Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2025:23
Faculty of veterinary medicine and animal science

The hunt for the perfect castration in horses

- techniques and tissue reactions

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DOCTORAL THESIS
Uppsala 2025

Acta Universitatis Agriculturae Sueciae
2025:23

Cover illustration: Photo by Linda Johansson

ISSN 1652-6880
ISBN (print version) 978-91-8046-458-1
ISBN (electronic version) 978-91-8046-508-3
https://doi.org/10.54612/a.41qeb963eg

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Print: SLU Grafisk service, Uppsala 2025

The hunt for the perfect castration in horses - techniques and tissue reactions

Abstract

Castration is frequently performed in stallions, primarily for husbandry purposes. To ensure animal welfare, any discomfort during the procedure must be minimised. However, castration techniques and peri- and postoperative management practices vary widely, and the quality of evidence regarding their associated complications is limited. This makes it challenging to make an informed decision regarding the most appropriate method. This thesis aimed to provide evidence-based guidance to veterinarians and horse owners in selecting the optimal castration technique for individual stallions.

Medical records were analysed to assess the castration techniques and perioperative medications used in Sweden. Additionally, the tissue reactions to a resorbable device, designed to facilitate ligation during standing castration, were evaluated using ultrasonography and histology. The extent of tissue trauma associated with a sutured castration technique was objectively measured using a biomarker sensitive to inflammation (SAA).

In ambulatory practice, standing castration using an open, un-sutured technique was the preferred method, and perioperative antimicrobial prophylaxis was associated with a reduced risk of surgical site infection (SSI). In hospital settings, a closed, sutured technique performed under general anaesthesia and sterile conditions was the most common approach. In this setting, antimicrobial prophylaxis did not reduce SSI rates.

The ligation device caused a mild and transient tissue reaction, but its mechanical properties require further refinement before clinical application.

Sutured castration induced an inflammatory response with low SAA-values, minor swelling, and no SSI, indicating that the technique caused minimal surgical trauma and represents a high-quality procedure.

Keywords: animal welfare, antibiotics, antimicrobial resistance, castration, equine, SAA, stallion, technique, tissue response

Jakten på den perfekta kastrationen för häst – tekniker och vävnadsreaktioner

Abstract

Hingstar kastreras ofta, främst av hanteringsmässiga skäl. För att säkerställa god djurvälfärd är det av yttersta vikt att operationen genomförs med minsta möjliga lidande för hästen. Kastrationstekniker samt peri- och postoperativa strategier varierar dock kraftigt, och det saknas kvalitativ evidens om komplikationsfrekvenser kopplade till ingreppet. Detta gör det svårt att fatta välgrundade beslut om vilken metod som är mest lämplig. Målet med denna avhandling är att ge ett evidensbaserat stöd till veterinärer och hästägare för att kunna välja den mest lämpliga kastrationsmetod för varje individuell häst.

Journaler analyserades för att bedöma vilka kastrationstekniker och behandlingsstrategier som används i Sverige. Vävnadsreaktionen kring ett självlåsande band, utvecklat för att underlätta ligering vid stående kastrationer, undersöktes med hjälp av ultraljud och histologi. En inflammationskänslig biomarkör (SAA) användes för att objektivt mäta graden av kirurgiskt vävnadstrauma orsakad av en kastrationsteknik med suturerade sår.

I fältmässig praktik kastrerades hästar oftast stående med en öppen teknik och öppna sår. Perioperativ antibiotika profylax minskade risken för sårinfektion. På hästsjukhusen användes främst en stängd, suturerad teknik, utförd under sterila förhållanden i allmän narkos. Under dessa förhållanden minskade inte risken för sårinfektion med profylaktisk antibiotika.

Ligeringsbandet orsakade en lindrig och övergående vävnadsreaktion, men dess mekaniska egenskaper behöver förbättras innan den kan appliceras kliniskt.

Suturerade kastrationer skapade en inflammationsreaktion med låga SAA värden, minimal svullnad och inga sårinfektioner. Detta tyder på att tekniken orsakar ett minimalt kirurgiskt trauma och att den representerar en högkvalitativ metod.

Keywords: antibiotika, antimikrobiell resistens, djurvälfärd, hingst, häst, kastration, SAA, teknik, vävnadsreaktion

To my family

"Scientific and medical definitions are tools. Even when we recognize them as imperfect or provisional, awaiting replacement by an improved version, they perform work that cannot be accomplished by less precise instruments."

Morris D.B. 2003

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Sjöberg I*, Lundgren J, Syrén K, Wattle O. Equine castration: A retrospective study of postoperative complications in Swedish field and hospital practices (*in manuscript*)

II. Sjöberg I*, Law E, Södersten F, Höglund OV, Wattle O. (2023). A preliminary investigation of the subcutaneous tissue reaction to a 3D printed polydioxanone device in horses. *Acta Vet Scand,* 65 (48),1-11 https://rdcu.be/eaXRX

III. Sjöberg I*, Horn I, Ljungvall K, Andersen PH, Sternberg-Lewerin S. (2025) Influence of antimicrobial prophylaxis in horses undergoing sutured castrations. (*submitted manuscript*)

IV. Sjöberg I*, Ljungvall K, Andersen PH. Serum Amyloid A response to sutured castration with scrotal approach in horses. (*in manuscript*)

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The contribution of Ida Sjöberg to the papers included in this thesis was as follows:

I. Compilation of and interpretation of data, drafting and revising of the manuscript.

II. Study design, acquisition and interpretation of data, manuscript preparation and critical revision of the manuscript.

III. Conception and design of the work, collection and interpretation of the data, drafting and revising of the manuscript.

IV. Conception and design of the work, collection and interpretation of the data, drafting and revising of the manuscript.

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Abbreviations

AN	Anaesthesia
CBL	Coldblooded horse
DV	District Veterinary Organisation
IZ	Inflammatory zone
NI	Non-infectious
PDO	Polydioxanone
PLGA	Poly(lactid-co-glycolic acid)
SAA	Serum amyloid A
SSc	Sum of surgical score
SSI	Surgical site infection
TIR	Tissue inflammatory reaction

1. Introduction

1.1 Castration of horses

Castration of stallions is believed to have been practiced by the Scythians as early as the Iron Age, and the procedure was mentioned by Aristotle around 350 BC (Wikipedia 2025). Castration means that the testicles are removed, and both historically and in modern times, primarily performed for husbandry purposes. Castrated horses, or geldings, are easier to manage and can be kept more safely with other horses. Today, the majority of stallions undergo castrations. Given the number that there are > 6 million horses in Europe (European Horse Network 2010), an estimate suggests that at least 100,000 horses are castrated annually in Europe alone. Thus, castration is the most common surgical procedure performed on horses.

1.1.1 Anatomy

The two testes and epididymides are oval shaped and positioned in the cranio-caudal direction within the scrotum. The scrotal cavity is separated by a sagittal septum, dividing the scrotum into left and right pouches. Each testis is enclosed by the vaginal tunic, which is derived from the abdominal peritoneum (Figure 1). The tunic consists of visceral and parietal layers, with a thin vaginal cavity between them that communicates with the abdomen. The spermatic cord originates from the internal inguinal ring, extends through the inguinal canal and attaches to the dorsal border of the testis. It comprises the ductus deferens (sperm duct), neurovascular supply to the testis, and vaginal tunics. Although closely related, the *musculus cremaster* lies external to the parietal tunic and is technically not a part of the cord.

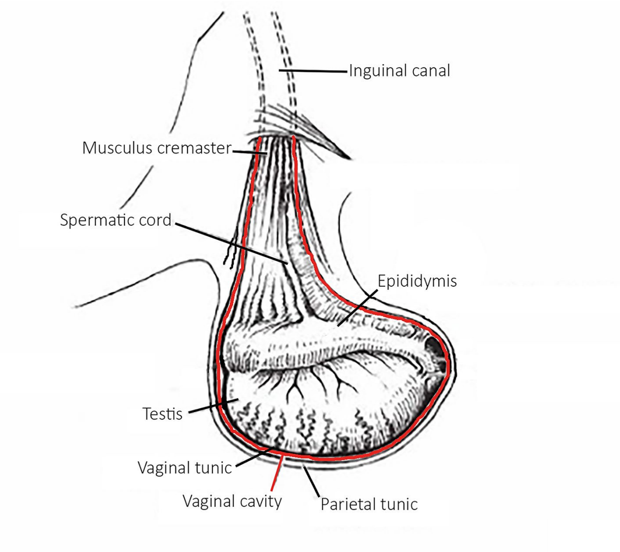

Figure 1. Schematic drawing of the testicle and its surrounding tissues adapted from Equine Surgery, 5 ed. (Schumacher 2019) with the vaginal cavity highlighted in red.

1.1.2 Techniques

Several castration techniques and approaches have been described in the literature. Additionally, the anaesthetic protocol could involve standing anaesthesia (e.g. sedation and local analgesics), or recumbent anaesthesia (e.g. intravenous or inhalation anaesthesia). Castrations with different anaesthetic protocols are further on referred to as standing or recumbent castration respectively. The choice of anaesthesia depends on technique and approach, as well as the size and temperament of the horse. Most surgeons agree that standing anaesthesia is not suitable for small ponies, or for individuals that do not tolerate testicular palpation despite sedation.

The testicles can be removed using open, closed or semi-closed techniques (Schumacher 1996). Briefly, the difference between the open and closed technique lies in the handling of the parietal vaginal tunic. In the open technique the parietal tunic is incised and left open following castration, creating a potential passage into the abdominal cavity (Baldwin 2024). In the closed technique, the parietal tunic is kept intact and removed together with the testis and distal end of the spermatic cord. The semi-closed (or half-closed) technique is a variation of the closed technique, in which the parietal tunic is incised to allow inspection of its contents before it is removed together with the testis. The literature regarding whether the closed technique involves proximal ligature of the spermatic cord or not is inconsistent; however, several authors have considered it necessary to seal off the communication to the abdomen (Shoemaker et al. 2004; Carmalt et al. 2008). A ligature must be placed aseptically, as the material can otherwise become a nidus for infection, which can be a particular concern during standing castrations.

The incisional approach for castration can be scrotal, inguinal or variations thereof. The scrotal approach can be used for both standing and recumbent castration, whereas the other approaches require recumbent patients. In the recumbent horse, when aseptic conditions can be maintained, the surgical incisions are preferably sutured (sutured castrations) (Lowe & Dougherty 1972; Cox 1984; Barber 1985; Sedrish & Leonard 2001). Otherwise, incisions are usually left open to heal by secondary intention (un-sutured castrations)(Heinze 1966).

Other techniques, such as immunological castration, laparoscopic removal of the testicles, or transection of the spermatic cord with the testicles left *in situ*, are beyond the scope of this thesis.

Table 1. Major complications and prevalence after castration.

Complication	Description	Prevalence	
		Sutured	Un-sutured
Swelling	Soft tissue swelling in the scrotum, prepuce or at the surgical site. The swelling may be secondary to haematoma, seroma or SSI. The presented prevalence include cases of mild swelling.	18-38% (Kummer et al. 2009; Torrent Crosa & Desjardins 2018)	4-51% (Carmalt et al. 2008; Bergstrom et al. 2022)
Haemorrhage	Intra- or postoperative bleeding from smaller vessels in the subcutaneous tissues or from the testicular artery. Haemorrhage originating from the subcutaneous tissue are usually mild to moderate and often ceases spontaneous, whereas that originating from the testicular artery is more severe and may be fatal.	0-8% (Robert et al. 2017; Koenig et al. 2019)	0-9% (Haucke et al. 2017; Rosanowski et al. 2018)
Local SSI	Appears within 30 days of surgery and involves only the skin or subcutaneous tissue at the surgical site, along with at least one of the following features: 1. Purulent discharge 2. Positive bacterial culture 3. Incision is deliberately opened by the surgeon because of pain, localised swelling or heat, unless a culture is negative.	0-10% (Robert et al. 2017; Koenig et al. 2019)	1-29% (Carmalt et al. 2008; Bergstrom et al. 2022)

Deep SSI (incisional or organ/space, e.g. funiculitis and peritonitis)	Appears within 30 days of surgery and involves deep soft tissue or organ opened or manipulated during surgery, along with at least one of the following features: 1. Purulent discharge 2. Spontaneous dehiscence or incision is deliberately opened by the surgeon because of pain, localized swelling or heat, unless a culture is negative. 3. An abscess or other evidence of infection found upon examination or reoperation.	0-7% (Robert et al. 2017; Koenig et al. 2019)	0-3.3% (Mason et al. 2005; Koenig et al. 2019)
Evisceration	Abdominal content, such as intestine or omentum, protrudes into the scrotal cavity. Intestinal evisceration is an acute life-threatening condition, especially in un-sutured castrations. Protrusion of the omentum is of less concern, although it requires proper intervention.	0% (Mason et al. 2005; Kummer et al. 2009)	0-8% (Shoemaker et al. 2004; Mason et al. 2005)

1.1.3 Complications

The reported complication rates after castration vary widely depending on the study design, technique and what is classified as a complication. A substantial inconsistency exists in the definitions of techniques and complications, making direct comparisons and definitive conclusions difficult (Rodden et al. 2024). However, with reported overall complication rates ranging from 2% to 23% (Mason et al. 2005; Kummer et al. 2009; Koenig et al. 2019), sutured castrations appear to have fewer complications than un-sutured castrations (range 8–60%) (Carmalt et al. 2008; Haucke et al. 2017; Rosanowski et al. 2018). Additionally, for the un-sutured castrations, fewer complications are reported for recumbent procedures (range 8–40%) (Carmalt et al. 2008; Kilcoyne et al. 2013; Bergstrom et al. 2022), than in standing procedures (range 16–60%) (Mason et al. 2005; Kilcoyne et al. 2013; Rosanowski et al. 2018). Most complications are considered mild and easily resolved; however, evisceration, haemorrhage, and severe infection can be life-threatening. A description of the major complications and their prevalence following sutured and un-sutured castration is presented in Table 1. The definition of surgical site infection (SSI), provided by the Centre of Disease Control and Prevention in USA (Mangram et al. 1999), is the most widely used in both human and veterinary medicine.

In rare cases, other types of complications may occur, such as penile damage, hydrocele, neuroma formation in the resected spermatic cord, or continued masculine behaviour.

1.1.4 Antimicrobial prophylaxis and surgical site infection

Surgical antimicrobial prophylaxis (SAP) has been employed to prevent postoperative SSI since the development of penicillin in the 1940s. Postoperative infections can be devastating for patients and impose a significant financial burden on both individuals and society (Stewart & Richardson 2019). The introduction of SAP and its ability to reduce the risk of SSI represented a major breakthrough in advanced surgery. The purpose of SAP is to reduce the bacterial load at the surgical site. To be effective, the choice of antimicrobial substance and the correct timing relative to the start of surgery are important factors (Southwood 2023). The expected bacterial pathogens guide the selection of the antimicrobial substance, with the goal of achieving maximal concentration of the substance in the tissue at the time of surgical intervention. However, antimicrobial drugs should not replace meticulous atraumatic and aseptic surgical techniques. Surgical wounds are classified as clean, clean-contaminated, contaminated or dirty (Table 2) (Stewart & Richardson 2019). Clean surgical procedures do not generally require SAP, unless the incidence of SSI exceeds 5% without SAP, or there is a risk of devastating consequences if deep SSI occurs in the area (Esposito 1999; Southwood 2023).

Table 2. Classification of surgical wounds (Stewart & Richardson 2019).

Classification	Criteria
Clean	Elective, primarily closed, and undrained Non-traumatic, uninfected No break in technique No inflammation encountered. Respiratory, alimentary, genitourinary tracts not entered
Clean-contaminated	Gastrointestinal or respiratory tracts entered without significant spillage Oropharynx entered Vagina entered Genitourinary tract entered in the absence of infected urine Minor break in technique
Contaminated	Major break in technique Gross spillage from the gastrointestinal tract Traumatic wound, fresh (<4 hours after trauma) Entry into the genitourinary tract or biliary tract in the presence of infected urine or bile
Dirty	Acute bacterial inflammation encountered Transection of ‘clean’ tissues for the purpose of surgically access the collection of pus Traumatic wound with retained devitalised tissues, foreign bodies, faecal contamination, and/or delayed treatment (<4 hours after trauma)

The risk of developing SSI during clean surgery is multifactorial and depends on factors such as the virulence of the endogenous skin flora, patient´s immune status, age, and the duration of surgery (Macdonald et al. 1994; Isgren et al. 2017; Robert et al. 2017). Castration is preferably performed in young and healthy stallions or colts (i.e. male horses under 4 years). In this context, proper aseptic preparation of the skin, minimal intraoperative and postoperative contamination, and a short surgical procedure are critical factors that can significantly reduce the risk of infection.

1.1.5 Antimicrobial resistance

The emergence of antimicrobial resistance (AMR) is recognised as one of the most important threats to human and animal health worldwide and indirectly to the global economy (World Health Organization 2015). Antimicrobial drug use, particularly overuse or misuse, drives resistance and motivates the restrictive use of SAP (Bratzler et al. 2013). To encourage the responsible use of antimicrobials, guidelines and policies on how and when to use SAP have been implemented in animal practice (The Swedish Veterinary Association 2013; *Antibiotics | BEVA* 2020). Although awareness of good antimicrobial stewardship is increasing, 48% of equine surgeons who responded to a British questionnaire still used SAP for clean surgeries (Wilson et al. 2023). This is believed to be due to the lack of guidelines for many surgical procedures in horses. Benzylpenicillin is one of the most commonly used substances for SAP (Stewart & Richardson 2019). Owing to regulations and the risk of negative side-effects, such as acute colitis (Cohen & Woods 1999; Båverud 2004), a limited choice of antimicrobial substances is available for equine surgeons; therefore, preserving the efficacy of benzylpenicillin is even more important. As discussed in Section 1.1.4, the definition of complications following castration, including SSI is inconsistent in the existing literature. Additionally, only a few studies have reported on sutured castration without using SAP (Laves et al. 2024; Riemersma et al. 2024), and none have focused specifically on postoperative complications. Before establishing evidence-based guidelines, additional knowledge about the risk of SSI is necessary.

1.2 Welfare of the horse

1.2.1 Surgical trauma

All surgical procedures induce some degree of tissue trauma, leading to inflammation and triggering pain response. Uncontrolled or excessive inflammation can exacerbate tissue damage, creating a negative feedback loop that may progress to systemic inflammation if not properly managed (Dobson 2015). Thus, atraumatic surgical techniques are essential to minimise excessive inflammation.

1.2.2 Pain

Human pain is defined as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al. 2020). Following key points accompany this definition:

- Pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors.
- Pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons.
- Through their life experiences, individuals learn the concept of pain.
- A person's report of an experience as pain should be respected.
- Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being.
- Verbal description is only one of several behaviours to express pain; inability to communicate does not negate the possibility that a human or a non-human animal experiences pain

Understanding pain in horses has received increased attention during the last few decades, alongside a growing focus on animal and horse welfare. Recognising pain is important to ensure adequate welfare, particularly when pain is induced by surgical procedures performed for husbandry purposes. Although domesticated for thousands of years, horses are prey animals by nature and may instinctively hide their pain. The expression of pain or discomfort is often disrupted or altered in the presence of humans (Torcivia & McDonnell 2020); hence signs tend to be subtle and difficult to detect even for experienced horse keepers and equine health-care professionals (i.e. veterinarians and nurses). Traditionally, the evaluation of pain in equine patients has relied on objective measures such as heart and respiratory rate, but these parameters fail to distinguish pain from other physiological or psychological stress (Manteca & Deag 1993). Multiple composite pain scales have been designed to combine physical parameters with observable behaviours associated with discomfort (Bussières et al. 2008; van Loon & Van Dierendonck 2015), as well as pain scales using facial expressions (Dalla Costa et al. 2014; Gleerup et al. 2015). Nevertheless, despite efforts to introduce objectivity into pain assessment, pain recognition is inherently subjective and time- consuming. Recently, automatic pain detection models have been developed, that may be the future for pain recognition (Broomé et al. 2022). However, more data are required on how horses express pain originating from different body parts and in various situations. Several studies have been conducted on pain behaviour after un-sutured castration (Love et al. 2009; Sanz et al. 2009; Dalla Costa et al. 2014; Olson et al. 2015); however, pain after sutured castrations has been sparsely described (Abass et al. 2018; Laves et al. 2024).

1.2.3 Other aspects

Today horses are primarily bred for sport, pleasure and work. Since domestication, humans have relocated horses from their natural open plains to confined environments, such as stalls, paddocks, and fenced pastures. In this 'new' environment a stallion's masculine, and often aggressive behaviour becomes problematic, making castration necessary for most male horses. Geldings can be kept with other horses without the risk of uncontrolled breeding, and with less risk of traumatic injury, both to themselves and other horses and, most importantly, the caretaker. Consequently, most stallions are currently isolated from other horses. Horse welfare is closely tied to the ability to express natural behaviours (Broom 1991), including social interactions with other horses. Isolation can cause stress for the horse, potentially triggering aggressive and stereotypical behaviour (de Oliveira & Aurich 2021), and imposing greater demands on the caretaker to adjust the environment to facilitate safe social contact. Given these aspects the option of not castrating a horse that is not kept for breeding purposes is debatable and, in many circumstances, not a viable alternative.

2. Aims of the thesis

The overall aim was to provide knowledge that can support veterinarians and horses owners in selecting an optimal castration technique for individual stallions and improving equine welfare.

The specific aims were:

- To investigate and describe the surgical techniques used for routine castration of stallions in Swedish equine ambulatory and hospital practices and to assess the incidence of reported postoperative complications.
- To investigate the perioperative and postoperative use of antibiotics and their association with complications post-castration.
- To investigate the subcutaneous tissue reaction in horses to a 3D printed self-locking ligation device made of polydioxanone (PDO).
- To evaluate the feasibility of using a novel self-locking ligation device for standing castration of stallions.
- To investigate the quality of a castration technique using serum amyloid A (SAA) as an objective indicator for postoperative inflammatory response.
- To determine if repeated measurement of postoperative SAA concentrations, following sutured castration, can be used to predict the development of SSI.

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3. Material and methods

This section summarises the materials and methods used in the four studies included in this thesis. A more detailed description is provided in each manuscript. All experiments and samples performed in this thesis were approved by the Uppsala Ethical Committee on Animal Experiments and conducted following national and institutional guidelines for the care and use of animals in research

3.1 Study design

Study I and Study III were retrospective studies that used medical records, to obtain an overview of common practices and complications of horse castrations in Sweden. In Study I, castration techniques, perioperative and postoperative medications and prevalence of complications were investigated and compared between ambulatory practice and hospital settings. In Study III, the influence of antimicrobial prophylaxis on complication rates in horses undergoing sutured castration was assessed. The castrations were performed using the same anaesthetic and surgical protocol; however, the prophylactic antimicrobial regimen was not standardised.

To investigate a potential new tool to improve castration methods, Study II was an experimental study comparing the tissue reaction to a 3D printed self-locking device (Figure 2) made of PDO, and poly(lactid-co-glycolic acid) (PLGA) sutures. The device, developed to facilitate a standardized ligation technique, was implanted into the subcutaneous tissue on the horse´s trunk. A double stranded PLGA suture, commonly used for suture ligation, was implanted in the same manner. In two other horses, a sham site was created to serve as a control for the inflammation caused by the surgical procedure.

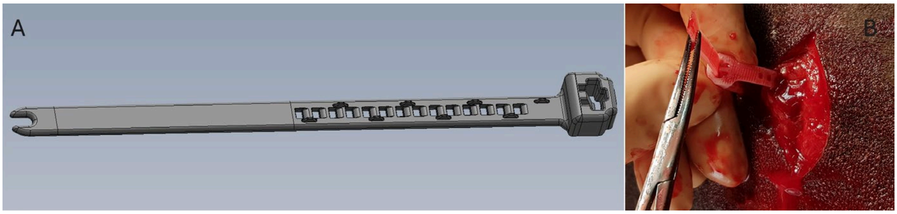

Figure 2. Schematic drawing of the ligation device (A) and implantation of the device in a horse (B). Images reproduced from Sjöberg et al. (Sjöberg et al. 2023).

Study IV was a single centre prospective clinical trial that investigated the SAA response as a proxy for inflammation after sutured castrations. Sutured castration was deemed the best practice for castrating horses, given the results from Study I and Study III. All castrations were performed under general anaesthesia following a standard anaesthetic and surgical protocol. The horses were pre-medicated with meloxicam, and treatment was continued for 5 days post-surgery. The horses were discharged after 2 days, and owners/trainers were instructed to follow the same management protocol for the postoperative period (2 weeks).

3.2 Study population

In Study I and Study III, the general inclusion criteria included records of healthy stallions with two descended testes admitted for routine castration. Records were excluded if the horse had any pathological urogenital condition or if sufficient data was lacking. In Study I, castrations were performed under ‘field conditions’ by veterinarians working for the District Veterinary Organisation (DV), at the premises of the horse, in 2019 (Group 1, n=521), or in the surgical theatre of three hospitals, between 2014 and 2020 (Group 2, n=554). In Study III, included records were of horses admitted for sutured castration at one equine hospital, performed by a single surgeon during 2016 - 2023 (n=220). Records of castrated horses that received prophylactic antimicrobials within 24 h post-surgery were excluded.

Ten adult horses of mixed age, breed and sex were used in Study II. They all belonged to the Department of Clinical Sciences in Uppsala, Sweden.

In Study IV, client-owned horses scheduled for sutured castration at an equine hospital between March 2023 and May 2024 were used. The included horses were aged between 6 months and 20 years, had both testes descended into the scrotum and had written informed consent from the horse owner or trainer (n=61). The exclusion criteria were as follows: castration performed using a different technique or in conjunction with another surgical procedure, pathological conditions of the testes, any known chronic illness, or horses that received any antimicrobial prophylaxis at the time of surgery.

3.3 Data collection

3.3.1 Study I and III

The collected data included demographics of the horses, perioperative and postoperative medication, and surgical techniques. Any notation in the records of postoperative complications was registered and categorised as shown in Table 3.

Table 3. Postoperative complications categorised according to definitions based on the major complications reported in Table 1.

Category	Definition
Non-infectious (NI)	Excessive scrotal swelling without fever (i.e. haemorrhage, seroma or hematoma), or prolapse of tissue (i.e. subcutaneous tissue or eventration)
Surgical site infection (SSI)	Excessive scrotal swelling with fever (>38.4°C), or purulent drainage from incision, devitalised and infected tissue at re-surgery, or positive bacteriological culture.
Anaesthesia (AN)	Any catastrophic event during anaesthesia or recovery, fever within 48h post-surgery without any clinical signs at the surgical site or, signs of respiratory disease or colic within 2 weeks post-surgery.

3.3.2 Study II

The appearance of the two implanted materials and surrounding tissue was monitored over time via ultrasonography following a set protocol, and both static and cine loop B–mode and colour Doppler images were saved for analyses. Implants and surrounding subcutaneous tissue were removed after 10 and 27 (±1) days for histological examination. Subcutaneous tissue were harvested from the sham sites after 10 days.

3.3.3 Study IV

Intraoperative variables were recorded to estimate the surgical trauma caused by the castration technique (Table 4). These variables were used to calculate the sum of surgical scores (SSc), which was used as a proxy for the total surgical trauma. Additionally, the duration of anaesthesia was registered, along with postoperative fever (>38.4°C) and swelling in the surgical area.

Table 4. Description of intraoperative variables.

Variable	Description
Suture material (mm^3)	Remaining suture materials were saved to enable calculation of the volume (length * suture size) of suture material left in the horse.
Ischemic cord (mm)	The size of the ischemic cord left in place was measured.
Weight of excised tissue (g)	The weight of the testis and the excised funiculi was measured.
Duration of surgery (min)	Duration of surgery was recorded from the time the testicles were locally anesthetised until the wound was protected with wound spray.
Recovery score (1–5)	The quality of the recovery was monitored via a surveillance camera system and scored according to Young and Taylor´s criteria (Young & Taylor 1993)
Haemorrhage (Yes/No)	Any excessive bleeding during surgery was noted.

Blood samples were collected prior to surgery for assessing baseline SAA concentrations and after 2 and 7 days to monitor the inflammatory response after castration.

Postoperative pain was evaluated using the Equine Pain Scale. In the first 2 days after surgery, assessment was performed by the staff working at the hospital, either a veterinarian or an experienced nurse. After the horse was discharged, the owners/trainers were asked to assess the pain, according to instructions provided in the Equine Pain Scale chart. Additionally, horses were monitored using video surveillance cameras (Ubiquiti Networks UniFi Protect G4 Instant, TP-Link Tapo C310 3MP, or TP-Link Tapo 320WS 4MP) and recordings between 10:00 PM and 02:00 AM were saved for analysis.

3.4 Data analysis

3.4.1 Ultrasonography and histological examination (Study II)

Ultrasonographic images were evaluated for implant remnants, fluid collection, echogenicity of the surrounding tissue and neovascularization. Tissue inflammation reaction (TIR) surrounding the two implants was assessed histologically based on the density of three different cell types (lymphocytes, macrophages, and neutrophils) and assigned a numerical grade using a modified Ehrlich–Hunt numerical scale (Table 5)(Ehrlich et al. 1973). The size of inflammatory zone (IZ) was semi-quantified at the lowest magnification (4x objective).

Table 5. Density grading of cells, modified from the Ehrlich–Hunt numerical scale (Ehrlich et al. 1973).

Grade	Density of cells
0	Absence
1	Bare scattering
2	Moderate
3	Dense aggregation

3.4.2 Serum Amyloid A analysis (Study IV)

Blood samples were centrifuged and the serum was stored at -80°C. To reduce inter-assay variability, all samples were analysed using the same batch of an immunoturbidometric assay (VET-SAA; Eiken Chemical Co. Ltd., Tokyo, Japan), at the Laboratory Department of Evidensia Specialist Animal Hospital, Strömsholm, Sweden. The detection range was 5–1600 mg/L, and all measurements below and above this range were assigned value of 4 mg/L and 1600 mg/L, respectively.

3.4.3 Pain assessment (Study IV)

In the Equine Paine Scale, the horse's facial expression is evaluated in combination with eight behavioural categories. Each category is assigned a score between 0 and 4, and individual scores are then summed to obtain a final score, with a maximum score of 36. A single score of 4 in either category, or a total score ≥10 was considered to be indicative of pain (Gleerup & Lindegaard 2016). In a subgroup of 29 horses, the video recorded material was analysed using an ethogram adapted from a two previously described ethograms (Pierard et al. 2019; Torcivia & McDonnell 2021). All behaviours defined in the ethogram were registered and analysed with respect to their total number and frequency.

3.5 Statistical analysis

Descriptive statistics describing the demographics of the included horse populations were used in all studies. Differences were analysed using Wilcoxon Rank test, or Fisher's Exact test. Statistical analyses were performed using the statistical software, JMP®Pro 16.0 (Study I–III), JMP®Pro 17.0 (Study IV) and Graph Pad Prism 9.4 (Study IV). Statistical significance was set at $p < 0.05$.

In Study I and Study III, Chi-square tests were used for univariable analyses to evaluate the effect of explanatory variables on the response variable, such as the occurrence of complication between and within Groups 1 and 2 in Study I. Additionally, in Study III, the effect of antimicrobial prophylaxis was further analysed by grouping castrated horses into a group that did not receive any antimicrobials (SAP-) and a group that received perioperative antimicrobials (SAP+).

In Study I, all variables were analysed in a multivariable regression model. A stepwise backward selection was performed, until only significant variables remained. In Study III, multivariable analysis was not performed because no significant associations were observed in the univariable analyses.

In Study II, Kaplan–Meier survival graphs were used to assess the median time to event for ultrasound findings. The median and interquartile range (IQR) were reported for TIR and IZ.

In Study IV, Wilcoxon Rank test was used to compare SAA and postoperative swelling at given time points. Continuous variables were log-transformed for normality, and age was treated as a categorical variable. Postoperative SAA concentrations as an outcome variable, was assessed for correlation with continuous variables using scatter plots, and Chi-square tests were used to explore the effects of categorical variables. Significant variables from these analyses were selected for a multivariable regression model using standard least squares tests, and SSc was forced into the model. A stepwise backward selection was performed until only significant variables remained in the model.

4. Results and Discussion

Castration of stallions is one of the most common surgical procedure performed in horses, primarily for husbandry purposes. For welfare reasons, we have an obligation as horse owners, veterinarians, and researchers, to ensure that the procedure causes minimal discomfort to the horse. Despite, or potentially because of, the wide variations in technique and perioperative and postoperative management, the evidence regarding various castration techniques and their associated complication rates remains limited. This lack of evidence makes it challenging to make informed decisions regarding the method to be used. This thesis aimed to provide more evidence-based knowledge to support veterinarians and horse owners in deciding the optimal castration technique for individual stallions.

In this thesis, information on castration techniques and perioperative management used in ambulatory and hospital practices in Sweden were obtained from medical chart reviews. In ambulatory practice, standing castration with an open, un-sutured technique was the preferred, whereas a closed, sutured technique was the method of choice in hospital settings. Perioperative antimicrobial prophylaxis in ambulatory practice was associated with a reduced risk of complications. However, antimicrobial prophylaxis did not reduce the rates of complications or SSI in sutured castrations performed under general anaesthesia and sterile conditions in a hospital setting. Furthermore, in the clinical trial, sutured castration under sterile conditions induced only a mild inflammatory response, with no complications requiring additional medical intervention.

4.1 Castration of horses in Swedish equine practices

Study I provided a background for the following studies in this thesis, providing retrospective information about techniques, perioperative management, and risk factors for complications following castration in Swedish clinical practices. Study III offered further insight into the effect of antimicrobial prophylaxis in horses undergoing sutured castrations based on the medical records from one equine hospital.

4.1.1 Surgical technique

In the records from ambulatory practice (Study I, Group 1), the surgical technique was described in 230 of 521 records, whereas this information was provided in all hospital records (Study I, Group 2 and Study III). For further analysis in Group 1, the same proportions were assumed for records where the technique was not described. In Group 1, standing castration with an open technique was dominant, whereas in Group 2, most horses were castrated in recumbency using a closed technique with sutured incisions (Table 6). In Study III, all castrations were sutured under general anaesthesia, using the same surgical technique, by the same surgeon.

Table 6. Recorded surgical technique in Group 1 (DV) and Group 2 (hospital A, B and C) and Study III (hospital D).

	Study I					Study III
Technique n (%)	DV n=230	A n=288	B n=206	C n=60	Total A,B,C n=554	D n=220
Open	194 (84)	0	0	0	0 (0)	0 (0)
Closed	0 (0)	288	157	58	503 (91)	220 (100)
Semi-closed	36 (16)	0	49	2	51 (9)	0 (0)
Sutured	0 (0)	218	205	50	473 (85)	220 (100)

4.1.2 Complication rates

Ambulatory practice (Group 1)

The overall complication rate in Group 1 was 35% (182/521), which is within the previously reported range after standing castration (Mason et al. 2005; Rosanowski et al. 2018). All registered complications in this group required veterinary attention. In other words, the complication rate did not encompass cases of mild swelling that resolved spontaneously or with increased exercise. The number of complications within the different categories is shown in Figure 3. Twenty-six percent were categorised as having SSI (133/521), which is consistent with findings of other studies (Mason et al. 2005; Rosanowski et al. 2018; Bergstrom et al. 2022). Colonisation of bacteria in open unprotected wounds is inevitable, and studies have shown that 100% of the wounds after un-sutured castrations are positive for bacterial cultures (Haucke et al. 2017). If proper drainage is not achieved for any reason (e.g. swelling, lack of exercise, or suboptimal site of surgical incision), colonisation may progress to multiplying microorganisms and wound infection. In Group 1, most complications were successfully treated by local veterinarians; however, 13 horses were referred to an equine hospital for treatment. No case of evisceration of intestines or omentum was reported. The risk of evisceration, after castration using the open technique is reported to be very low (Mason et al. 2005; Kilcoyne et al. 2013). The exception seems to be for certain breeds, such as draft horses and Standardbreds, where the incidence has been reported to be as high as 8% (Shoemaker et al. 2004). In this study population, the number of Standardbreds was low (n=28) and although there were more coldblooded horses (CBL) (n=71), proper draught horses are uncommon in Sweden.

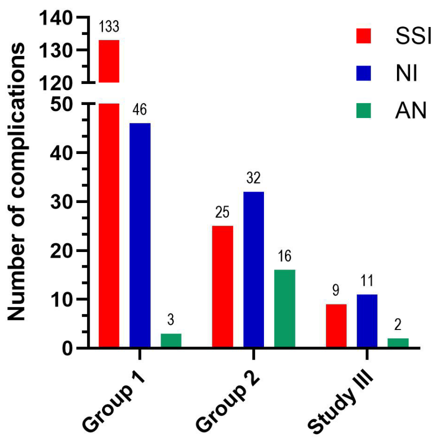

Figure 3. Number of complications in each category in Study I (Group 1 and 2) and Study III.

Hospital setting

For horses castrated in a hospital setting, the complication rates were 13% (73/554) in Study I, and 10% (22/220) in Study III (Figure 3), with SSI incidence of 4.5% and 4.1%, respectively. Horses with severe postoperative swelling, that did not respond to medical treatment and/or drainage of the surgical site under standing anaesthesia, underwent reoperation (n=9 in study I and n=4 in study III). A few registered complications did not require medical intervention, but resolved spontaneously or with increased exercise (n=7). Complication rates for sutured castration range between 2% and 23%, varying across studies and based on the criteria used to define a complication. For example, one study reported that 38% of the horses had a swelling at the surgical site, of which 37% was considered to be mild (Kummer et al. 2009). Nevertheless, the reported incidence of surgery-related complications was 2.1%, only taking into account the cases that required surgical intervention. As noted in several reviews on castration, this inconsistency makes it challenging, if not impossible, to compare of complication rates between studies (Baldwin 2024; Rodden et al. 2024). Despite this, authors repeatedly attempt such comparisons, requiring readers to remain aware of the substantial discrepancies.

4.1.3 Risk factors

The risk factors calculating odds ratios (OR) for complication and SSI were analysed using multivariable model in Study I.

Breed

In Group 1, CBL had a higher risk of complications and SSI than most other breeds ($p < 0.05$). The reason for this higher risk is unknown, but a speculation is that these breeds are not sufficiently sedated to remain still during the procedure. In heavier breeds, estimating the correct weight can be challenging, potentially leading to under-dosing of sedative drugs. Additionally, a common belief is that coldblooded horses are more sensitive to sedation than other breeds, which may further contribute to under-dosage. Excessive movement during castration has been proposed as a risk factor for SSI (Hodgson & Pinchbeck 2019). In the hospital setting, breed was not associated with complications or SSI.

Age

Most castrated horses in Group 1 were aged ≤2 years (315/521), indicating that castration of older horses in ambulatory practice is avoided. Older age is considered a risk factor for complications (Kummer et al. 2009; Robert et al. 2017; Hodgson & Pinchbeck 2019), and horses aged ≥5 years in both groups in Study I, had a three times higher risk of complications than younger horses ($p < 0.05$). Older age may predispose for intra- or postoperative haemorrhage (Kummer et al. 2009; Robert et al. 2017; Hodgson & Pinchbeck 2019), which may be reflected in the increased risk of complications in study I. However, in Study III age was not associated with complications at all. This may indicate the presence of unknown confounding factors for age-associated risks.

Hospital unit

In Study I, complication rates varied across the hospitals, ranging from 5–17%. The risk of complications was more than four times higher in hospital B than in hospital C ($p < 0.05$). This increased risk was primarily due to the significantly higher rate of anaesthesia-related complications in hospital B. No catastrophic (i.e., fatal) events occurred during anaesthesia or recovery and complications consisted of postoperative fever and colic. Due to the study´s reliance on pre-existing medical records, it is possible that these types of complications were overlooked at hospital C, because no anaesthesia related complications were registered there. No differences in SSI rate were noted between hospitals.

4.1.4 Antimicrobial prophylaxis

Perioperative administration of benzylpenicillin was associated with a lower risk of complications and SSI in Group 1, with OR of 0.6 and 0.5 respectively ($p < 0.05$). The use of prophylactic antimicrobials in horse castrations remains a topic of debate, with no clear consensus. However, it is important to distinguish between un-sutured and sutured castrations, as their wound healing processes differ significantly. In the author´s opinion, this discrepancy is often overlooked in the current discussions. Un-sutured castration, which at best can be classified as a clean-contaminated procedure, may benefit from prophylactic antimicrobials to prevent complications (Busk et al. 2010; Haucke et al. 2017; Hodgson & Pinchbeck 2019). The results of Study I support the use of a perioperative dose of a first-line antimicrobial, but prolonging the use of antimicrobials did not reduce the risk of complications. In Group 2, all patients received perioperative benzylpenicillin, regardless of whether the incisions were sutured or un-sutured. As sutured castrations performed in a surgical theatre, with standard aseptic preparation, should be classified as clean surgeries, this practice does not align with the Swedish policy on antibiotic use (The Swedish Veterinary Association 2013). In a small pilot study, bacterial growth after routine aseptic preparation prior to suture castration was investigated. Of the five bacterial samples, only one tested positive, yielding a single colony of *Staphylococcus aureus* (unpublished results). This result indicates that aseptic preparation sufficiently disinfects the surgical area and supports the classification of the procedure as a clean surgery. However, information regarding the prevalence of SSI following sutured castration, without antimicrobial prophylaxis is limited. This gap was addressed in Study III, in which the influence of antimicrobial prophylaxis was investigated in horses undergoing sutured castration.

The medical records of sutured castrations performed under general anaesthesia in a hospital setting were categorised based on whether they received surgical antimicrobial prophylaxis (SAP+; n=136) or not (SAP-; n=84). No association was observed between the groups and complication rates, with an incidence of 7.1% (6/84) in SAP- group and 11.8% (16/136) in SAP+ group ($p = 0.36$). Furthermore, the probability of SSI was not associated with the use of SAP, with an incidence of 3.6% (3/84) in SAP- group compared to 4.4% (6/136) in SAP+ group ($p = 1.0$). Most previous studies reported an incidence of SSI between 0% and 2.1% (Mason et al. 2005; Kummer et al. 2009; Robert et al. 2017; Torrent Crosa & Desjardins 2018). However, in one study five of 30 horses (17%) developed SSI, and two required a second surgery under general anaesthesia to remove infected tissues (Koenig et al. 2019). All horses in these studies received SAP and many continued prophylactic antimicrobial treatments for several days. Benzylpenicillin was used in all the studies, typically in combination with gentamicin (Kummer et al. 2009; Torrent Crosa & Desjardins 2018; Koenig et al. 2019), and one study reported the use of third- or fourth-generation cephalosporins (Robert et al. 2017). This SAP regimen does not align with the current standards of care, and if continued, may contribute to an increase in antimicrobial resistance. Few studies have described sutured castration without antimicrobial prophylaxis (Laves et al. 2024; Riemersma et al.

2024); however, none have focused on postoperative complications. Although a larger sample size in Study III would have allowed for a more robust statistical analysis, I believe that the study contributes to the existing knowledge on sutured castration without SAP. Together with the results of Study IV, these findings support and provide evidence for the Swedish national guidelines for antibiotic use in equine practice (The Swedish Veterinary Association 2013) against the use of SAP in sutured castrations.

4.2 The self-locking ligation device

The device was designed as a flexible, partially perforated band, connected to a locking case. The appearance was similar to that of a cable tie but it was made of resorbable PDO (Figure 2). It was intended to facilitate ligation of the spermatic cord, with easy application, and when tightened with a special tool, the applied force could be standardised. Although PDO is frequently used in equine surgery, (Anderson et al. 2015; Trela et al. 2016; Bertuglia et al. 2023), inflammatory tissue reactions are not triggered solely by the material itself. The shape and surface characteristics of the material also contribute to the magnitude of the tissue reaction (Matlaga et al. 1976). Therefore, the subcutaneous tissue reaction to the device was investigated before its use in a clinical trial (Study II). On ultrasonography, the device was initially seen as a hyperechoic structure with strong acoustic shadowing. Over time the device acquired a more amorphous and fragmented appearance with less acoustic shadowing, which remained visible after 4 months. This was unexpected, because a similar device implanted in dogs was fully resorbed within 4 months (Höglund et al. 2014). In the present study, complete resorption was not observed before 7 months after implantation. On histological examination, a transient foreign body reaction of similar extent was seen around both the PDO device and PLGA suture (Figure 4). The magnitude of the reaction was highly related to the type of surrounding tissue, as materials implanted adjacent to subcutaneous fat resulted in a more prominent reaction, than tissue consisting of muscle fibres. One explanation could be that fat tissue is delicate and more prone to degeneration and necrosis than muscle fibres, or that the clearance of hydrolytic debris from the device is less in fat than in muscle, contributing to a greater tissue reaction (Böstman et al. 1990; Atkinson et al. 1998).

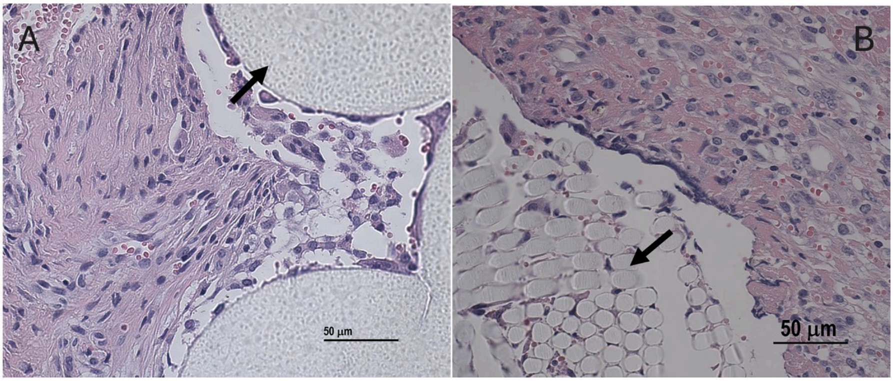

Figure 4. Histologic images of the PDO device (A) and the PLGA suture (B), 10 days after implantation. Arrow pointing at the implanted material.

When the device was removed 10 days after subcutaneous implantation, macroscopic inspection revealed that it was already fragmented. This suggests that the mechanical properties of PDO deteriorated more rapidly than anticipated. This early fragmentation was not consistent with previous findings of the same material (Metz et al. 1990; Kearney et al. 2014; Fatkhudinov et al. 2019). However, other studies have reported that PDO fibres degrade faster in living tissue than in saline (Kimura et al. 2003). When the degradation of the device was compared in vitro and in vivo, it was found that in vivo aging had a different impact on the mechanical properties of the device, with an earlier and more severe loss of tensile strength and decreased elasticity (Adolfsson et al. 2021). The authors suggested that the 3D printing process and anatomic location could influence the degradation process, and highlighted the importance of in vivo testing of a device before its clinical application. Considering its intended use as a ligation device, early fragmentation warrants further adjustments to both the material and 3D printing process before the device can be used in a clinical setting.

4.3 Objective assessment of the quality of castration

Study IV investigated the postoperative SAA response in serum following sutured castration, using a scrotal approach. The response pattern corroborated with previous reports (Pollock et al. 2005; Jacobsen et al. 2009), with a significant increase in concentrations after 2 days, followed by a normalisation after 7 days. The magnitude of the maximum median concentration (39.5mg/L) corresponded to the level described for minimal surgical trauma (Jacobsen et al. 2009), and was similar to that described for an inguinal approach (Laves et al. 2024; Riemersma et al. 2024). Since Study III concluded that SAP was not indicated for sutured castration, none of the horses included in Study IV received SAP. Despite this, no cases of SSI were observed in Study IV.

4.3.1 Variables associated with Serum amyloid A

The group consisting of CBL, Icelandic horses and ponies had significantly higher SAA concentrations on day 2 than warmblood horses and Standardbred trotters. This group consisted of horses with heterogeneous characteristics, including both the smallest and the largest breeds. A common observation in this group was that the horses had more fat deposited in their subcutaneous tissues. Fat is a delicate tissue that is sensitive to necrosis and stimulates inflammation (Sjöberg et al. 2023). Theoretically, this inflammation may have contributed to higher SAA-levels. However, further studies focusing on specific breeds are required to confirm this hypothesis.

On day 2, SAA did not correlate with SSc either in the univariable or multivariable models. Notably, a weak but significant correlation was observed on day 7. This could indicate that the factors contributing to SSc were associated with surgical trauma and a prolonged inflammatory response, resulting in slower decrease in SAA concentrations. However, the results should be interpreted with caution, as the severely skewed distribution of SAA concentrations could not be fully corrected with log-transformation; therefore, outliers may still have an effect on the model.

A weak correlation was observed between longer anaesthesia time and higher SAA concentrations on day 2 ($p < 0.05$). Knowledge on the effect of general anaesthesia on SAA concentrations in serum is scarce. A few studies including small groups of horses have indicated that SAA could rise to concentrations of 200–500 mg/L after general anaesthesia alone (Stowasser-Raschbauer et al. 2013; Rossi et al. 2019). In both studies, major inter-individual variations were observed, indicating that anaesthesia could be a confounding factor in the evaluation of the SAA response to surgical trauma.

4.3.2 Complications

Swelling of the scrotum and/or prepuce was the most common complication, occurring in almost 50% of the cases and 13% of the horses had an episode of fever for less than 24 hours. Similar to previous reports on sutured castrations (Cox 1984; Kummer et al. 2009) scrotal swelling was generally mild, and only one horse had severe swelling of the prepuce. Mild to moderate swelling of the incisional site, scrotum and prepuce is by many authors not recorded at all, or not included in the overall complication rate (Rodden et al. 2024). Some degree of swelling and fever within the first 48 hours after surgery are generally regarded as normal physiological response to the surgical trauma. Postoperative fever has a reported incidence of 2.5–21.4% following sutured castration under general anaesthesia (Kummer et al. 2009; Robert et al. 2017; Laves et al. 2024). However, temperature thresholds used to define fever vary substantially between the studies, with some classifying fever as a temperature of >38.4°C, and others using >39.5°C as a threshold (Kummer et al. 2009; Laves et al. 2024). According to a review on equine castrations, haemorrhage, swelling, fever, pain and some degree of tissue protrusion are often considered acceptable within equine practice or regarded as normal post-castration findings (Rodden et al. 2024). The author also highlights that these complications would not be considered normal in other species, such as dogs or humans.

Thus, the question arises; how should complications be defined? Should 'normal' physiological responses be classified as complications or only events that require medical or surgical intervention? These questions have also been raised in human surgery, prompting efforts to standardise the definition of postoperative complications (Dindo & Clavien 2008; Katayama et al. 2016). Complications were purposed to be defined as "any deviation from the ideal postoperative course" and should be distinguished from sequelae, which refer to conditions inherent to the procedure. If future studies on castrations adopt the definitions and report postoperative events as sequelae or complications, it may lead to a better understanding of what is inherent to the procedure and what constitutes a true complication. Yet another important aspect is postoperative pain, and its recognition in newly castrated horses. Considering the welfare of horses, this essential knowledge must be improved.

4.4 Welfare aspects

The castrated horses in Study IV were evaluated for pain using the Equine Pain Scale (unpublished data). None of the horses had a single score of 4, or reached a total score of 10, indicating that the observed pain was not alarming and did not require medical attention. The horses were also monitored using video surveillance cameras during the night to observe their behaviour in the absence of humans. The recorded material was analysed and described in two master (MSc) theses, focusing on lying - down behaviour and changes over time post castration (unpublished results Bonow 2025; Hultén 2025). Postoperative pain in humans reduces sleep quality. In horses, stress or pain reduces their time spent in recumbence (Glade 1986; Bertone 2006), but no study has investigated how pain affects their sleep patterns. Results from the MSc studies indicated that horses lie down for shorter periods and less frequently during the hospitalization than when they returned home. This finding was consistent with other studies showing that new environments alter horses´ behaviour (Oliveira et al. 2025). Furthermore, when the horses did lie down, they slept for longer periods on day 6 than on day 1. However, despite this increase in sleep duration, the frequency and total number of registered behaviours peaked on day 6. The ethogram documented all behaviours expressed by the horses without interpreting whether they were related to pain. Further studies are required to determine whether these findings are due to changes in the environment or related to pain experienced by the horse.

The decision of surgical technique appears simple, if only complications and the risk factors are considered. The complication rates after sutured castrations in this thesis were one third of the rates after standing castrations. Fewer complications cause less pain in horses, which is beneficial from a welfare perspective. Furthermore, from a One Health perspective sutured castrations do not normally require the use of antimicrobials, and therefore do not contribute to growing antimicrobial resistance. However, there are some other aspects that must be considered when choosing an optimal castration technique, for both horses and their caretakers.

4.4.1 Cost of castration

The cost of standing castration is approximately one-third that of a sutured castration in a hospital setting. According to Mason et al. (2005), this remains true even when factoring in the costs of treating complications. This conclusion may hold true when considering the average cost of complications in all horses undergoing standing castration. However, if 35% of standing castrations require a revisit by the treating veterinarian, even if it is ‘just’ for a mild complication, the cost immediately doubles. For 2.5% of standing castrations that requires further advanced treatment at a referral hospital, the cost may increase by 10 to 15 times (based on personal experience and figures from Swedish insurance companies and DV). Therefore, owners must be aware of this financial risk and prepare accordingly. In contrast, severe complications can also occur in sutured castrations, but the risk is lower in this case. Considering the current total costs of keeping a horse, the cost of castration is relatively low. For ethical reasons, this should not be the deciding factor in selecting technique of castration.

4.4.2 Management aspects

When examining the reported complication rates, recumbent un-sutured castrations, appears to have a slight advantage over standing castrations (7–40% vs. 6–60%, respectively). However, due to the lack of standardised definitions for complications this advantage is far from conclusive. Notably, publications on standing castration originate primarily from Scandinavia and Great Britain and its closely related former colonies (e.g. Hong Kong) (Mason et al. 2005; Busk et al. 2010; Rosanowski et al. 2018). In addition to environmental factors, such as weather conditions and stable yard facilities, regional geographic tradition may also influence the choice between standing or recumbent castration.

Horses castrated in a hospital setting have to be transported which, along with the exposure to a new environment, trigger a stress-response, particularly in young, untrained horses (Tadich et al. 2015; Oliveira et al. 2025). Stress may impair immune function and predispose horses to infections, which is a suboptimal condition prior to a surgery. Furthermore, it is recommended to keep a sutured castrated horse in a smaller, restricted area with controlled walking exercise during the first 10-14 days after surgery, which may have negative effects on the horse. In contrast, un-sutured castrations are encouraged intensive movement as early as 24 hours post-surgery, to facilitate drainage and prevent early wound closure. These factors could impact the horse´s welfare to such an extent that they outweigh the increased risk of complications associated with standing castration and must be considered when selecting the technique.

4.5 Additional aspects of material and methods – benefits and limitations

4.5.1 Retrospective studies

The study design in both Study I and Study III, were retrospective medical record reviews. As the records in such a review are not primarily written for research purposes, the quality and quantity of data may be insufficient. This can lead to unreliable or missing data, and potentially introduce a selection bias if records need to be excluded. This problem was encountered in Study I, and to compensate for the missing data, an online survey was distributed to veterinarians working at DV to collect information on how horse castrations were performed. Additionally, open interviews with operating surgeons at the hospitals were conducted to supplement the records, where necessary.

The reported complications in both studies relied solely on notations in the medical records. This, most certainly, resulted in the underreporting of complication rates. Mild complications that did not cause concern to the owner/caretaker or resolved spontaneously, as well as complications treated by another veterinary facility were missed. Considering the time frame of the included records, a standardised follow-up in which the owners were contacted did not seem relevant. Additionally, this may not have been possible because of the data protection regulations.

In Study III, the effect of SAP was investigated using two groups of medical records. An optimal study design would have been a double blinded prospective trial, where horses were randomised to receive antimicrobials or not. However, before conducting such a clinical trial, we need to have prior knowledge of expected outcomes for the control or treatment group. With a retrospective study design, we contributed to this knowledge without exposing new patients to unknown risk factors. Based on what we found here such a trial would need more than 200 horses in each group to reach statistical power, requiring either a study period over several years or a multicentre study.

4.5.2 Pilot horses

In Study II, a pilot horse was used to evaluate the feasibility of inserting the ligation device into the subcutaneous tissue and to determine whether there were any clinical signs of a major tissue reaction during the first 3 days. In hindsight we should have followed the pilot horse for a longer period. If we had been able to predict early fragmentation and a longer resorption time, the research protocol could have been adjusted to optimize the timing of samples for macroscopic and microscopic examination and the duration of ultrasonographic follow-up. In Study IV, a 4-week interval between the first and second horse, allowed for adjustments to some practical issues in the research protocol, such as changing the type of surveillance camera and recognising the need for detailed logistical planning for follow-up visits.

4.5.3 Sampling

Ideally, the tissue surrounding the implant in Study II should have been excised with a greater margin, than that performed. In some samples, the full extent of the tissue reaction could not be evaluated, because it extended beyond the resection margins. However, with the device fragmented into several pieces *en bloc* resection was challenging.

In Study IV, it would have been interesting to investigate if SAA concentrations peaked on day 2, or continued to increase until day 3. However, financial and logistical constraints prevented an extension of the horses´ hospitalisation.

5. Conclusions

- Surgical antimicrobial prophylaxis is not indicated for sutured castrations performed under standard aseptic conditions in a hospital setting.

- The serum amyloid A response after a sutured castration with a scrotal approach, indicates a low intensity of surgical trauma, unrelated to the intraoperative characteristics of the surgery.

- Standing castrations in ambulatory practice, using the open technique exhibit an SSI rate of 26%, which is at least three times higher than for sutured castrations in hospital setting.

- A single dose of benzylpenicillin administered before standing castration reduced the risk of SSI with 50%.

- From a One Health perspective sutured castrations contribute minimally to antimicrobial resistance, given that this procedure normally does not require the use of antimicrobials and the prevalence of surgical site infection is low.

6. Future perspectives

This thesis contributes to the understanding of how castrations are performed in Swedish equine practices and situates these techniques, anaesthetic protocols and complications within an international context. Furthermore, the work provides insights into the use of antimicrobial prophylaxis, which can serve as a foundation for future guidelines. However, the research question of the 'optimal castration technique' has not yet been fully answered.

Welfare aspects, such as pain and stress following castration using different techniques needs further attention. Before we can comprehend what is pain- or stress- related, we need more knowledge about horses' normal behaviour, with a specific focus on younger horses and the effect of changes in their environment.

Serum amyloid A concentrations were mildly increased in most horses after sutured castration and never reached levels of disease. However, for unknown reasons, a high inter-variability in the response was observed in the group consisting of Icelandic horses, coldblooded horses and ponies. Furthermore, the effect of general anaesthesia on SAA has only been sporadically reported in horses. A randomised controlled study, explicitly investigation these factors, is needed before SAA can be used as predictor of surgical complications.

Although standing castrations are associated with higher risk of complications than sutured castrations under general anaesthesia, they still have a place in equine surgery under special circumstances, such as for horses not accustomed to transportation or when it is not possible to keep the horse under controlled exercise during convalescence. A self-locking device, could be a valuable aid for standing castrations, facilitating a standardized ligation and potentially reduce complications. Therefore, further development of this device is warranted.

Finally, relating back to the aim “to provide knowledge that can support veterinarians and horse owners in selecting an optimal castration technique for individual stallions and improve equine welfare” the work presented in this thesis needs to be effectively disseminated to equine veterinary colleagues and their clients.

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Popular science summary

Castration of stallions is the most common surgical procedur performed on horses. Already in the Iron Age, stallions were castrated for husbandry purposes, as castrated horses, geldings, have a more docile temperament. A gelding is easier to manage and can safely be kept with other horses. To ensure animal welfare, any discomfort during the procedure must be minimised.

Castration methods in horses vary widely, in terms of technique, and management before and after the surgery. The procedure can be performed either in the horse´s home environment by veterinarians working in ambulatory practice or at a hospital in a surgical theatre. The reported complication rates vary considerably among methods and inconsistencies in defining complications make it challenging to identify the best approach. Therefore this thesis aimed to provide veterinarians and horse owners with evidence-based guidance in selecting an optimal castration technique for individual stallions.

Background information on how castrations were performed in Swedish equine practices was summarised from medical records. A review of the records revealed that standing castration with an open, un-sutured technique was the preferred method in ambulatory practice. Additionally, castrated horses that received antibiotics before surgery had a lower risk of developing wound infection. In hospital settings, most castrations were performed under general anaesthesia using sterile, closed and sutured techniques. In this setting, antibiotic administration did not reduce the risk of infection. Complication and infection rates were significantly higher for castrations in ambulatory practice than that in hospital settings. Given the emerging threat of antimicrobial resistance is a global concern for both human and animal health, surgical techniques that minimise or eliminate the need for antibiotics should be prioritised.

All surgical procedures induce some degree of trauma, leading to inflammation and pain. In a clinical trial, we observed that the sterile sutured castration technique induced only a mild inflammatory response, indicating minimal surgical trauma.

In summary, this thesis concludes that sutured castrations performed under standard sterile conditions in a hospital setting are best from a One Health perspective. However, from an animal welfare standpoint, the decision should consider the individual horse´s ability to tolerate the management aspects of the castration, such as transportation and restricted movement.

Populärvetenskaplig sammanfattning

Hingstkastration är den vanligaste operationen som görs på hästar. Redan på järnåldern kastrerades hästar för att få ett mer lätthanterat djur. En kastrerad hingst, d.v.s. en valack, har ett lugnare temperament och kan lättare hållas tillsammans med andra hästar utan oönskade konsekvenser. För att säkerställa god djurvälfärd måste onödigt lidande för hästen undvikas i samband med operationen.

Kastrationsmetoderna varierar kraftigt avseende teknik och behandlingsstrategier före och efter operationen. Hästen kan kastreras i sin hemmiljö eller på ett hästsjukhus. Den rapporterade komplikationsfrekvensen skiljer sig markant åt mellan olika metoder och tydliga definitioner i vad som är en komplikation saknas. Detta medför att det är svårt att avgöra vilken metod som är mest lämplig. Den här avhandlingen syftar därför till att ge evidensbaserat stöd till veterinärer och hästägare, att bestämma lämpligaste kastrationsmetod för den individuella hästen.

Bakgrundsinformation om hur kastrationer utförs i svensk hästpraktik sammanfattades från patientjournaler. Denna genomgång visade att stående kastrationer utförda med en öppen teknik där såren lämnades öppna var vanligast när hästen kastrerades i sin hemmiljö. Antibiotika som gavs innan operationen minskade risken komplikationer och sårinfektion. På hästklinikerna användes framförallt en sluten teknik där såren syddes. Operationen utfördes under allmän narkos under sterila förhållanden. I denna miljö hade förebyggande antibiotika ingen effekt på risken för komplikation eller sårinfektion. Förekomsten av komplikationer, i synnerhet sårinfektioner, var signifikant högre för hästar som kastrerades i hemmiljö jämfört med de som kastrerades på hästklinikerna. Den ökade resistensutvecklingen mot antibiotika är ett globalt hot mot både människors och djurs hälsa, och därför bör operationsmetoder som inte är beroende av antibiotika prioriteras.

All kirurgi skapar ett visst mått av vävnadsskada, vilket leder till en inflammationsreaktion och smärta. I en klinisk studie kunde vi se att den sterila kastrationstekniken med sydda sår endast orsakade en lindrig inflammationsreaktion, vilket talar för att operationen orsakade mycket lite vävnadsskada.

Sammanfattningsvis kan den här avhandlingen konkludera att sydda kastrationer utförda under normal sterila förhållanden på hästklinik är den bästa metoden ur ett ”One Health” perspektiv. För att upprätthålla en god djurvälfärd, bör dock hänsyn även tas till den enskilda hästens förmåga att acceptera yttre omständigheter runt kastrationen, som t. ex transport och begränsad rörlighet under konvalescensen.

Acknowledgements

The work included in this thesis was performed at the Department of Clinical Sciences, faculty of Veterinary Medicine and Animal Science, at the Swedish University of Agricultural Sciences, Uppsala, Sweden. Financial support for the work presented in this thesis was generously provided by the Swedish Animal Welfare Society. Travel grants was kindly provided by The Royal Swedish Academy of Agriculture and Forestry (KSLA), and from Gymn. dir. Stina Johansson´s and Gunnar Philipson´s Scholarships.

I am truly grateful to all people working in different hospitals and departments that in some way has been involved in this project. You have all contributed to this thesis and helped me along the journey, as well as friends, colleagues and family. No one mentioned, no one forgotten!

I would like to express my gratitude to my supervisor group:
Sanna Sternberg-Lewerin, my main supervisor who bravely stepped in and made it possible for me to finish this project. **Pia Haubro Andersen** and **Kalle Ljungvall**, you have both contributed to invaluable support during all this time. I would also like to thank **Ove Wattle** for initiating this project, and **Odd Höglund** for always being enthusiastic and encouraging.

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https://doi.org/10.1186/s13028-023-00710-0

Acta Veterinaria Scandinavica

RESEARCH Open Access

A preliminary investigation of the subcutaneous tissue reaction to a 3D printed polydioxanone device in horses

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Abstract

Background A 3D printed self-locking device made of polydioxanone (PDO) was developed to facilitate a standardized ligation technique. The subcutaneous tissue reaction to the device was evaluated after implantation in ten horses of mixed age, sex and breed and compared to loops of poly(lactic-co-glycolic acid) (PLGA). In two of the horses, the implants were removed before closing the skin. The appearance of the implants and surrounding tissue was followed over time using ultrasonography. Implants were removed after 10 and 27 (± 1) days for histologic examination.

Results On macroscopic inspection at day 10, the PDO-device was fragmented and the surrounding tissue was oedematous. On ultrasonographic examination, the device was seen as a hyperechoic structure with strong acoustic shadowing that could be detected 4 months post-implantation, but not at 7 months. Histology revealed a transient granulomatous inflammation, i.e., a foreign body reaction, which surrounded both PDO and PLGA implants. The type and intensity of the inflammation varied between individuals and tissue category.

Conclusions The 3D printed PDO-device caused a transient inflammatory reaction in the subcutaneous tissue and complete resorption occurred between 4 and 7 months. Considering the intended use as a ligation device the early fragmentation warrants further adjustments of both material and the 3D printing process before the device can be used in a clinical setting.

Keywords Additive manufacturing, Equine, Ligation, Resorbable device, Tissue reaction

Background

Biodegradable synthetic polymers have been used in medical devices since the 1980s in both human and veterinary medicine. One of the major advantages with a resorbable device over a non-resorbable device is that it does not require a second surgery. However, it is essential that the device provides sufficient mechanical support during the tissue regeneration process and eventually degrades to non-toxic products with little or no harm to the body. Foreign materials implanted in tissue will trigger an inflammatory response. The type and extent of the response is influenced by the properties of the material

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and the tissue [1–3] as well as the dimensions, shape and surface characteristics of the material [4–7]. A transient foreign body reaction has been seen with resorbable polymers, but after complete resorption of the materials only scar tissue remains [8–10]. Polydioxanone (PDO) is a resorbable polymer commonly used in equine surgery [11–13]. The polymer is biodegradable and biocompatible and has good flexibility [14], leading to favourable handling characteristics. Although biodegradable polymers are continuously investigated, research is almost exclusively in laboratory animals, aiming towards the use in humans [15–19]. In horses however, there are only a few published studies investigating the subcutaneous tissue reaction to implanted resorbable polymers [20, 21].

Self-locking, non-resorbable, polyamide cable-ties have been used in different surgical procedures, i.e. ligation of the spermatic cords in dogs [22], ovariectomy [23] and ligation of mesenteric vessels [24] in horses. Such self-locking devices are easy and quick to apply and can be tightened around the desired tissue, even when access is limited. However, when leaving non-resorbable material in situ, there is a long term risk of persisting granulomas, adhesions, fistulas and even rejection of the implant [23, 25, 26]. Promisingly, it has been demonstrated that an injection moulded self-locking device, made of resorbable polymers, had adequate clinical performance for ligation in dogs [27, 28] and pigs [29]. This device, made of polydioxanone, has been used when castrating stallions with cryptorchidism laparoscopically [30]. A similar device capable of ligating larger vessels or vascular tissue could be a valuable aid when performing standing castrations in horses. However, the manufacturing process of injection moulding is not flexible with respect to the possibility of modifying the design of the device.

Additive manufacturing, i.e., 3D printing, is a technology that enables the manufacturing of complex structures using a layer-by-layer approach, and it is believed to revolutionize industrial production by decentralising it [31]. The possibility to 3D print resorbable polymers has opened up a wide range of opportunities that, with high flexibility, could customize the design for different applications and transform digital models into unique and on-demand surgical devices. The 3D printing of PDO with the fused filament fabrication (FFF) technology has been successfully demonstrated, and its physical, mechanical and morphological properties were studied before and after degradation in vitro [14, 32]. A scaffold of 3D printed PDO has also been implanted in the distal femur of rabbits with good results [33]. Recently, a 3D printed PDO-device designed for standardized ligation of spermatic cord in horses was developed with features similar to a cable tie. The degradation profile and the mechanical properties of this device have been evaluated in vivo and in vitro [34], but in vivo studies regarding the tissue reactions to 3D printed PDO are still limited.

The objective of the study was to investigate the subcutaneous tissue reaction in horses to a 3D printed self-locking device made of PDO. We hypothesized that the tissue reaction would be similar to that of a traditional resorbable suture material made of a poly(lactid-co-glycolic acid) (PLGA).

Methods

Animals

Ten clinically healthy horses (seven Standardbred trotters, one Warmblood and two Shetland ponies) of mixed sex and mean age of 15.4 years (range 9–22) were used in the trial (Table 1). The horses were part of the teaching heard at the Swedish University of Agricultural Sciences and were housed in individual stalls and turned out for <8 h during the day in a 20 m × 20 m paddock. They were fed haylage and water was available *ad libitum* in both stall and paddock.

This study was conducted in accordance with an ethical approval from the Uppsala Animal Ethics Committee. Six of the horses were also included in a concurrent research study investigating recovery from general anaesthesia in healthy horses.

Table 1 Characteristics of the 10 horses and the randomised location of the implanted materials

Horse	Sex	Age (y)	Breed	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5a	Loc 5b
#1	M	9	STB	PLGA	PDO	PDO	PLGA		
#2	M	9	STB	PDO	PLGA	PLGA	PDO	PDO	
#3	M	17	STB	PDO	PLGA	PLGA	PDO	PDO	
#4	M	10	SWB	PLGA	PDO	PDO	PLGA	PDO	
#5	G	12	STB	PDO	PLGA	PLGA	PDO	PDO	
#6	G	15	STB	PLGA	PDO	PDO	PLGA	PDO	
#7	G	21	SP	PLGA	PDO	PDO	PLGA		PDO
#8	G	22	SP	PLGA	PDO	PDO	PLGA		PDO
#9	M	19	STB	C					
#10	M	20	STB	C					

C, Control; G, gelding; Loc, location; M, mare; PDO, Polydioxanone; PLGA, Poly(lactid-co-glycolic acid); SP, Shetland pony; STB, Standardbred trotter; SWB, Swedish Warmblood; y, years

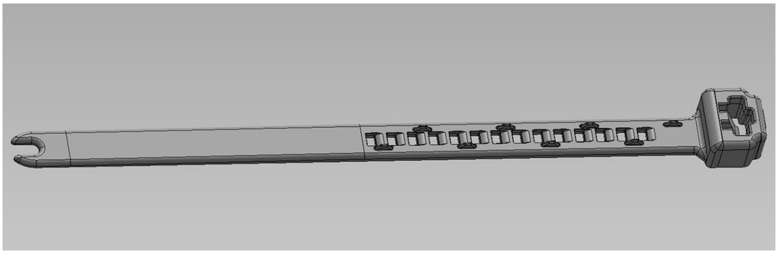

Fig. 1 A schematic drawing of the 3D printed device. The device consists of a locking head connected to a partly perforated flexible band

Table 2 3D printing parameters of the polydioxanone device adapted by Poly-Med

3D printing parameters	
Filament material	Dioxaprene100M
Printing temperature	165 °C
Fan cooling	80%
Extrusion width	0.3 mm
Nozzle size	0.25 mm
Infill percentage	100%
Infill angle	0; 90 °
Infill pattern	Rectilinear
Shell/perimeter	2
Layer height	0.15 mm
First layer height	0.15 mm
Bottom solid layer	0
Top solid layer	1

Materials
The PDO-device (Dioxaprene, Poly-Med Inc., SC, USA) (Fig. 1) was designed as a flexible, partly perforated band (84×4.5×0.9 mm) connected to a locking case (6.4×8.0×4.2 mm) and 3D printed according to the parameters in Table 2. The device, packed in breathable bags, was sterilised with ethylene oxide and dried under vacuum for at least 7 days, then placed inside sealed aluminium foil pouches. As a comparison, we used 20 cm of PLGA (Polysorb USP 2, Covidien, Medtronic, CT, USA). This amount of material corresponds to the amount normally used for ligating spermatic cords when castrating stallions at our university animal hospital.

Surgical procedure
The surgical procedures were performed by the first author (IS), either under general anaesthesia (n=6) according to a standard protocol [35], or under routine sedation (n=4) [36]. For the sedated horses, local infiltration anaesthesia with mepivacaine 20 mg/mL was administrated as an inverted L-block at least 10 cm from the skin incision.

The surgical areas, location 1–5 (Fig. 2), were clipped and aseptically prepared with chlorhexidine soap and ethanol. Via an approximately 8 cm skin incision, a subcutaneous pocket was created in a cranio-dorsal direction with haemostatic forceps. Within the pocket, and at least 5 cm from the incision an implant was attached as follows: The tip of the flexible PDO-band was introduced through the locking case and a self-locking loop was formed encircling subcutaneous tissue (Fig. 3). The loop was tightened and approximately 40 mm flexible band protruding from the locking case was cut off. A loop of double stranded PLGA was placed in a similar way and tied with a surgeon´s knot. The skin was closed with USP 2–0 polypropylene sutures. In the first horse, which served as a pilot, this procedure was repeated

Fig. 2 Surgical areas illustrated and location 1–5 denoted with a red circle where implants were inserted subcutaneously in the horse. **a** Left side showing location 1, 2 and 5a. **b** Right side with location 2, 4 and 5b

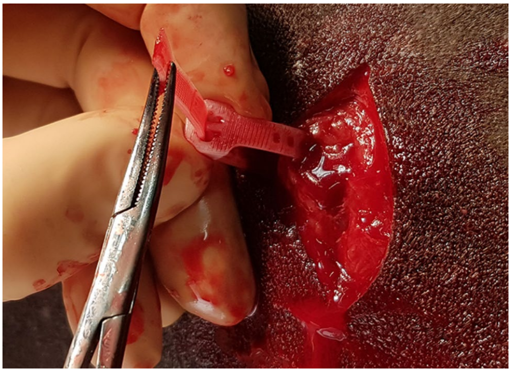

Fig. 3 The 3D printed device encircling a piece of fascia, with the free end passed through the locking head

in four locations on the left and right side of the trunk, implanting either a PDO-device or a PLGA loop in random order (Table 1). In Horse #2–8, an additional PDO-device, intended for long-time follow up with ultrasound, was implanted at location 5a or b (Fig. 2). In Horse #9 and #10, an incision was made at location 1 as described above, but before closing the skin, the implant was removed. Thereby sham sites where created to serve as controls for inflammation caused by the surgical procedure.

The wounds were protected with a sterile gauze and adhesive foam for at least 48 h post-operatively. The horses were treated with meloxicam 0.6 mg/kg SID IV on the day of surgery and *per os* for three consecutive days. They were monitored daily, with special attention given to signs of inflammation at the surgical areas, until skin sutures were removed 10–14 days after surgery and thereafter in conjunction with ultrasound examinations.

Ultrasonography

Following routine preparation, i.e. clipping and applying alcohol and/or ultrasound gel, all surgical sites in Horse #1–8 were scanned by a resident in veterinary diagnostic imaging (EL), using a LOGIQ e (General Electrics, Sweden) ultrasound machine with a variable frequency 8–12 MHz linear transducer. According to a set protocol, ultrasound examinations were performed pre-surgery to rule out any pre-existing abnormalities and at 2, 7 and 14 days, 3 and 4 weeks and at 2, 3 and 4 months post-surgery. The areas were examined for remnants of the implants, which were represented by a hyperechoic structure with or without acoustic shadowing, fluid collection, tissue reaction (i.e., hyperechoic tissue) and neovascularization. The implants and surrounding tissues were evaluated transversely, with the probe in a vertical dorso-ventral orientation, and longitudinally, with the probe in a horizontal cranio-caudal orientation. Both static and cine loop B-mode and colour Doppler ultrasound images (gain 20, scale 3 to -3 cm/s) were saved for analysis and evaluated independently by two of the authors (EL and IS).

Since some implants were removed for histologic examination, there were only eight PDO-devices and eight PLGA loops left in situ 4 weeks post-implantation. If the implant was still visible at 4 months, additional examinations were performed at 7 and 8 months post-implantation.

Sample collection

Samples (S) of the implants and surrounding tissue were harvested *en bloc* via a 3 cm skin incision over the implant site, identified by palpation or with ultrasound when the implant was not possible to palpate. The implant and approximately 1 cm of surrounding tissue were excised sharply with a scalpel blade and Mayo scissor. In the pilot horse (Horse #1) two samples, one PLGA and one PDO (S1 and S2) were excised at 14 days post-implantation (d14) and were macroscopically inspected. Due to these results, S1 and S2 was excised at d10 (±1) in the following seven horses. A third sample (S3) of the PDO implant was excised at d14 in Horse #2 and at d27 (±1) in the remaining six horses. In Horse #9 and #10, subcutaneous tissue from the sham site was excised in a similar fashion as for the implants at d10. The two ponies (Horses #7 and #8) were not going to be accessible after 4 months, which necessitated the collection of additional samples (S4) from location 5b at d120 removing the remains of the PDO-device (Fig. 4). All samples were excised under sedation and local anaesthesia or under general anaesthesia according to the previously described protocols. After gross inspection, the implants and the surrounding tissues were fixated in 4% formaldehyde (Unimedic Pharma AB, Solna, Sweden) and prepared for histology according to routine protocol.

Histology

Paraffin embedded sections, 4 µm thick and stained with haematoxylin and eosin, were evaluated with light microscopy (Nikon Eclipse 80i, BergmanLabora AB, Danderyd, Sweden) by a pathologist. The tissue inflammatory reaction (TIR) immediately surrounding the implant was assessed by the density of three different types of cells (lymphocytes, macrophages and neutrophils) and assigned a numerical grade from 0 to 3 using a modified Ehrlich-Hunt numerical scale (0 – Absence, 1 – Bare Scattering, 2 – Moderate, 3 – Dense Aggregation). The size of inflammatory zone (IZ) surrounding the implant was semi-quantified using the lowest magnification (4x objective).

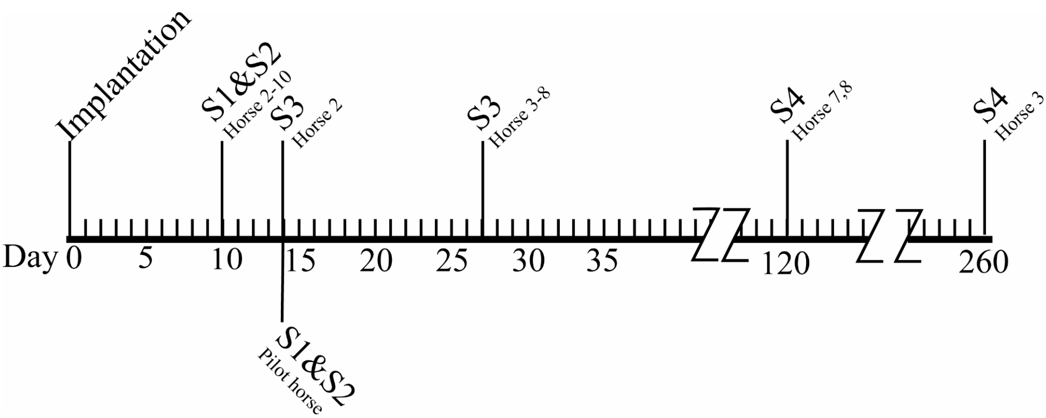

Fig. 4 Timeline displaying the days post implantation when samples (S) were excised for histology. S1 Poly(lactid-co-glycolic acid); S2-4 Polydioxanone. The pilot horse was sampled at one occasion, Horses #2, 4–6, 9 and 10 at two, and Horses #3, 7 and 8 at three occasions

Statistical analysis

Data were analysed in JMP Pro version 16.0.0 (SAS Institute, NC, USA) and a Kaplan-Meier survival graph was used to extract the median time to event for the ultrasound findings. Descriptive data were presented for TIR and IZ as median and interquartile range (IQR).

Results

Surgical procedure

The implants were inserted subcutaneously as planned (Figs. 2 and 3), but the tip of the flexible band had expanded during the 3D printing process and needed trimming before introduction into the locking case. Recovery from sedation, anaesthesia and surgery was uneventful and all wounds healed within 10–14 days without intervention or any clinical signs of excessive inflammation.

When the PDO-implant from the pilot horse was harvested for histologic preparation at d14, the device was fragmented and the surrounding tissue was oedematous. Macroscopic inspection of the PDO-implants removed at d10 and d14 in Horse #2 had a similar appearance, therefore samples of implants and surrounding tissue were excised at d10 in the following six horses (Fig. 4). Meanwhile, the PLGA loop was intact and the tissue was denser and integrated with the material. After 27 days, the PDO-device was even further fragmented making it difficult to harvest the biopsy *en bloc*.

Ultrasonography

Over time a range of changes, "events", were seen on ultrasonographic examination of the 3D printed device. The median time when these events were first registered were extracted from a survival plot (Fig. 5). During the first week, the locking case and loop of the PDO-device were seen as hyperechoic structures with strong acoustic shadowing. Within 2 months, the device gradually obtained an amorphous or fragmented appearance with less acoustic shadowing. After 3 months, the device was seen as an oval shaped, smoothly outlined and well defined structure. At 7 months post-implantation, the tissue had regained its pre-surgical appearance in all horses except one, where a rounded, mildly shadowing, hyperechoic structure could still be seen (Fig. 5d).

During the first 3 weeks, the PLGA-implants were seen as two hyperechoic focal regions, or as thin hyperechoic lines with strong acoustic shadowing. At 4 weeks, the remnants of the implants were difficult to distinguish from surrounding tissues and at 2 months post-implantation, the tissue had regained its pre-surgical appearance.

Regardless of the type of implant, a separation between the subcutaneous tissue and muscle fascial planes was seen at d2. The separation was initially filled with homogenously hypoechoic tissue, which subsequently increased in echogenicity during the following 4 weeks. Neovascularization surrounding the implants was observed in three of the eight horses at different time spans, ranging from d7 to 7 months (Fig. 5e). In Horse #6, this occurred at d7 in locations 3–5a (Fig. 2), but at 3 weeks a vessel was only seen in location 5a, where it persisted for the remaining examination period. In Horses #2 and #4, a small vessel was seen growing into the implant at location 5a, which was visible between 3 weeks and 3 months and from 3 months and onward respectively.

Due to practical reasons, ultrasonography was not performed at d2 in Horses #2, #7 and #8. Since the PDO-implant was fragmented when it was removed for histologic evaluation at d14 in Horse #1, the ultrasound protocol was preponed from d14 to d10 for the remaining horses.

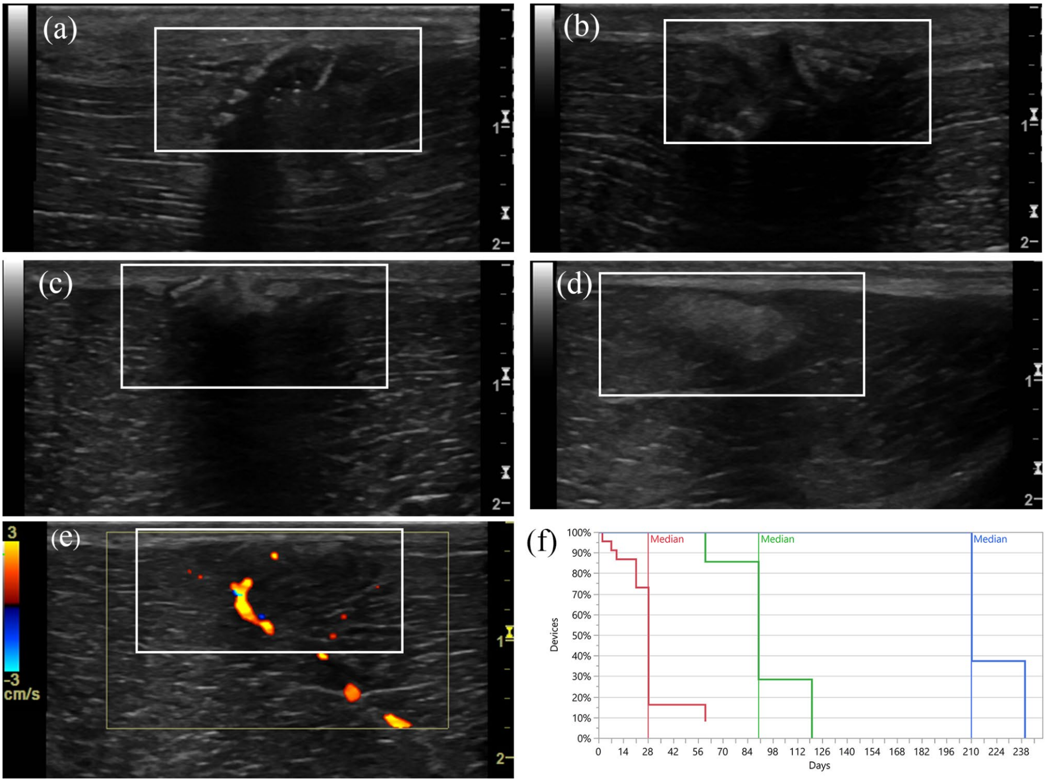

Fig. 5 Images depicting ultrasonographic changes of the polydioxanone (PDO) device over time and a graph illustrating the median time of the events. **a** At day 2 the locking case and loop of the device was seen as a hyperechoic ring-like structure with a tail, causing strong acoustic shadowing. **b** At day 28 the device had an amorphous, fragmented look. **c** After day 90 most devices had a rounded homogenous, mildly hyperechoic shadowing appearance. **d** A rounded, smoothly outlined structure was still seen in Horse #3 after 7 months. **e** In Horse #6, neovascularization was observed with colour Doppler in the region of the device at day 90. **f** Kaplan-Meier Survival Plot describing the median time when the PDO-device was fragmented (red), had lost its original shape (green) and was no longer visible with ultrasound (blue)

Histology

The inflammatory reactions in the tissue surrounding both the implants were characterized by chronic granulomatous inflammation containing elements of foreign body reaction, mainly concentrated around the implants. The inflammation was dominated by macrophages mixed with a variable ratio of neutrophils ranging from 5 to 40%. In one horse (#6), an eosinophilic response surrounded the PLGA implant. The TIR grades and the proportion of the IZ at d10 and d27 are presented in Table 3. The type and intensity of the inflammation varied between individuals and different categories of tissue, regardless type of implant. The implants adjacent to adipose tissue showed a more prominent inflammation with degeneration and necrosis of the adipocytes, whereas in areas of muscle tissue, the inflammatory response was less abundant (Fig. 6a-c). In the two control wounds, there was a mild chronic inflammatory reaction with areas of fibrotic tissue and neovascularization (Fig. 6d). As time progressed, the inflammatory reaction matured and was subjectively more organized (Fig. 7a). In a majority of horses, macrophages represented ≥ 90% of the inflammatory cells at d27. However, in one horse (#8) a 20% infiltration of lymphocytes was present. After 4 months, the signs of chronic inflammation were replaced by granulation tissue encapsulating the remnants of the PDO-device (Fig. 7b). In Horse #3, where a persistent hyperechoic region was seen with ultrasound, a tissue sample of the region at d260 revealed a thin band of fibrotic tissue without any inflammatory component or remnants of the device.

Table 3 Tissue inflammatory reaction (TIR) and inflammatory zone (IZ) around the implants

	TIR (grade)			IZ (%)		
	d10		d27	d10		d27
Horse	**PLGA**	**PDO**	**PDO**	**PLGA**	**PDO**	**PDO**
#2	1	2		50	40	
#3	1	3	1	15	25	10
#4	2	1	1	100	15	15
#5	2	3	1	85	85	10
#6	2	2	1	60*	20	10
#7	1	2	2	30	90	15
#8	2	2	1	50	50	35¤
Median(IQR)	**2** (1–2)	**2** (2–3)	**1** (1–1)	**50** (30–85)	**40** (20–85)	**12,5** (10–20)

The tissue inflammatory reaction in samples collected at day (d) 10 and 27 was assessed with histology using a modified Ehrlich-Hunt numerical scale from 0–3. The inflammatory zone was semi-quantified as a proportion of the total field view using the lowest magnification (4x objective)

* 4% of the inflammatory reaction were represented of eosinophils

¤ 20% of the inflammatory reaction were represented of lymphocytes

IQR, Inter quartile range; PDO, Polydioxanone; PLGA, Poly(lactid-co-glycolic acid)

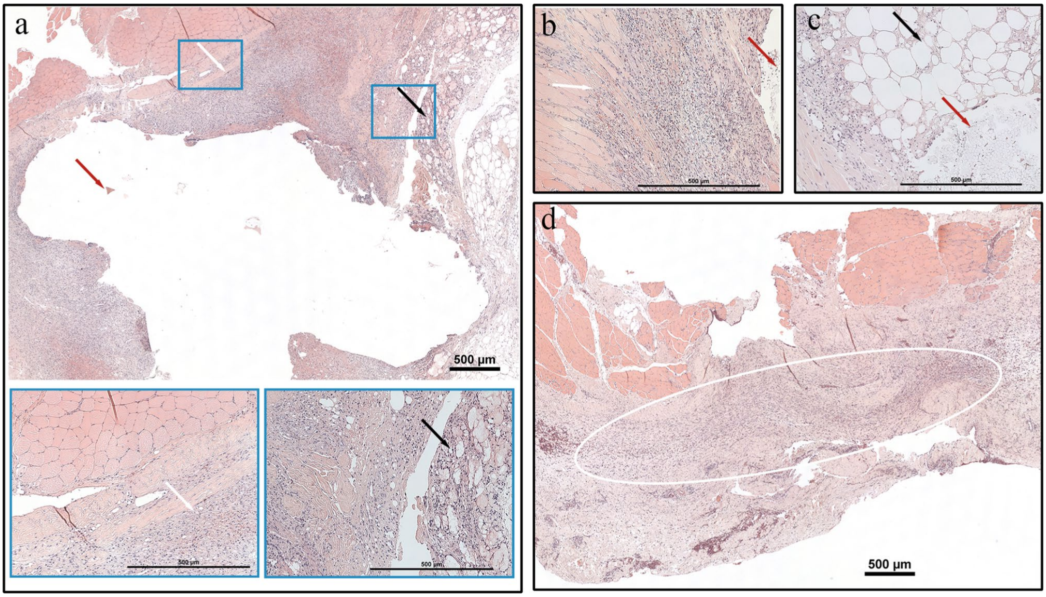

Fig. 6 Photomicrographs with haematoxylin and eosin staining that show implanted material or its lumen (red arrows) and surrounding tissue reaction at day 10. **a** Polydioxanone device magnified x4 in the top frame and x20 in the 2 bottom frames. **b, c** Poly(lactid-co-glycolic acid)magnified x20. **d** Control wound magnified x4. Surrounding the implanted material a more restricted inflammation was seen in areas adjacent to the muscle tissue (white arrows), whereas there was a more prominent inflammation in the adipose tissue with steatitis (black arrows). In the control wound, there was a mild chronic inflammatory reaction (white oval). Bar scale = 500 µm

Discussion

The 3D printed PDO-device did not cause any clinical signs of inflammation in the subcutaneous tissue, although complete resorption was not seen prior to 7 months post-implantation. The device however, was already fragmented 10 days after implantation, which suggests that its mechanical properties decreased faster than anticipated. On histologic examination, there was a transient foreign body reaction, but with great variation between the different regions within one sample.

Ultrasonography was used to follow the appearance of the implants and surrounding tissue reaction over time. Initially both the PDO-device and the PLGA loop were seen as hyperechoic structures with strong acoustic

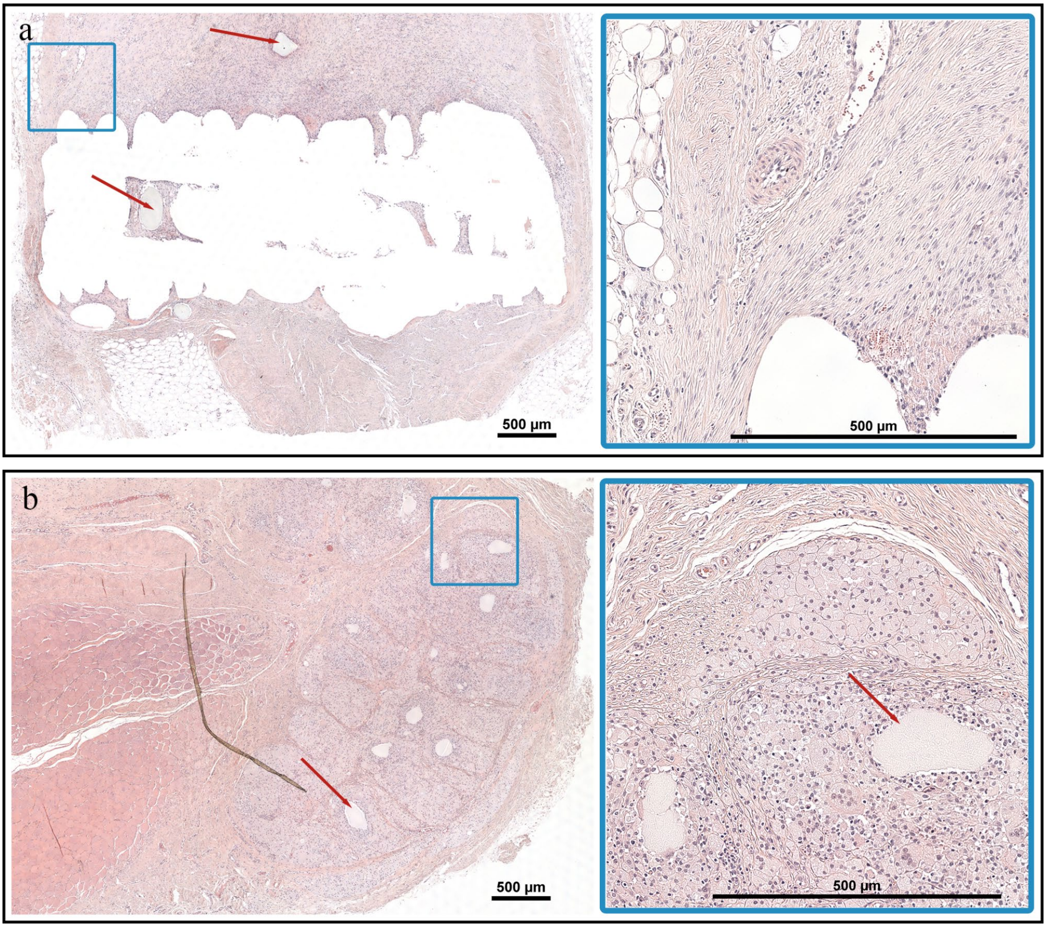

Fig. 7 Photomicrographs with haematoxylin and eosin staining of the progressing tissue reaction to the polydioxanone (PDO) device (red arrow). **a** Sample collected at day 27. **b** Sample collected at day 120. Blue square to the right magnified x20. Over time, the inflammation around the PDO-device became more mature and organized with decreasing numbers of inflammatory cells and a more compact, and less cellular, connective tissue. At day 120 there was granulation tissue encapsulating the remnants of the PDO-device. Bar scale = 500 µm

shadowing. The hypoechoic tissue separation surrounding the implants was likely oedema or seroma formation related to the surgical procedure, and the space was filled with granulation tissue within 4 weeks. This corroborates the results presented in a similar study of resorbable polymers implanted in horses [20]. All but one PDO-device had an intact appearance on ultrasound during the initial 4 weeks. As the devices exhibited strong shadowing properties, and some parts of the device often shadowed other parts, a full evaluation of the entire device was not always possible. This could be the reason why fragmentation was not observed on ultrasonography earlier. In contrast to the findings from Höglund et al. [37], all remaining devices were still visible 4 months post-implantation. A plausible explanation could be that the devices in the current study were larger, which resulted in a greater amount of material to be absorbed, or access for imaging was easier since the devices were implanted subcutaneously. In regard to factors such as vascularization and transport capacity, it should be noted that comparisons with previous studies must always be made cautiously as there are differences between the tissues and species studied [2, 38]. Ideally, additional ultrasound examinations should have been performed at 5 and 6 months to better pinpoint the exact time when the device was no longer visible. This was not possible since the horses used in this study were not accessible during this period. On the other hand, the exact resorption time of a material is difficult to assess with ultrasound [20, 37]. This difficulty was further demonstrated in this study where remnants of the device could not be found on histologic examination, even though the preceding ultrasound images showed a hyperechoic structure with mild acoustic shadowing.

Histologically both materials caused low-grade inflammatory reactions which were confined to the surrounding tissue, which is in agreement with previous studies [18, 37, 39]. The magnitude of the reaction was highly related to the type of surrounding tissue, where materials implanted adjacent to subcutaneous fat resulted in a more prominent reaction, than tissue consisting of muscle fibres. This was not anticipated, but likely a spill over effect initiated by the handling of tissue during surgery, as adipose tissue is more prone to undergo degeneration and necrosis than muscle fibres. If clearance of polymeric hydrolytic debris is less in fat than muscle, then that will also contribute to a greater tissue reaction [2, 3]. In Horses #7 and #8, the remains of the PDO-devices were excised at 4 months, giving us additional information on the resorption progress of the device. This further demonstrated the transient inflammatory response and was in agreement with the findings from Carvalho et al. [21], where another type of polymer was implanted in a horse. PLGA was used as a control material since it is commonly used for ligating spermatic cords in equines. It has favourable handling characteristics and a limited, transient tissue reaction in laboratory animals [39]. The histological findings were in agreement with previous studies [39, 40], although one horse had an eosinophilic inflammatory response around the PLGA implant, which could be a normal individual variation.

Implants 3D printed with the FFF technique will expand while cooling down on the printer bed. The material was processed through a heated printer nozzle and deposited onto molten layers. The produced volume may, to some extent, "flow out" beyond its intended shape, which probably caused the difficulty in fitting the tip into the locking case. Improving the original design by tapering the tip could be a simple adjustment using 3D printing technology.

The early fragmentation of the device was unexpected and not in accordance with some previous in vitro [19, 41] and in vivo [16, 19] studies of the same material. However, it has been shown that polydioxanone fibres degraded significantly faster in living tissue than in saline solution [7]. When Adolfsson et al. [34] compared in vitro and in vivo degradation of the tested device, it was found that in vivo aging had a completely different impact on the mechanical behaviour of the devices, which resulted in an earlier and more severe loss of tensile strength and decreased elasticity. The authors suggested that the 3D printing process and anatomic location could influence the degradation process, and highlighted the importance of the in vivo testing of a device before clinical application.

Study limitations

Firstly, the creation of a surgical wound triggers a tissue response, which may interfere with the response to the implanted material. In this study, an effort was made to limit this interference by positioning the materials away from the skin incision, and by comparing the results to sham wounds. Additionally, the aim was to evaluate a device intended to be used in a surgical environment. Secondly, the tested device is proposed to be used for ligation of the spermatic cord when castrating horses standing. However, this area is difficult to access and requires that the horse is a stallion, why the device was implanted subcutaneously on the trunk of the horses instead. Resorption time may differ between different anatomical locations [2], an important discrepancy that always needs to be acknowledged when using implants. Thirdly, the tissue reaction varied greatly between different regions within one sample, which was not expected and made the histologic scoring difficult. Scoring tissue reaction is challenging, as there are always elements of subjectivity and to the authors' knowledge, there is no gold standard protocol. The histological examination could not be blinded since remnants of the implants could be seen in the sections. The large individual variation resulted in low statistical power, which is why descriptive data was presented.

Conclusions

The 3D printed PDO-device only caused a transient foreign body reaction in the subcutaneous tissue, similar to that of PLGA. Complete resorption of the device occurred between 4 and 7 months, but function was lost already before 10 days post-implantation. Continued adjustments of the design of the device, polymer material and the 3D printing process is warranted before the device can be used in a clinical setting. A different resorbable polymer or a PDO composite aimed to strengthen the device and prevent early fragmentation might be a solution in the quest for a functional device. This study also reveals the limitations of ultrasonographic examination when it comes to evaluating the resorption process of implants and the surrounding tissue reaction.

Abbreviations

FFF	Fused filament fabrication
IZ	Inflammatory zone
PDO	Polydioxanone
PGLA	Poly(lactid-co-glycolic acid)
S	Sample
TIR	Tissue inflammatory reaction

Acknowledgements
We thank J. Ingman DVM, EBVS for her expertise in ultrasonography and J. Rydén for his help with the statistical analysis. We will also like to thank R. Quell for proofreading and English language editing the manuscript. This work was in part (3D printing prototyping) conducted within the Additive Manufacturing for the Life Sciences Competence Center (AM4Life). The authors gratefully acknowledge financial support from Sweden's Innovation Agency VINNOVA (Grant no: 2019-00029).

Authors' contributions
OW, OVH, IS and EL designed the experiment. IS and EL performed the acquisition and interpretation of data and drafted the manuscript. FS and OW contributed to the interpretation of data. OW and OVH revised the drafted manuscript. All authors have read and approved of the final version of the manuscript.

Funding
This project was supported by a grant from Uppsala's Competence Centre of Additive Manufacturing for the Life Sciences.
Open access funding provided by Swedish University of Agricultural Sciences.

Data Availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
OVH is the inventor of the self-locking device and founder of company Resorbable Devices AB. The company holds the associated patents but the inventor performed neither the analysis nor interpretation of data. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication
Not applicable.

Ethics approval
This study was conducted in accordance with ARRIVE (Animal Research: Reporting of In Vivo Experiments) and an ethical approval was obtained from the Uppsala Animal Ethics Committee, Dnr 5.8.18–18803/2018.

Prior publication
Some of the data included in this article have previously been published as an abstract in the Proceedings of the ACVS Surgery Summit 2020, Virtual Session, DOI: https://doi.org/10.1111/vsu.13506.

Received: 11 April 2023 / Accepted: 29 October 2023
Published online: 20 November 2023

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Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2025:23

Castration is frequently performed in stallions for husbandry purposes. Evidence-based guidance is needed to select the optimal castration technique. Complication rates for standing castrations using an un-sutured technique are three times higher than for sutured castrations under general anesthesia. The use of benzylpenicillin before standing castration may reduce complications. From a One Health perspective, sutured castrations contribute minimally to antimicrobial resistance, as this procedure does not require the use of antimicrobials and the prevalence of surgical site infection is low.

Ida Sjöberg received her graduate education at the Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala. Her undergraduate degree in veterinary medicine was obtained at the Faculty of Veterinary Medicine and Animal Science, SLU.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

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ISSN 1652-6880
ISBN (print version) 978-91-8046-458-1
ISBN (electronic version) 978-91-8046-508-3