an actual time in surgery of about 20 minutes. The inguinal procedure may not even be more expensive than a field castration which requires travelling, on site preparations and p.o. patient checks and treatments, which encourages many local practitioners to refer castrations of mature horses to the authors clinic.

An inguinal approach may be applied in practice as well, and has been practiced by the first author, if recumbent restraint and intra-operative contamination can be controlled. An inguinal approach will technically be impossible or extremely complicated in the standing horse, which leads to the recommendation that the standing castration should only be applied after full consent of the owner concerning a significant more traumatic surgery (this paper) associated with a considerable higher complication rate than a recumbent castration (Mason, 2015).

Acknowledgement

We thank Dr. Monika Heinzel-Gutenbrunner of MH Statistical Consultancy (http://www.Statistik-Beratung.net) for her professional statistical analysis of the data. Dr. Heinzel-Gutenbrunner is statistician on the Philipps-University of Marburg, Germany.

References

1. Adams SB, Fessler JF: Noninvasive inguinal cryptorchidectomy. In: Atlas of equine surgery.WB Saunders, 2000, ISBN 0-7261-4643-3: 215-218
2. Bergstrom GT, Frey M, Rao S, Bass LD. (2021) Comparison of p.o. inflammatory response in horses undergoing elective castration treated pre-operatively with Ceftiofur crystalline free acid or Procaine penicillin. Equine veterinary Education (2021), DOI:10.111/eve.13444
3. Birrell JR, Schulman ML, Alma, E. Botha AE, Ganswindt A, Fosgate GT, Bertschinger HJ. (2020) Vaccination against GnRH as a prelude to surgical castration of horses. Equine Vet J. DOI: 10.1111/evj.13411
4. Bowen, M. (2013) Antimicrobial Stewardship: a time for change. Equine Vet. J. 45, 127-129.
5. Busk P, Jacobsen S, Martinussen T. (2010) Administration of perioperative Penicillin reduces p.o. Serum Amyloid A response in horses being castrated standing. Veterinary Surgery 39, 638-643.
6. Haucke K, Kuhn M, Lübke-Becker A, Mählmann K, Lischer C (2007) Two regimes of perioperative antimicrobial prophylaxis for equine castration: Clinical findings, acute phase proteins and bacterial cultures. Journal of Equine Veterinary Science. DOI: 10.1016/j.jevs.2017.07.001
7. Jacobsen S, Jensen JC, Frei S, Jensen AL and Thoefner MB (2005) Use of Serum Amyloid A and other acute phase reactants to monitor the inflammatory response after castration in horses: a field study.

Equine Vet. J. **37** (6) 552-556.

8. Keller H, Elker M, Schad D. 1996) Hengstkastration mit inguinale Schnittführung und primären Wundverschluß. (Castration of the stallion using an inguinal incision and primary wound closure). Prakt. Tierarzt 77 (10): 890-895.
9. Kilcoyne I, Watson JL, Kass PH and Spier SJ. (2013a) Incidence, management, and outcome of complications of castration in equids: 324 cases (1998–2008). J. Am. Vet. Med. Ass. 242, 820-825.
10. Killcoyne I (2013b) Equine castration: A review of techniques, complications and their management. Equine vet. Edu. 25 (9), 476-482
11. Kummer M, Gygax D, Jackson M, Brettschart-Wolfensberger R, Fürst A. (2009) Results and complications of a novel technique for primary castration with an inguinal approach in horses. Equine Vet J 41 (6): 547-551
12. Love J, Taylor PM, Clark C, Whay HR and Murell J (2009) Analgesic effect of Butorphanol in ponies following castration. Equine Vet J. 41 (6) 552-556. DOI: 10.2746/042516409X391024
13. Mason BJ, Newton JR, Payne RJ and Pilsworth RC (2005) Costs and complications of equine castration: a UK practice-based study comparing ,,standing nonsutured" and ,,recumbent sutured" techniques. Equine vet.J. 37 (5) 468-472
14. May, K.K. and Moll, H.D. (2002) Recognition and management of equine castration complications. Comp. Cont. Educ. Pract. Vet. **24** , 150-162.
15. Mezerova J, Krupil Z, Schillova B, Zert Z. (2004) Kastration des Hengstes – primare Wundheilung und Komplikationen. (Castration of the stallion - Primary wound healing and complications). Prakt.Tierarzt**85** (1), 28-36.
16. Moll HD, Pelzer KD, Pleasant RS et al (1995): A survey of equine castration complications. J. of Equine Vet Sci **15** , 522-526.
17. Riemersma DJ. (2005) Complication rate of castration of the horse using an inguinal approach in 554 cases. Proc. ECVS 14th Ann. Sci. Meeting: 162-165.
18. Rutgers LJE, Merkens HW. (1983) Ervaringen met de halfbedekte castratie van de hengst (Experience with the open/reclosed castration of the stallion). T.v.D.. 108 (18): 705-711.
19. Sedrish SA, Leonard JM. (2001) How to perform a primary closure castration using an inguinal incision. Proc. AAEP **2001** ; 47:423-425.
20. Schumacher J, Schumacher J, Spano JS, et al. Effect of castration on peritoneal fluid in the horse. J Vet. Int. Med. **2** , 22-25.
21. Turner AS, McILWraight CW. (1989) Cryptorchidectomy by the noninvasive inguinal approach. In: Techniques in large animal surgery, 2nd ed.Lea & Febiger, ISBN 0-8121-1177-X: 185-191
22. Valdez H, Taylor TS, Mc Laughlin SA, et al (1979) Abdominal cryptorchidectomy in the horse using inguinal extension of the gubernaculum testis. J AM Vet Med Assoc 174:1110

Table 1 Results Serum Amyloid A 51 horses subdivision in 3 groups

Nr. of horses Group mean Age (sd) mean SAA SAA range mean Sum of Weight

26 Negative 2.15 (1.08) <3 0 - 2 547 (229)

17 Moderate 3.12 (1.54) 24 7 – 69 553 (196)

8 High 6.63 (6.70) 535 111 – 1160 655 (270)

Legend of Table 1

Data of horses divided in three groups of post-operative Serum Amyloid A (SAA) depending on SAA value: Negative, Moderate and High, presenting number of horses, mean age and Standard deviation, mean value and range of SAA, Sum of Weight of excised tissue and standard deviation for each of the three groups.

Table 2 Four horses with extreme SAA values

Breed Age SAA pre-OP SAA post-OP Remarks

Standardbred 3 yr 75 mg/l 514 mg/l Possible orthopaedic injury

Riding pony 2 yr < 3 mg/l 747 mg/l Violent character, unilateral Cryptorchid

Welsh Cob 9 yr < 3 mg/l 1110 mg/l p.o. unilateral incisional infection

Warmblood 4 yr < 3 mg/l 1160 mg/l Incisor removal, tetanus vaccination

Legend of Table 2

Additional data of four horses with extreme high values of p.o. Serum Amyloid A (SAA): presenting breed, age (in year, yr), pre-operative SAA values, post-operative SAA values and some possible correlations.

Table 3 3rd day p.o. SAA Closed Inguinal Approach versus Field Castration

Literature: N1 s1 Mean SAA t df P-value P Cohen's d

Jacobsen (2005) 1 11 68 458 mg/l 4.75 60 0.00000666 <0.001 2.75

Jacobsen (2005) 2 7 67 550 mg/l 4.75 56 0.00000726 <0.001 2.71

Busk (2010) 1 24 370 543 mg/l 6.18 73 0.00000002 <0.001 1.42

Busk (2010) 2 26 428 708 mg/l 7.94 75 0.00000000 <0.001 1.75

Haucke (2017) 1 24 151 664 mg/l 10.27 73 0.00000000 <0.001 1.98

Haucke (2017) 2 23 216 729 mg/l 10.50 72 0.00000000 <0.001 2.48

Bergstrom (2021) 1 33 479 608 mg/l 6.43 82 0.00000000 <0.001 1.34

Bergstrom (2021) 2 32 608 570 mg/l 5.35 81 0.00000040 <0.001 1.11

Legend of Table 3

Post-operative mean Serum Amyloid A (SAA) values of 51 horses castrated by the Closed Inguinal Approach in comparison with mean SAA values of eight groups of horses castrated in Field Castrations (FC) using a paired T-test. Representing author of respective FC study, number of horses in FC study (N1), standard deviation (s1), mean SAA (mean1), test value (t), degrees of freedom (df), calculated probability (P-value), significance level (P), Cohen's d.

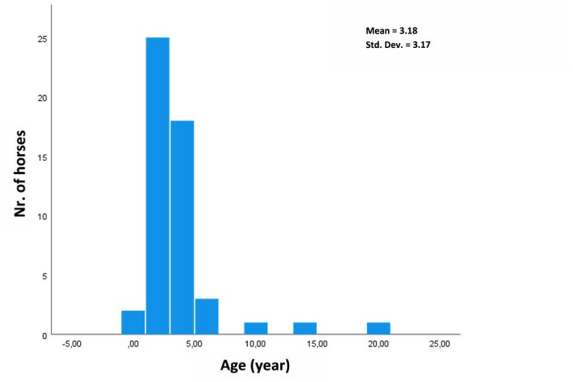

Fig.2

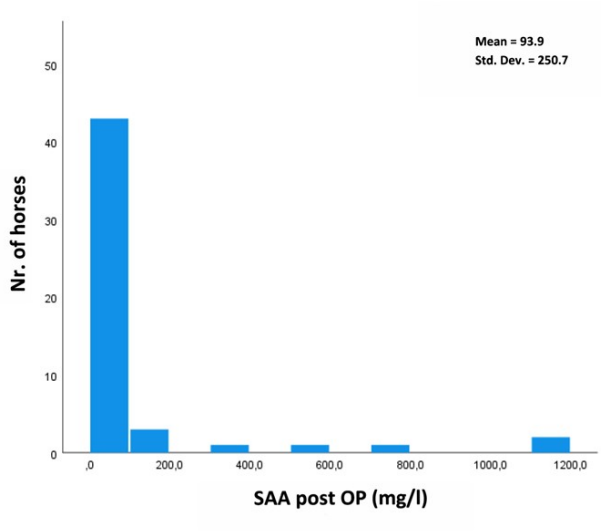

Fig. 4

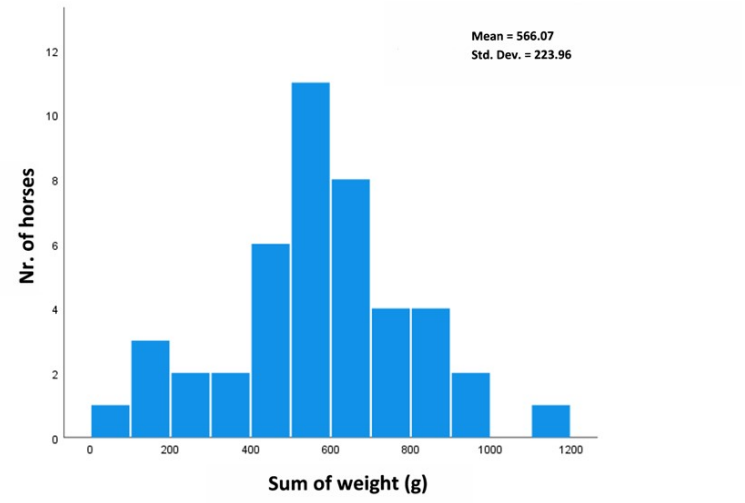

Fig. 3

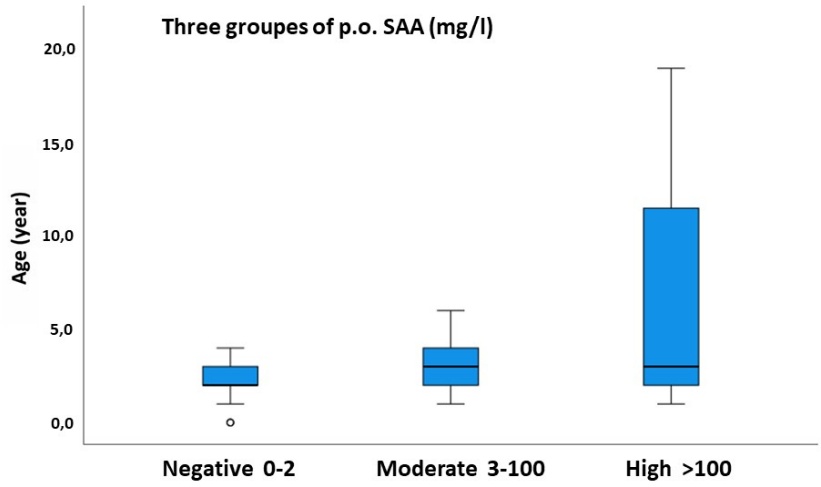

Fig. 6

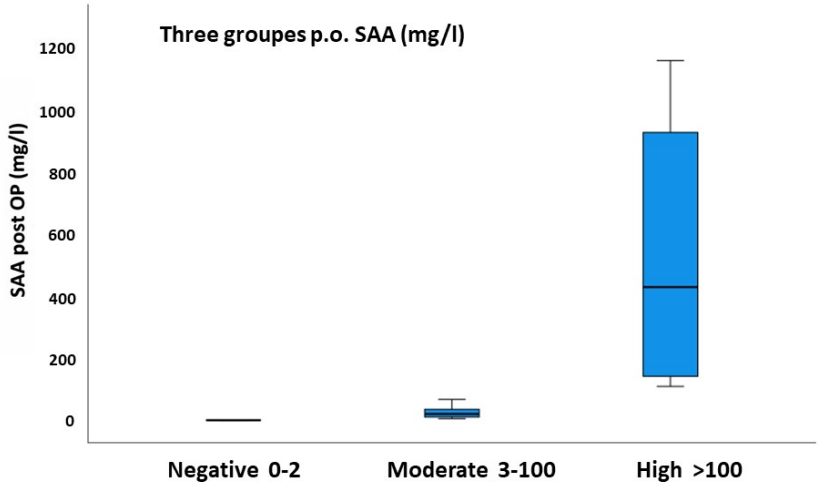

Fig. 7

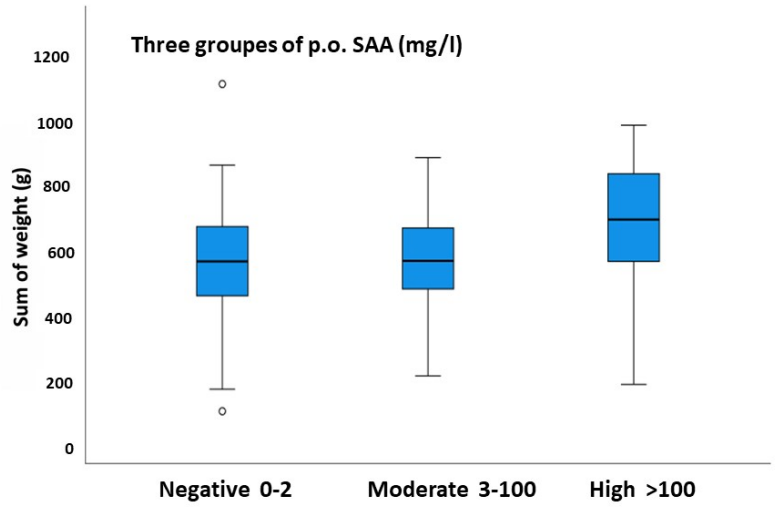

Fig. 5