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FEASABILITY STUDY AND EVALUATION OF THE EFFICACY, SAFETY, AND SHORT AND LONG TERM FOLLOW UP OF A NEW TECHNIQUE FOR SURGICAL CASTRATION IN ADULT BOARS

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FEASABILITY STUDY AND EVALUATION OF THE EFFICACY, SAFETY, AND SHORT AND LONG TERM FOLLOW UP OF A NEW TECHNIQUE FOR SURGICAL CASTRATION IN ADULT BOARS

Presented by

Toufic AKL

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ABSTRACT

INTRODUCTION

Surgical castration of adult boars proves to be more challenging than that of piglets and despite it being less often necessary, it is still common practice. Classical techniques of orchiectomy require the use of ligatures and/or emasculator which can lead to an increased risk of surgical wound contamination and subsequent infection. For this aim, we devised a novel technique termed "sutureless castration" consisting of an open castration where the vas deferens is ruptured from the epididymis and tied into several square knots with the vascular portion of the spermatic cord.

STUDY 1:

A pilot study was conducted on 101 adult boars undergoing routine castration aiming to determine the feasibility and of the novel technique and power analysis was conducted to determine the sample size necessary for future studies. The boars were castrated by classical castration (G1; n=41) or sutureless castration (G2; n=60) and age (A), weight (W), and left and right deferent duct diameter and tensile strength (DDTS) were recorded. Pearson's linear correlation coefficient was conducted on a homogeneous pool of 10 boars per group to determine the correlation between DDTS-A and DDTS-W. All the surgical procedures were successful, and no record of immediate postsurgical complications or mortality was noted. R value found for the pooled boars was 0.99 and 0.96 for DDTS-A and DDTS-W respectively. Power analysis showed that a significant level of accuracy determining the DDTS-A/W can be reached with a total sample of 388 deferent ducts.

STUDY 2:

A clinical follow up study was conducted on 403 boars castrated by the sutureless technique. Age, weight, deferent duct diameter and tensile strength were recorded, and DDTS-A and DDTS-W were analyzed by a Pearson correlation. A pool of 50 animals were randomly selected for inclusion in the short and long term follow up. For short term follow up, general and physical examination was conducted on days 1 to 4 and subsequently on day 7 and day 14. Data recorded included wound healing, gait stiffness, feeding behavior, other complications noted by the breeder and eventual death. Long-term follow up consisted of carcass inspection at slaughter and recording the presence of scar tissue, abscess, granuloma, and adherences at the surgical site and adjacent area. All surgeries were successful and correlation between DDTS-A and DDTS-W was strongly significant (R= 0.91 and 0.87). For short-term follow up, all animals showed some degree of swelling with 72% of them being only mild. The incidence of gait stiffness was

36% and that of myiasis 12%. A single case of abscess formation near the surgical site was recorded. Regarding long-term follow up, 26% of the animals had small subcutaneous scrotal granulomas which were considered to be unrelated to sutureless castration.

CONCLUSION

This novel technique of castration was validated for the use in adult boars and showed a success rate comparable to that of the classical technique. Additional studies should be conducted to determine its' potential superiority in the matter of surgical time, cost efficiency and teachability.

KEYWORDS

Boar; surgical castration; sutureless technique; pilot study; clinical follow-up

RIASSUNTO

INTRODUZIONE

La castrazione chirurgica dei verri adulti si rivela più impegnativa di quella dei suinetti e, nonostante sia meno necessaria, è ancora una pratica comune. Le tecniche classiche di orchiectomia richiedono l'uso di legature e/o emasculatore che possono aumentare il rischio di contaminazione della ferita chirurgica e successiva infezione. Per questo motivo, abbiamo inventato una nuova tecnica denominata castrazione sutureless consistente in una castrazione aperta in cui il dotto deferente viene rotto dall'epididimo e legato in diversi nodi piani con la porzione vascolare del funicolo spermatico.

STUDIO 1:

È stato condotto uno studio pilot su 101 verri adulti sottoposti a castrazione di routine con l'obiettivo di determinare la fattibilità della nuova tecnica ed è stata condotta un power analysis per determinare la dimensione del campione necessaria per studi futuri. I verri sono stati castrati mediante castrazione classica (G1; n=41) o sutureless (G2; n=60) e sono stati registrati l'età (A), il peso (W) e il diametro del dotto deferente sinistro e destro e la forza di trazione (DDTS). Il coefficiente di correlazione lineare di Pearson è stato condotto su un pool omogeneo di 10 verri per gruppo per determinare la correlazione tra DDTS-A e DDTS-W. Tutte le procedure chirurgiche hanno avuto successo e non è stata rilevata alcuna registrazione di complicanze o mortalità post-chirurgiche immediate. Il valore R trovato per i verri raggruppati era 0,99 e 0,96 rispettivamente per DDTS-A e DDTS-W. La power analysis ha mostrato che un livello significativo di accuratezza che determina il DDTS-A/W può essere raggiunto con un campione totale di 388 condotti deferenti.

STUDIO 2:

Uno studio clinico di follow-up è stato condotto su 403 verri castrati con la tecnica sutureless. Sono stati registrati età, peso, diametro del condotto deferente e resistenza alla trazione e sono stati analizzati DDTS-A e DDTS-W mediante una correlazione di Pearson. Un pool di 50 animali è stato selezionato a caso per l'inclusione nel follow-up a breve e lungo termine. Per il follow-up a breve termine, l'esame generale e fisico è stato condotto nei giorni 1-4 e successivamente nei giorni 7 e 14. I dati registrati includevano aspetti della ferita, rigidità dell'andatura, comportamento alimentare, altre complicazioni rilevate dall'allevatore e eventualmente, il devesso del soggetto. Il follow-up a lungo termine consisteva nell'ispezione della carcassa alla macellazione e nella registrazione della cicatrizzazione dell’incisione, ascessi, granulomi e aderenze nel sito chirurgico e nell'area adiacente. Tutti gli interventi chirurgici hanno avuto successo e la correlazione tra DDTS-A e DDTS-W è stata fortemente significativa (R = 0,91 e 0,87). Per il follow-up a breve termine, tutti gli animali hanno mostrato un certo grado di tumefazione con il 72% di loro solo lieve. L'incidenza della rigidità dell'andatura è stata del 36% e quella della miasi del 12%. È stato registrato un solo caso di formazione di ascessi in prossimità del sito chirurgico. Per quanto riguarda il follow-up a lungo termine, il 26% degli animali presentava piccoli granulomi scrotali sottocutanei considerati non correlati alla castrazione sutureless.

CONCLUSIONE

Questa nuova tecnica di castrazione è stata convalidata per l'uso in verri adulti e ha mostrato una percentuale di successo paragonabile a quella della tecnica classica. Ulteriori studi dovrebbero essere condotti per determinare la sua potenziale superiorità in materia di tempo chirurgico, efficienza dei costi e insegnamento.

PAROLE CHIAVE

Verro; castrazione chirurgica; castrazione sutureless; studio pilot; studio clinico follow-up

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LIST OF ABBREVIATIONS

SpC: Spermatic cord

GnRH: Gonadotrophin releasing hormone

IGF-1: Insulin-like growth factor-1

IV: intravenous

SC: subcutaneous

IM: intramuscular

CA-T: correlation between age and tensile strength

CW-T: correlation between weight and tensile strength

CI: confidence interval

GENERAL INTRODUCTION

Among all meat producing domestic farm animals, swine are particular in that their meat can produce an offensive odor known as boar taint which is especially more prominent in mature boars due to the production of androstenone, a steroid produced by male pigs at puberty and that is partially responsible for the undesired odor (Squires & Bonneau, 2014). With the exception of England, Ireland, Spain, the Netherlands, and a few other countries where rearing entire male pigs is becoming the standard, castration (regardless of the method used) has been the solution that the remaining swine producers have resorted to in order to eliminate the occurrence of boar taint (Hegerová & Juhás, 2021). However, despite being more considerate towards the welfare of pigs, rearing uncastrated boars has several disadvantages over castration on an economical and farm management level. Economically speaking, although feed efficiency is considerably better than that of barrows (Xue, et al., 1997), uncastrated boars have to be slaughtered at a much lower live weight in order to reduce the risk of boar taint with the latter remaining insufficiently low even at weights as low as 75 kg for some species (Squires & Bonneau, 2014). Although some studies have reported successfully rearing pigs to a live weight of 100-110 kg while maintaining a low risk of boar taint, this is still almost half of the target body weight of castrated barrows (Hegerová & Juhás, 2021). Regarding farm management, other than the fact that rearing entire pigs requires a high amount of expertise in order to provide an adequate diet that reduces the risk of boar taint (Hegerová & Juhás, 2021; Squires & Bonneau, 2014); mature uncastrated boars tend to exhibit aggressive male behavior and be more difficult when it comes to handling and restraint, which has been shown by studies to be the cause of an increase in the prevalence of lameness and cutaneous lesions in the animals and ultimately reverts back to an economic loss for the farmer since some of the pork meat will have to be culled (Holinger, et al., 2015).

For these reasons, castration is still considered a viable method of eliminating boar taint and the procedure is routinely performed in a considerable number of swine farms (McGlone, 2013). However, the method used for castrating these animals vary widely and along varies the concern towards animal welfare, cost-efficiency, and success rate of the procedure. Currently, castration of swine is comprised of 3 main categories: immunocastration, surgical castration with the use of anesthesia and/or analgesia and surgical castration without the use of anesthesia or analgesia. Immunocastration is the use of a vaccine consisting of 2 doses of a synthetic GnRH analogue administered at least 4 weeks apart to trigger the production of antibodies against the animal's endogenous GnRH. The first dose of the vaccine is only considered to prime the immune system without triggering any immune response against endogenous GnRH or testicular function and therefore the animal may exhibit sexual behavior and aggressiveness at puberty. The second dose, which should be given at least 4 weeks prior to slaughter, triggers the immune response therefore inhibiting testicular function and by extension reducing the risk of boar taint (Font-i-Furnols, et al., 2012; Pinna, et al., 2015). Surgical castration is generally performed at an early age with most references suggesting the ideal time for castration of piglets being between 1 and 14 days of age since restraint is easier at this time and less stressful (Carroll, et al., 2006). Although the common consensus is that surgical castration is painful at all ages (von Borell, et al., 2009) and despite several countries imposing the use of anesthesia and/or analgesia for the castration of piglets (Hegerová & Juhás, 2021), some still consider the use of pain mitigation as only necessary in piglets older than 14 days of age (Tucker, et al., 2020). The use of pain relief during castration mainly concerns piglets and premature boars, nevertheless, adult boars undergoing surgical castration also fall into the same category although this situation is not sufficiently discussed due to its scarcity. In fact, the castration of adult pigs is mostly an issue for show pig and small-scale farmers who often rely on natural insemination and therefore require the constant presence of entire boars in their flock. This is particularly the case of porcine farmers in Sardinia where the intervention of a veterinarian is often required for the surgical castration of boars prior to their slaughter.

Contrary to surgical castration of piglets, adult boar castration proves to be a more challenging task due to the difficult handling and restraint of adult boars which necessitates the use of general anesthesia along with the complications that may arise from it, as well as the larger diameter of the spermatic cord which increases the risk of hemorrhage, the most common complication of surgical castration. Another concern of surgical castration is the risk of surgical wound contamination and infection which is particularly higher in adult boars since hemostasis measures are necessary which implies more manipulation and even the potential use of ligatures and/or an emasculator (Anderson & Mulon, 2019). For this reason, we were tempted to attempt a surgical technique that can achieve hemostasis in adult boars while reducing the need for extensive manipulation or the introduction of foreign bodies. Being inspired by a castration technique that is today considered routine in feline surgery, the technique which will hence forward be referred to as sutureless castration was devised. This technique consists of an open castration where the vas deferens is separated from the rest of the testis and hemostasis is achieved by means of throwing 3 to 5 square knots using these two structures. This thesis aims to describe the surgical technique of sutureless castration and discuss the pilot and feasibility studies conducted leading to the repeatability study and short and long term follow up of animals that were castrated by this technique in order to determine the occurrence of post-surgical complications.

CHAPTER I: LITTERATURE REVIEW

1. Introduction

In the following section of the thesis, we will discuss the elements necessary for the understanding of the surgical techniques developed in the following parts of this manuscript. Therefore, an overview of the reproductive system of the boar will be given with particular emphasis on the anatomy, histology and physiology of the testis, epididymis, and spermatic cord as well as the adjacent structures relating to them. The accessory genital glands, penis and urethra and prepuce will not be discussed hereafter due to their lack of involvement in surgical castration.

2. Applied anatomy of the reproductive system of boars

The male reproductive system of swine consists of 3 parts: the paired testicular structures, accessory genital glands (*i.e.* prostate gland, vesicular gland, and bulbourethral gland) and the penis as seen in **Figure 1**. The testicular structures include the testis, epididymis, deferent duct (vas deferens or ductus deferens) and their investments. The function of the male reproductive system is the formation, stocking, and delivery of spermatozoa (Bonet, et al., 2013). The testicles of the boar are presented in a perineal or subanal position with the testes being angled dorso-caudally towards the anus (Cerveny, et al., 2004; Dyce, et al., 2010). The tail of the epididymis which is located caudally to the testis (Schatten & Constantinescu, 2007) is readily palpable through the scrotum as a nodule located dorsally on the testis (Kuster & Althouse, 2007).

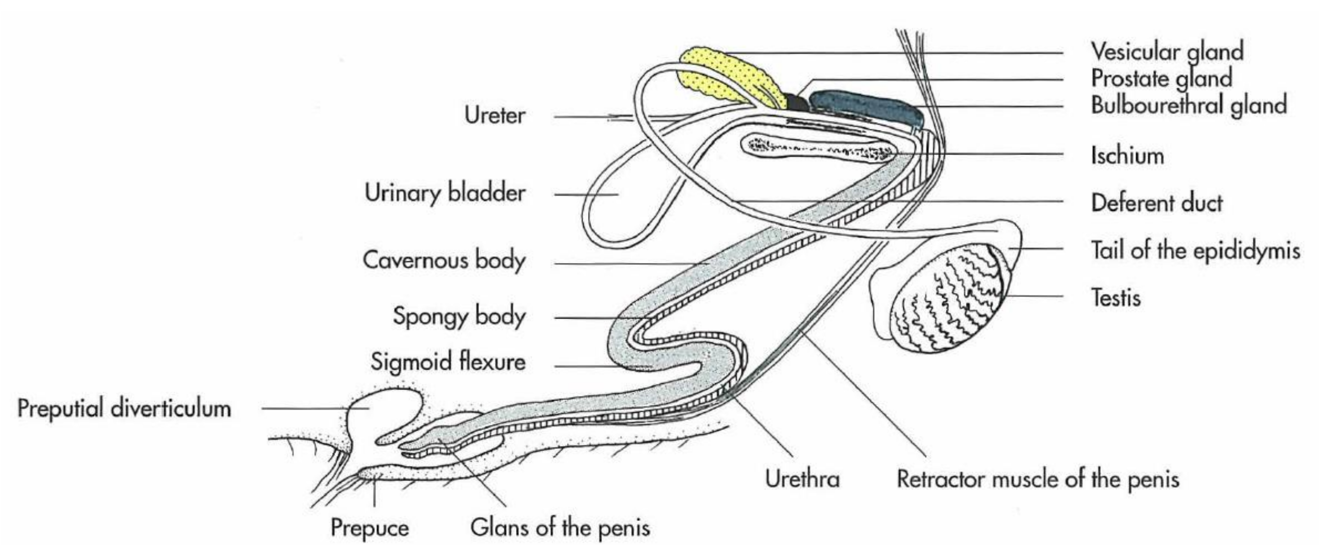

Figure 1: Schematic representation of the reproductive system of boars (Cerveny, et al., 2004)

2.1. Testis

The testes are the site of spermatogenesis and are also responsible for the production of the male steroidal hormone testosterone. They are located within the scrotal sac with the left testicle hanging slightly lower than the right one. Testis weight and ratio compared to a boar's weight varies depending on the swine species with each one reaching between 310 and 360 g in mature animal therefore not exceeding 0.45% of bodyweight (Bonet, et al., 2013). The most important increase in size and weight occurs between the 4th and 6th month of a piglets' life with the mature size being reached at 8 months of age (Kuster & Althouse, 2007).

The prenatal development of the testis occurs in the abdomen and takes origin at the gonadal primordium, medially to the mesonephros of the fetal lumbar area. Testicular migration into the scrotal sac starts at around the 60th day of gestation and should be completed around the 90th day, therefore piglets are born with both testes fully descended (Dyce, et al., 2010). This process can be divided into 2 phases: the transabdominal descent and the inguinoscrotal descent. The first phase, which is independent of the presence of androgens, is characterized by the expansion of the gubernaculum testis, a cord attaching the testis to the preformed vaginal process which is located within the scrotum. The expansion of the gubernaculum results in the dilation of the inguinal canal through which it passes and the sliding of the testes to the inguinal ring. The second phase is characterized by the regression of the gubernaculum under the influence of androgens, thereby pulling the testes through the inguinal ring and into the scrotum. Failure of this process to take place results in a retained testicle, a condition known as cryptorchidism. The gubernaculum in swine extends into the tunica dartos of the scrotum and therefore pulling the scrotum distally from the cryptorchid boar results in the exposure of the retained testis (Bonet, et al., 2013; Cerveny, et al., 2004).

The tunica albuginea is the most superficial layer of the testis and consists of an 800-950 µm thick fibrous capsule rich in collagen and fibroblasts but poor in elastic fibers and through which the testicular artery and vein run. The tunica albuginea overlays an even thicker layer of connective tissue (1400-1650 µm), the tunica vascular, which is also well vascularized and folds inwards forming the testicular septa separating the different lobules of the testis (Bonet, et al., 2013). These pyramid shaped lobules are the original site of spermatogenesis. They are composed of contorted seminiferous tubules located at the base of the lobule and extending all through it until they straighten toward the apex forming the aptly named, straight seminiferous tubules that open at the mediastinum of the testis converging all the tubules together into the rete testes or testicular network. The latter runs through the mediastinum finally converging into the efferent ducts before entering the head of the epididymis (Cerveny, et al., 2004).

2.2. Epididymis

The epididymis is tightly attached to the tunica albuginea of the testis and contains the epididymal duct which is highly contorted and has a length of 17-18 m. The epididymis can be divided into 3 parts: a head, body, and tail (Cerveny, et al., 2004). The tail is located caudally to the testis, exceeding its caudal apex and is palpable through the scrotum while the head of the epididymis is located at the cranial apex of the testis with a cranio-ventral orientation and slightly exceeding the testis (Schatten & Constantinescu, 2007). The head is the result of the efferent ducts converging in the apex of the testis to form the beginning of the epididymal duct. It is attached to the tail of the epididymis through the body which is the longest structure of the epididymis. Although they are tightly attached, the space that can be created by detaching the body of the epididymis from the testis is called the epididymal bursa and is of surgical importance. The tail is attached by the ligament of the tail of the epididymis to the vaginal tunic, a fold of the abdominal peritoneum that passes through the inguinal canal and is invaginated in the scrotum, enveloping the testis and spermatic cord. The ligament of the tail of the epididymis is particularly developed in swine, with fibers extending from it and being incorporated into the deep layer of the scrotum. The tail is also attached to the testis through the proper ligament (Cerveny, et al., 2004).

2.3. Vas deferens

The vas deferens or deferent duct (also known as ductus deferens) rises from the tail of the epididymis at the caudal end of the testis and travels through the spermatic cord to eventually reach the urethra. At its' epididymal end, the vas deferens starts as an undulating structure, slightly continuing the same contorted aspect seen in the epididymal duct, however as it passes across the medial border of the testis and into the spermatic cord, it gradually straightens as can be seen in **Figure 2** (Cerveny, et al., 2004). The vas deferens is particularly long in swine due to the perineal localization of the testes and their dorso-caudal orientation, which is the same as that of the tail of the epididymis. This orientation also explains the almost horizontal direction that the vas deferens takes as it leaves the testis to reach the urethra after passing through the inguinal ring, which has a very oblique caudoventral orientation in swine thus facilitating the occurrence of scrotal hernia (Schatten & Constantinescu, 2007).

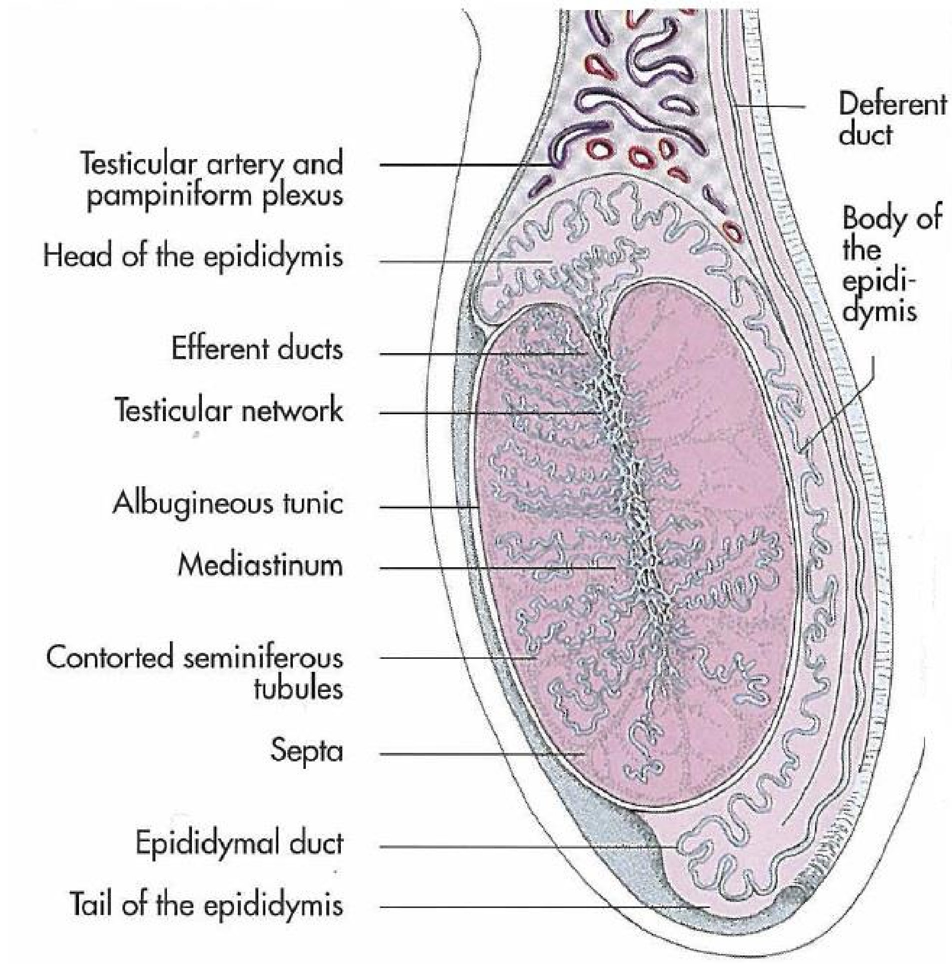

Figure 2: schematic representation of a transverse section of the testis, showing the different structures of the testicular parenchyma, the epididymis and deferent duct (Cerveny, et al., 2004)

2.4. Testicular vascularization and innervation

The testes are vascularized by the testicular artery, a direct branch of the abdominal aorta that runs along the abdominal wall and through the inguinal canal. The testicular artery passing in a highly convoluted manner near the vas deferens supplying it with blood through the rami ductus deferentis, branches into the rami epididymales at the level of the epididymis. Venous return starts with the pampiniform plexus, a mesh-like structure around the arterial coils that eventually converge into the testicular vein that drains into the caudal vena cava. On the other hand, the external pudendal artery and vein supply and drain blood from the investments of the testis. Lymphatic drainage occurs in the lumbar aortic and medial iliac lymph nodes. Parasympathetic fibers deriving from the vagal nerve and the pelvic plexus and sympathetic fibers arising from the caudal mesenteric plexus and the pelvic plexus reach the testis through the inguinal canal and insure its' innervation (Cerveny, et al., 2004).

2.5. Investments of the testis

The investments of the testicles are the different layers of skin, muscle, fascia, and membranes that envelope the testis, epididymis, and vas deferens as well as their vascularization and innervation. These are commonly divided into 2 sections: the scrotum and the vaginal process. The scrotum is further subdivided into several parts, the most external of which is the scrotal skin which is practically hairless in boars and is firmly interconnected with the tunica dartos, a subcutaneous fibromuscular layer surrounding the left and right testes joining together at the middle of the scrotum to form the scrotal septum separating the testes. The tunica dartos is involved in the regulation of testicular temperature through the contraction of the smooth muscle fibers forming it (Cerveny, et al., 2004; Schatten & Constantinescu, 2007).

The external spermatic fascia lies underneath the dartos tunic and results in the detachment of the superficial fascia covering the external abdominal oblique muscle. It is followed more deeply by the cremaster muscle, which is in turn a detachment of the internal abdominal oblique muscle into the inguinal canal and is covered by the cremasteric fascia (Cerveny, et al., 2004).

The vaginal process is formed by the invagination of peritoneum and transverse fascia through the inguinal canal and into the scrotum. It consists of the internal spermatic fascia and the parietal tunica vaginalis the former being a continuation of the transverse fascia while the latter that of the parietal peritoneum. On the other hand, the visceral tunica vaginalis is the continuation of the visceral peritoneum and it lies underneath the parietal tunic, separated by a minimal amount of peritoneal fluid within a space termed the vaginal cavity (Cerveny, et al., 2004).

At the level of the vas deferens, the visceral tunica vaginalis envelops the testicular vessels and nerves as well as the deferent duct thus forming the spermatic cord. The spermatic cord is surrounded by the vaginal process which at this level is called the vaginal canal and the space between the two which is the continuation of the vaginal cavity becomes tighter and is known as the vaginal opening as the spermatic cord runs through the inguinal canal (Schatten & Constantinescu, 2007).

The parietal and the visceral lamina of the tunica vaginalis are linked through the mesofuniculus. The mesorchium which can be seen in **Figure 3** is a fold of the visceral vaginal tunic enveloping the testicular and epididymal blood and lymph vessels and innervation; and is divided into a proximal part extending between the vaginal canal and the epididymis, and a distal part between the epididymis and the testicle with the limit between the two being termed the mesepididymis (Schatten & Constantinescu, 2007).

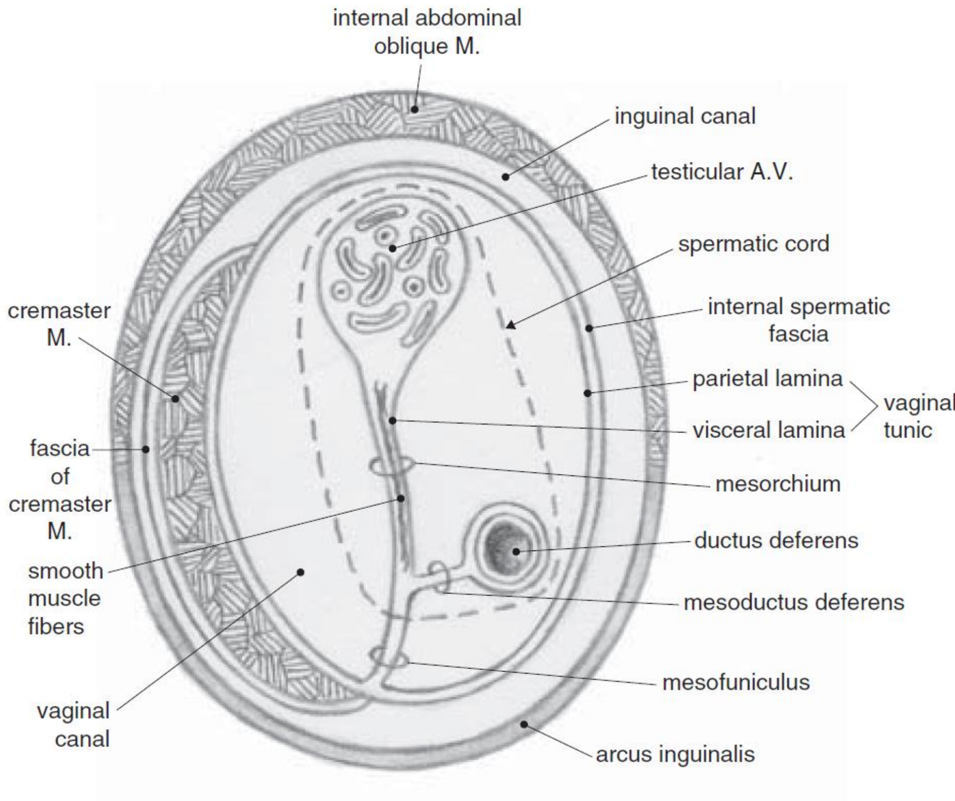

Figure 3: Schematic representation of a transverse section of the spermatic cord (Schatten & Constantinescu, 2007)

3. Histology and physiology of the reproductive system of boars

The male gonads or testes have the essential function of producing sperm, although they function in a second place as important endocrine glands, releasing the androgenic steroidal hormone testosterone, which is responsible for the exhibition of male sexual characteristics (Klein, 2013). This hormone is synthesized and secreted in the testicular stroma by the Leydig cells while the Sertoli cells found in the parenchyma synthesize activin and inhibin (Bonet, et al., 2013).

Inside the scrotal sac of an adult healthy boar, the left testicle hangs slightly lower than the right. Nevertheless, there should be no noticeable difference between the weight of the two testes (Pinart, et al., 2001).

The seminiferous tubules are responsible for the production of the spermatozoa through a process consisting of 3 phases: spermatogenisis, spermiogenesis and spermation. The process of spermatogenisis and spermiogenisis starts at the age of 3 months in boars while spermatozoa will only start appearing in the seminiferous tubules at the age of 4 months. Puberty would only begin at the age of 5 months and testes size, ejaculate volume and concentration will start increasing significantly at the age of 6 months until reaching their peak and stabilizing around 18 months of age (Bonet, et al., 2013).

Spermatozoa travel from the seminiferous tubules to the epididymis through the testicular network and efferent duct. At the level of the epididymis, spermatozoa undergo a complex process of maturation and will be stored in the tail of the epididymis until eventual ejaculation (Yeste, et al., 2012). Ejaculated spermatozoa travel through the deferent duct to the urethra where the secretions of the annex glands are found. Finally, semen travels through the urethra to the penis where it will be ejaculated (Klein, 2013).

Prostatic secretions contribute for 55-75% of the volume of ejaculate, followed by bulbourethral gland and seminal vesicle secretions at 10-25% and 15-20%, respectively. The contribution of the testes and epididymis is the lowest, accounting for only 2-5% of the ejaculate volume (Bonet, et al., 2013).

Pork meat can produce an undesirable odor termed boar taint, when skatole and androstenone accumulate in it. Androstenone is a steroid of testicular origin that has a typical urinal ammonia smell. It is produced by the Leidig cells of mature boars and has a highly lipophilic nature which explains its' high affinity to adipose tissue where it accumulates (Gower, 1972). In fact, after androstenone leaves the testicle and reaches the systemic circulation it gets mainly stocked in adipose tissue and in the salivary glands where it acts as a pheromone stimulating sexual behavior in the sow (Bonneau, 1982).

Skatole on the other hand is produced by the intestinal microflora and has no obvious physiological function but rather a toxic one for almost all the other animals although boars are resistant to its toxicity. It is the byproduct of bacterial breakdown of the amino acid tryptophane and is mostly excreted through feces, however a part of it gets absorbed by blood and is then metabolized in the liver. However, in the process of hepatic metabolism of skatole is inhibited by steroidal hormones such as androstenone and therefore, high levels of the latter lead to an increased accumulation of skatole (Zamaratskaia & Squires, 2009).

The main aspect determining boar taint in meat is the equilibrium between production and catabolism of androstenone and skatole. This equilibrium is influenced by various intrinsic and extrinsic factors most notably, nutrition and genotype. Surgical castration can prevent the formation of androstenone, however, when performed on prepubescent boars it can lead to disadvantages in weight gain since it halts the synthesis of testicular steroids such as testosterone and estrogen therefore negatively affecting the growth of lean muscles and food conversion rate. Castration of adult boars avoids the disadvantages previously mentioned and reduces the risk of boar taint. Indeed, since the adipose tissue storage of androstenone is reversible, the elimination of its' origin results in the decline of its' level gradually until the disappearance of the boar taint (Bonneau, 1982).

CHAPTER II: A CRITICAL REVIEW OF SURGICAL CASTRATION IN ADULT FARM ANIMALS

1. Introduction

The ideal in routine husbandry procedures consists of 3 major points: cost-efficiency, time-efficiency, and success rate. A fourth point has also been gathering growing interest in the field of husbandry: animal welfare and pain mitigation following routine procedures. In fact, studies on consumer preferences have consistently proved the willingness of consumers to pay up to 25% more for a product respecting animal welfare (Bozzo, et al., 2019; Dransfield, et al., 2005). Pursuing these ideals has been the aim of many studies regarding husbandry procedures such as castration which tend to be painful a procedure with varying cost, procedure length, and success rate depending on the conditions in which it is performed such as animal age and technique used among many others. These techniques can be divided in 2 major groups: surgical and non-surgical. The latter can be chemical, *i.e*., chemical castration, *i.e.* immune castration and intratesticular injection of chemo-sterilant substances such as sodium chloride and calcium chloride; or physical, *i.e.*, Burdizzo castration, rubber ring castration and banding. The efficacy of these techniques is variable depending on several animal factors and many studies compare these methods to each other (Robertson, Kent, & Molony, 1994). However, all studies agree that these procedures are always painful and are preferably performed on young animals in order to ensure the best success rate with the least amount of pain (Guatteo, et al., 2012).

In both ruminants and swine, castration is generally recommended at an early age for it being less stressful, expensive, time consuming, and painful for the animals (Anderson & Mulon, 2019; Ewoldt, 2008). Nevertheless, some farmers might choose to grow entire males for varying reasons, such as the desire to benefit from the faster growth rate that is seen in entire bulls and boars (Ames, 2014; Anaruma, et al., 2020). Therefore, it is not uncommon for veterinarians to find themselves facing a situation where they are required to castrate adult farm animals, which is a more complicated surgery as it requires some form of anesthesia, and depending on the situation, the implementation of some hemostatic measures prior to the excision of the testis.

Whereas young farm animal castration is well documented in textbooks and scientific publications, that of adult swine, bovine, ovine and caprine is less discussed. For that reason, we found it to be interesting to conduct database research allowing to collect all current knowledge on the different techniques used for the castration of the adult boar, bull, ram, and buck with the objective of comparing these techniques to each other when possible in regards to the occurrence of post-surgical complications, animals welfare and cost-efficiency, among other factors that may be of interest for the veterinary practician help him to decide which method to adopt in different situations.

2. Swine castration

Pork meat is subjected to monitoring under European regulation (EC No 854/2004 of the European Parliament and of the Council of 29 April 2004) to ensure the absence of boar taint, a foul meat odor that is caused mostly by the presence of skatole, which results from the breakdown of tryptophane by the intestinal microflora; and androstenone, a steroid produced by the testes of mature boars that is released in the systemic circulation. The latter accumulates in the salivary glands, but also most importantly in adipose tissue along with skatole, where the two compounds have a synergic effect leading to boar taint (Bonneau, 1982).

Castration highly reduces the risk of boar taint since fat tissue storage of androstenone is reversible and in the absence of the testes, its levels start declining until reaching one that does not produce any significant boar taint (Bonneau, 1982). This can be accomplished either by surgical castration, otherwise known as orchiectomy or by pharmaceutical castration which encompasses immunocastration, where a vaccine sensitizing the immune system against GnRH is administered in 2 doses; chemical castration, where necrotizing chemical compounds are directly injected in the testes; and finally hormonal castration where the hypothalamic-pituitary-adrenal axis is inhibited through the administration of exogenous hormones (Bonneau & Weiler, 2019).

However, orchiectomy, which is usually performed on newborn piglets, has been at the center of debates regarding animal welfare issues leading numerous pork producers to shift towards the rearing of entire boars. Nevertheless, this has several economic and farm management disadvantages since these animals are slaughtered at a much lower bodyweight than castrated pigs and the persistence of aggressive male behavior often leads to significant issues of lameness and cutaneous lesions within the farm (Hegerová & Juhás, 2021; Holinger, et al., 2015). This may explain why so many European farmers still endorse castration, with orchiectomy more specifically being routinely performed in more than 80% of swine farms of most European countries as seen in **Figure 4** (Higuera, 2019).

When performed on newborn piglets, orchiectomy is considered to be a rather simple procedure, however veterinarians often find themselves castrating adult boars which tends to be more complicated due to several factors such as: the difficulty of handling and restraining boars; the necessity for sedation and/or general anesthesia as well as analgesia; the need for hemostasis measures prior to the excision of the testes; and the increased risk of postsurgical complications such as excessive hemorrhage, inguinal hernia and surgical wound contamination and infection (Anderson & Mulon, 2019). Therefore, the techniques used for the surgical castration of piglets differ significantly from the ones used on mature boars.

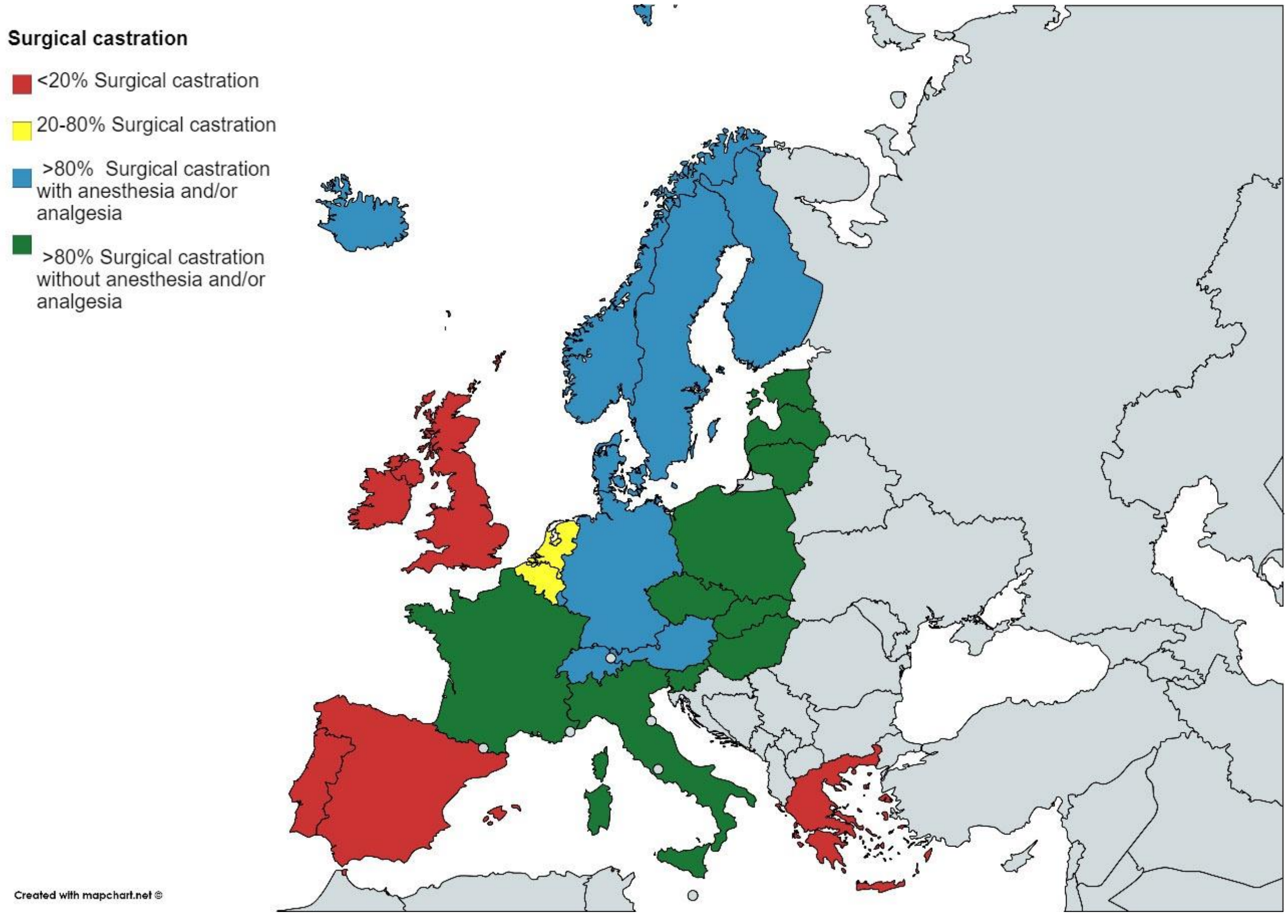

Figure 4: Map showing the percentage of swine farms that perform surgical castration in different European countries (Higuera, 2019)

2.1. Database Research

Following a complete database research on CABI, Web of Science, PubMed and Scopus using the keywords ((swine) OR (pig) OR (hog) OR (boar)) AND ((surgical AND castration) OR (orchiectomy) OR (orchidectomy)), a total of 2473 records were found which were then screened for relevance by the title and abstract according to our inclusion criteria. The inclusion criteria consisted of 1) the animal species: commercial pigs, therefore pet pigs were excluded; 2) the age of the animals: older than 2 months; 3) text containing a description of surgical castration (whether brief or detailed) in healthy, non-cryptorchid animals. In the beginning of the research, the criteria were limited to original articles accurately describing surgical castration of boars older than 5 months of age (*i.e.* beginning of puberty), however, due to the scarcity of such records, the age limit of the animals had to be lowered and the restriction on the type of published records had to be removed. Thereafter, 4 textbooks and one original article found through cross references and external resources were added to the results of the database research. Despite lowering the threshold of our inclusion criteria, only 9 records were ultimately eligible for inclusion in the full-depth study. These records were subjected to full text screening and relevant information was analyzed and extracted. Information regarding surgical technique were divided into 4 categories; 1) approach to the tunica vaginalis; 2) site of incision; 3) hemostasis technique and 4) closure of surgical incision. This data was reported into a summary table (**Table 1**), allowing us to easily identify all the different techniques that have been used for orchiectomy in adult boars.

2.2. Surgical Techniques

2.2.1. Approach towards the tunica vaginalis

The tunica vaginalis or vaginal tunic is an extension of the abdominal peritoneum passing through the inguinal canal and enveloping the testes and spermatic cord within the scrotal sac. Similarly to the peritoneum, the vaginal tunic consists of 2 laminae: the visceral lamina which is in direct contact with the testis and the parietal lamina adjacent to the scrotum and its underlying structures, with the 2 laminae being separated by the vaginal cavity filled with a modest amount of peritoneal fluid reducing the friction between the wall and the enclosed testicular structures (Cerveny, et al., 2004).

Surgical castration of farm animals can be performed with 2 different approaches regarding the opening of the tunica vaginalis. In the closed technique the parietal tunic is not opened and therefore is excised along with the spermatic cord and testis, while it is completely opened at the level of the testicle in the open technique and the testis is detached from the ligament of the tail of the epididymis and prolapsed from the parietal tunic in order to allow for the emasculation of the vessels and ductus deferens separately. Both techniques can be used on cattle (Petherick, et al., 2014a; Yamada, et al., 2021a), small ruminants (Melches, et al., 2007; Oyeyemi & Akusu, 1998) and equine (Searle, et al., 1999). A third technique exists known as semi open castration where a small vertical incision is made through the parietal tunica vaginalis over the spermatic cord in order to allow for the emasculation of the exteriorized vascular structures prior to emasculating the entire cord, although no publications mention the use of this technique in adult farm animals, but only in horses (Searle, et al., 1999).

In adult boars, the open and closed techniques are equally used (Anderson & Mulon, 2019; Lehmann, et al., 2017b) although little explanation is given in published records as to the advantages and disadvantages of each one in this animal species. The choice of approach to the tunic vaginalis is usually made according to the surgeon's preference. Indeed, contradicting notions as to which method should be used in old pigs are found in textbooks. Some consider open castration to be the preferred method for castrating adult boars (Dyce, et al., 2010) probably due to the need for direct access to the vascular portion of the spermatic cord and subsequent ligation and/or emasculation therefore achieving proper hemostasis. On the other hand, other textbooks consider the closed technique to be more suitable for boars (Anderson & Mulon, 2019). Unfortunately, no published studies were found comparing closed and open castration in adult swine or the postoperative complications of each technique. However, it is commonly known that the inguinal ring in boars is very large compared to other animal species and this predisposes to one of the most common complications of open castration which is inguinal hernia (Anderson & Mulon, 2019; Holtgrew-Bohling, 2020; Salcedo-Jiménez, et al., 2020). It is actually recommended to inspect the spermatic cord while still enveloped by the tunica vaginalis in closed castration, and after stripping it from the adjacent fascia, to ensure the absence of herniated viscera (Callan, et al., 2017). Nevertheless, some surgeons performing open castration opt for the suturing of the vaginal tunic after the excision of the testis in order to reduce the risk of inguinal hernia (Lehmann, et al., 2017b) or even closing the external inguinal ring when the boar has a preexisting inguinal hernia (Salcedo-Jiménez, et al., 2020). The closure of the external inguinal ring in the pet pigs that were included in the aforementioned study, was associated with a significantly higher risk of postsurgical edema, most likely due to the excessive manipulation.

2.2.2. Site of incision

2.2.2.1. Scrotum

The scrotum is the most obvious site of incision for orchiectomy since it is the structure that grants the fastest access to the testes and spermatic cord. The scrotal approach is used in both adult (Hughes & Berger, 2018) and newborn pigs (Pérez-Pedraza, et al., 2018) as well as other large animals such as cattle, horses, sheep, and goats. In adult boars, two 4 to 6 cm incisions (one over each testis) are most often used in the scrotal approach of orchiectomy (Huber et al., 2013). These incisions are parallel to the median raphe and should be made as ventrally as possible in order to allow easier drainage (do Prado, et al., 2018). On occasion, it is recommended to excise the median raphe at the end of the surgery of heavyweight boars (*i.e.* weighing more than 135 kg) to allow for better drainage (Ames, 2014). A single incision scrotal approach has been reported in piglets and consists of a horizontal incision extending from one testicle to the other. In this study, blood samples were collected at 5 moments extending from 24h prior to 48h after the surgery, from 60 five-day-old piglets being castrated either by the two incision or by the single incision technique, with or without analgesia. The samples were analyzed for pH, hematocrit, glucose, electrolytes, and other parameters and showed that piglets castrated with the single incision technique tended to exhibit a higher level of postsurgical metabolic acidosis than those castrated with a double incision. This was estimated to be caused by the dissection of a larger amount of connective tissue in the single incision method making it more invasive (Pérez-Pedraza, et al., 2018).

2.2.2.2. Parainguinal

The parainguinal or prescrotal approach refers to the area located between the inguinal ring and the scrotum. When the pig is in dorsal recumbency, the testis can be pushed cranially into the inguinal area and an incision is made over the relocated testis. This inguinal incision allows access to the testis and spermatic cord and is mostly used in newborn pigs castrated while being held upside down from their hind limbs by a veterinary technician (Callan, et al., 2017). The reason this approach is sometimes recommended in piglets is probably due to the better drainage of the surgical opening given its' anatomical positioning. This approach may also provide a better visualization of the full length of the spermatic cord and inguinal ring, allowing more adequate inspection for inguinal hernia and the resection of the spermatic cord closer to the inguinal canal which could be closed if necessary. It has only been mentioned in adult boars on a single occasion where 7 months old boars were castrated by accessing the testes through a 3 cm median parainguinal incision and the effect of the procedure on tissue growth was assessed by taking blood samples on a period extending up to 7 weeks after the surgery. The most notable difference in the parameters studied between castrated and uncastrated animals was in the IGF-1 serum levels which were higher in castrated boars as compared to non-castrated, although this difference did not translate into a significant difference in live body weight of the two groups (Umapathy, et al., 1997). The pre-scrotal approach has also been described in pet pigs undergoing routine castration surgery which allowed the examination of the inguinal ring and its' subsequent closure when it was deemed too wide or in the case of preexisting inguinal hernia (Salcedo-Jiménez, et al., 2020). An inguinal approach where the incision is made directly over the inguinal ring, is also used for the castration of pigs with cryptorchidism since this allows the direct identification of the gubernaculum and subsequent extraction of the abdominal testis through the inguinal incision (Scollo, et al., 2016).

2.2.3. Hemostasis technique

Depending on the age and weight of the boar, some form of hemostasis may be necessary. The lack of implementation of hemostasis can lead to potentially fatal hemorrhage even in relatively small sized pigs. In fact, this has been previously recorded in a 7-weeks-old swine that was castrated with a knife without any hemostatic measures leading to fatal excessive hemorrhage in the pelvic canal and abdomen (Callan, et al., 2017). Nevertheless, little to no hemostasis may still be acceptable in newborn piglets which can be safely castrated by directly cutting the spermatic cord with a sharp instrument (Viscardi, et al., 2020) or simply by manually tearing (Anderson & Mulon, 2019) or scarping it with a scalpel or surgical knife until it breaks which still provides some sort of hemostasis due to the elastic nature of blood vessels (Ames, 2014). More extensive hemostasis measures have also been described in piglets aged between 1 and 24 days where an encircling ligature was placed around the spermatic cord of the animals during castration (White, et al., 1995).

Hemostasis can be achieved by three main methods which may be used together or separately in older pigs. Twisting and crushing the spermatic cord rely on the elasticity of the testicular artery and vein to achieve hemostasis and are usually coupled with the ligation of the spermatic cord especially in heavyweight boars. Twisting has been described in 2 textbooks and is achieved by continuously rotating the exteriorized testis until the spermatic cord is tightly compressed near the external inguinal ring. In these records, the use of 2 transfixation (Anderson & Mulon, 2019) or circumferential ligatures (Callan, et al., 2017) concomitantly to twisting was recommended. While surgical sutures have been used traditionally for the castration of adult boars (Hughes & Berger, 2018; Lehmann, et al., 2017b), a recent study has shown the possibility of using small sized nylon clamps, otherwise known as zip-ties, to achieve preventive hemostasis as seen in **Figure 5** (do Prado, et al., 2018). These clamps have previously been successfully used in cattle (Silva, et al., 2009) and in horses (Silva, et al., 2006) undergoing routine surgical castration. In their clinical study, do Prado et al. (2018), compared the effect of double ligation of the spermatic cord with either a nylon clamp or surgical nylon suture during open castration of 3 months old boars. Their novel technique outperformed surgical suture ligation in terms of surgical time (1.7 min Vs 2.68 min with $p<0.05$) and blood loss (0.81 mL Vs 1.9 mL with $p<0.05$), while showing no difference in terms of weight gain and inflammatory response up to 7 days after the procedure (do Prado, et al., 2018).

Finally, crushing the spermatic cord, which is usually achieved using an emasculator (**Figure 8**), has been described both as a complement to ligation (Baars, et al., 2013) or as a stand-alone hemostatic measure (Umapathy, et al., 1997). The emasculator should be applied over the spermatic cord as close as possible to the inguinal ring and in the case of concomitent ligation, distally from the ligatures. It is maintained closed over the spermatic cord for 30 seconds at least (Ames, 2014). In the closed castration of adult boars, the use of ligation is always recommended since the main advantage of this technique is reduced risk of inguinal hernia which can only be achieved by the closure of the vaginal cavity and this can only be secured by proper ligation of the tunica vaginalis, emasculation alone not providing sufficient closure (Anderson & Mulon, 2019).

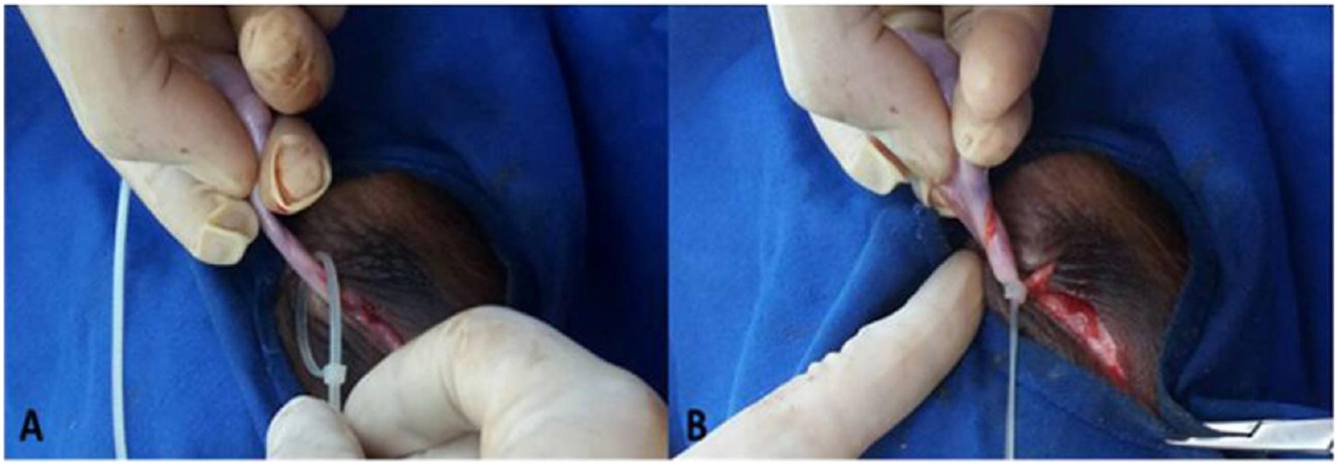

Figure 5: orchiectomy of 3 months old boars using nylon clamps to achieve hemostasis; A- the nylon clamps are placed around the spermatic cord that has been stripped from the tunica vaginalis; B- the clamp is tightened until and another clamp is placed similarly on the same testis distally to the first one (do Prado, et al., 2018)

2.2.4. Closure of the surgical incision

When castrating farm animals with the scrotal or parainguinal approach, the surgeon has the choice to leave the wound to heal by secondary intention or alternatively to allow for primary closure by suturing the surgical incision. Regardless the animal species, most references agree that under field conditions the wound is preferably left open for secondary intention healing in order to ensure proper drainage in case of infection (Callan, et al., 2017). In horses however, primary closure is often described and despite being three times more expensive than castration with secondary healing, studies have shown it to be less associated with evisceration and to allow accelerated healing and shorter convalescence (Crosa & Desjardins, 2018; Maxwell, 2009). In adult boars, primary closure is not recommended unless asepsis during the surgery was maintained which is rarely the case in field conditions. This explains the fact that most studies describing primary closure of the scrotal incision were ones conducted on boars kept in a research facility where the sanitary conditions are probably superior to those found in the field whether during or after the surgery (Hughes & Berger, 2018; Lehmann, et al., 2017b).

3. Bovine castration

Unlike swine, where castration is necessary prior to slaughter in order to reduce the risk of boar taint as previously mentioned; bovine castration is mainly performed in order to increase meat quality by improving fat deposition and to reduce aggressivity in bulls (Amatayakul-Chantler, et al., 2013). This procedure is usually performed in calves at an early age, with the general recommendation being to castrate animals at most immediately after weaning or at a weight less than 225 kg. Not only is the castration of young calves easier to perform due to the limited need for excessive restraint, anesthesia, and hemostasis; but it is also considered to be less stressful to the animal when compared to postpubescent castration (Mellor, et al., 1991). However, when compared to steers, bulls have a higher growth rate which has led some researchers to suggest castrating bulls after the age of 14 months, thus benefiting from the rapid growth of bulls and greater muscularity, while also gaining the higher meat quality developed in castrates (Anaruma, et al., 2020; Knight, et al., 1999). Therefore, it is not uncommon for a veterinarian to be requested to castrate adult bulls.

The pendulous aspect of the scrotum in ruminants allows the use of variable methods of castration that do not require incising the scrotum for direct access to the spermatic cord, as is necessary for swine, which have a perineal scrotum (Cerveny, et al., 2004). These methods are commonly known as bloodless castration techniques and rely on the principle of compromising the testicular vascularization, thus causing ischemic necrosis of the testis. This can be achieved by the transcutaneous crushing of the spermatic cord at the level of the scrotal neck with a Burdizzo emasculatome (**Figure 6**) or by placing with an elastrator or callicrate bander (**Figure 6**) a constricting rubber ring or latex band at the base of the scrotum and leaving it until necrosis is achieved (Gilbert, et al., 2017). Although some of these techniques can be used in older bulls, with the callicrate bander being in fact designed for that exact purpose (Gilbert, et al., 2017; Knight, et al., 2000), they may cause more pain and swelling than surgical castration, which is why many veterinarians still prefer surgical castration for heavy weight bulls (Coetzee, et. al., 2010; Stafford & Mellor, 2005).

Figure 6: Some of the tools used for bloodless castration include the Burdizzo castrator (**A** and **B**), bander (**C**) and elastrator (**D**); Burdizzo castrators come in different sizes according to animal size with the one seen in image **B** being used for calves and small ruminants while the larger castrators seen in image **A** being reserved for larger bulls; similarly, the elastrator (**D**) is used for applying the elastic rings mainly for small animals whereas the Callister bander (**C**) can be used for all animals despite it being designed initially for older ones

3.1. Database Research

Following a complete database research on CABI, Web of Science, PubMed and Scopus using the keywords ((bull) OR (cattle) OR (calf) OR (calves) OR (bullock)) AND ((surgical AND castration) OR (orchiectomy) OR (orchidectomy)), a total of 1652 records were found which were then screened for relevance by the title and abstract according to our inclusion criteria. The inclusion criteria consisted of 1) the animal species: bovine; 2) the age or weight of the animals: older than 6 months or weighing more than 200 kg; 3) text containing a description of surgical castration (whether brief or detailed) in healthy, non-cryptorchid animals. A total of 35 original articles were found to meet these criteria. These records were subjected to full text screening and relevant information was analyzed and extracted. Information regarding surgical technique were divided into 4 categories; 1) approach to the tunica vaginalis; 2) site of incision; 3) hemostasis technique and 4) closure of surgical incision. This data was reported into a summary table (**Table 2**), allowing us to easily identify all the different techniques that have been used for orchiectomy in adult bulls. Farm management and surgery textbooks were used as a reference for common practices of orchiectomy in adult bulls but were not included in the list of records meeting the criteria of the database research.

3.2. Surgical Techniques

3.2.1. Approach towards the tunica vaginalis

As previously mentioned for boars, both open and closed castration have been known to be used in adult bulls, with no exact consensus as to which technique is superior. Some textbooks indeed recommend the use of the open technique for bulls weighing more than 225 kg (Ames, 2014) which may be attributed to the need for a direct access to the testicular vasculature for hemostasis, whereas others only describe the closed technique (Baird, 2013) and a few others describe both techniques without suggesting any advantages or disadvantages for each (Gilbert, et al., 2017). In fact, among the records found during our database research, none studied the difference between the open and closed methods of castration in adult bulls. Out of the 35 records that met our criteria for inclusion, 12 mentioned using the open technique, 8 mentioned using the closed technique, and the rest, 14/35, didn't specify which approach was employed.

On the other hand, a technique not requiring the opening of the tunica vaginalis, while also not being categorized as neither a closed castration nor a bloodless castration, has been suggested. This technique known as pinhole castration, involves the *in situ* ligation of the spermatic cord by passing a hypodermic needle through the scrotal neck while the spermatic cord is held tight on the lateral side of the scrotum. A surgical suture is then passed through the needle and the latter is removed. The spermatic cord is then moved to the opposite side of the scrotum, *i.e.* the median raphe, thus partially encircling it by the suture. After reintroducing the needle again through the same holes and passing the cranial end of the suture through it, the spermatic cord becomes fully encircled by the suture and the needle can be removed and ligation finished by tying a tight knot (**Figure 7**). This technique has been first experimented on young calves (48-56 days of age) (Ponvijay, 2007), but has since then been successfully used to castrate bulls of 1 year of age or older (Okwee-Acai, et al., 2016).

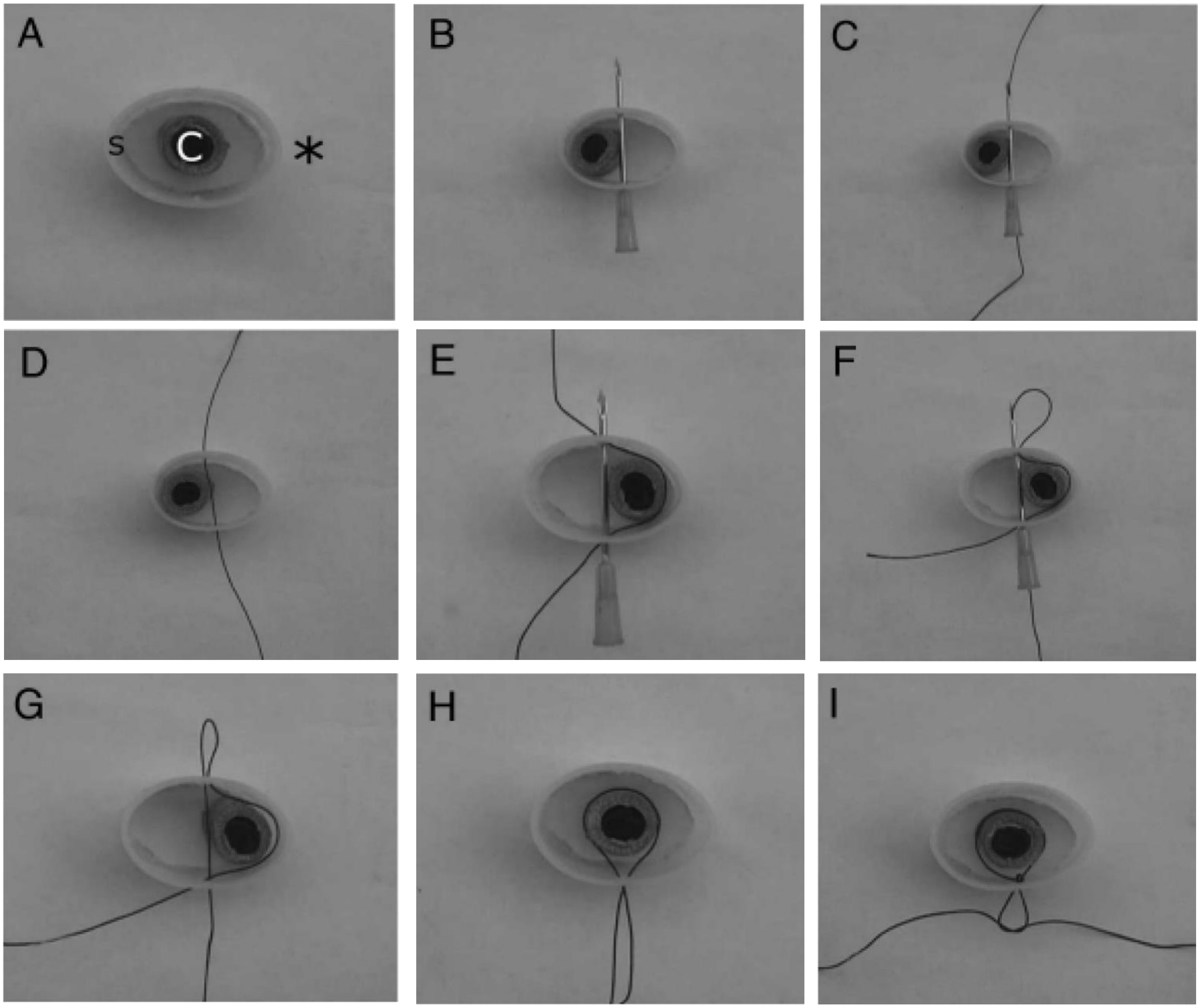

Figure 7: pinhole castration was first described by Ponvijay (2007) in calves aged 48-56 days; A- the spermatic cord (C) can be seen in the middle of the scrotal neck (S) with the median raphe being marked by the asterisk; B- the spermatic cord is manipulated to the lateral side of the scrotal neck and a hypodermic needle is passed through the scrotum; C- a surgical suture is passed through the needle; D- the needle is withdrawn leaving the suture passing through the scrotum; E- the spermatic cord is manipulated to the side of the median raphe and is now half covered by the suture, at which point the needle is reinserted through the same holes; F- the suture is passed again through the needle; G- the needle is finally withdrawn; H- and the suture is pulled tight thus covering the entire spermatic cord at which point; I- a few square knots are thrown, leaving the spermatic cord ligated in situ

In the study first describing pinhole castration, no complications were observed, and all calves showed testicular atrophy accompanied by complete ischemic necrosis which was confirmed by histology

(Ponvijay, 2007). However, when this procedure was studied in older bulls (1 year of age), 5% of the animals did not seem to show any testicular atrophy, although 94% of these cases were later confirmed to be cases of hydrocele, which was therefore reported for the first time as a possible complication of pinhole castration in adult bulls (Okwee-Acai, et al., 2016). In fact, pinhole castration appears to be less effective in animals with large spermatic cords, with a similar failure to achieve complete atrophy observed in 1-2 year old donkeys castrated using this technique (Abou-Ahmed, et al., 2012). Nevertheless, pinhole castration remains a cost and time-efficient method of castrating young bovines and small ruminants, with its' efficacy being more questionable in large sized animals (Okwee-Acai, et al., 2016).

3.2.2. Site of incision

Unlike in boars, where a parainguinal approach is possible, the scrotum is the only viable option for access to the testis and spermatic cord in cattle. However, several methods are recognized allowing the opening of the scrotum. A scalpel can be used to make a horizontal incision of the scrotum (Baird, 2013) or a longitudinal one on the lateral side (Silva, et al., 2009) or on the caudal side, parallel to the median raphe (Fernandes, et al., 2016; Ferreira, et al., 2015). The horizontal caudoventral incision is thought to have the advantage of allowing better surgical wound drainage (Bertagnon, et al., 2017; Devant, et al., 2012). Alternatively, a Newberry knife (**Figure 8**) can be used to open the scrotum. In this technique, the surgeon places the Newberry knife over the middle of the scrotum after placing some tension over it to retract the testis dorsally. Then, with a rapid motion, the surgeon pierces the scrotum and pulls the knife distally, thus creating a cranial and caudal flap of the scrotum. Finally, the distal third of the scrotum can be completely excised with a scalpel, a method which is thought to improve drainage (Gilbert, et al., 2017). Between the original articles included in our study, the majority of the authors (10/35) reported the use of the Newberry knife for opening the scrotum, while caudoventral incision, longitudinal incision and scrotal excision were reported 2, 4 and 5 times respectively. On 9 other occasions, the authors failed to mention the specific method used for incising the scrotum, or did not mention scrotal incision entirely.

In a comparative study between longitudinal incision and excision of the scrotum, it was found that the former caused less severe pain in the postsurgical period, which was explained by the author by the fact that scrotal excision affects a larger number of nerves and leads to more bleeding. However, surgical technique did not seem to affect the prevalence of complications, although 22 bulls developed abescesses, of which, 14 were castrated by scrotal excision, a method that is commonly considered to be favorable for wound drainage and reduce the risk of such complications (Silva, et al., 2009). In another study comparing Newberry knife incision to scrotal excision, it was found that scrotal excision indeed causes significantly more edema and increased maximal scrotal temperature in the postsurgical period when compared to Newberry knife incision. However, wound healing between days 21 and 35 after the surgery seemed to be more favorable in bulls castrated by scrotal excision, although at the end of the experiment both groups a showed similar wound healing index (Marti, et al., 2017).

3.2.3. Hemostasis technique

3.2.3.1. Traction

In young calves, minimal hemostasis is generally accepted during surgical castration. This may be achieved by simply cutting the spermatic cord with a scalpel, or scraping it until it breaks, or even by manually applying gentle traction until rupture occurs. The latter causes enough vasospasm to prevent hemorrhage in small calves but is not usually recommended for older bulls (Gilbert, et al., 2017). However, traction was found to be used on several occasions in mature bulls of varying ages in the records included in our study. Indeed, bulls as young as 6-8 months (Laurence, et al., 2018) and as old as 20 months have been castrated by using traction as the sole method of hemostasis, although excessive hemorrhage occurred in 1.5% of the latter animals, and spermatic cord funiculitis had a prevalence of 3% (Amatayakul-Chantler, et al., 2013). In other studies, bulls as old as 6-8 months and 7-10 months were respectively castrated by severing (Van der Saag, et al., 2018b) or scraping (Petherick, et al., 2014b) the spermatic cord with a scalpel. These techniques are usually not recommended for bulls of this age, but the authors did not report the occurrence of complications. Traction was used in 7/35 records that were included in this review.

3.2.3.2. Twisting

The twisting of the spermatic cord is a successful way of ensuring its closure and therefore hemostasis as previously mentioned for boars. This method is frequently described for mature bulls and can be done by attaching a Henderson castrator or a metal hook (**Figure 8**) to the testis and rotating the testis 10-20 times either manually (only for the Henderson castrator) or using an electric drill, until the spermatic cord is coiled and ruptures (Gilbert, et al., 2017). Castration by twisting was practiced in 7 studies that were included in this review. In a study comparing the effect of Henderson castration or emasculation on the feeding behavior of weaned calves, it was found that the choice of technique did not affect average daily gain, and both techniques elicited similar inflammatory response which was characterized as a short term response, as opposed to the delayed one that was found in calves castrated by banding in that same study (Warnock, et al., 2012).

3.2.3.3. Crushing

The crushing of the spermatic cord also relies on the elastic nature of the testicular vasculature, which under the effect of compression using an emasculator (**Figure 8**) becomes secured from the risk of excessive hemorrhage. Crushing is commonly used on adult (Gilbert, et al., 2017) as well as young animals, with calves as young as one week of age being castrated using an emasculator (Meléndez, et al., 2018b). Following database research, 11 records were found to mention the use of crushing as a method applied to achieving hemostasis in adult bulls. In a study comparing crushing with traction, calves of 6 months of age did not show any significant difference at the level of cortisol concentration or plasma substance P. Although all of the previous parameters seemed to indicate a lower level of inflammation, stress, and pain in 8-week-old claves that were subjected to the same procedure, infrared thermography and heart rate variability did not show any significant differences between the two castration techniques. On the other hand, electroencephalogram measurements showed a greater desynchronization in 6 months-old bulls castrated with an emasculator, which suggests a higher level of arousal (Dockweiler, et al., 2013).

3.2.3.4. Ligation

Ligation is an established method of hemostasis used in different types of surgery. In regard to its' use in bovine, it is mainly recommended for adult bulls (Gilbert, et al., 2017), although it has also been described in calves younger than 2 months of age (Pieler, et al., 2013). This technique was in fact used by 8 out of the 35 records meetings the criteria of inclusion in this review. Traditionally, ligation is done by tying surgical suture material into an encircling ligature, or preferably a transfixation ligature since the latter has a reduced risk of slippage (Gilbert, et al., 2017). This method may be used alone (Machado, et al., 2018) or in combination with other hemostasis techniques such as emasculation (Bertagnon, et al., 2017) or traction (Anaruma, et al., 2020) for supplemental hemostasis. Similarly to what was mentioned previously for boars, non-traditional techniques of ligating the spermatic cord have emerged in the last decade, with plast zip-ties or "nylon clamps" being the best documented with 3 published records mentioning the successful use of this technique in adult bulls (Fernandes, et al., 2016; Ferreira, et al., 2015; Silva, et al., 2009). In the first published record describing the use of nylon clamp ligation in 18-months old bulls, postsurgical complications that were encountered included hemorrhage (2.3%), myiasis (17.8%), abscess formation (13.1%), funiculitis (11.9%) and granuloma (4.2%) (Silva, et al., 2009). However, a more recent large scale study on bulls of similar weight only noted the occurrence of myiasis and at an even lower rate (3.09%) (Fernandes, et al., 2016). Finally, the comparison of nylon ties and Chromic Catgut sutures for the ligation of the spermatic cord of bulls between the ages of 12 and 15 months, revealed no difference in hematocrit, total protein, plasma fibrinogen, or leukocyte count, thus confirming the viability of nylon clamps as a cost-efficient alternative for surgical suture ligation (Ferreira, et al., 2015).

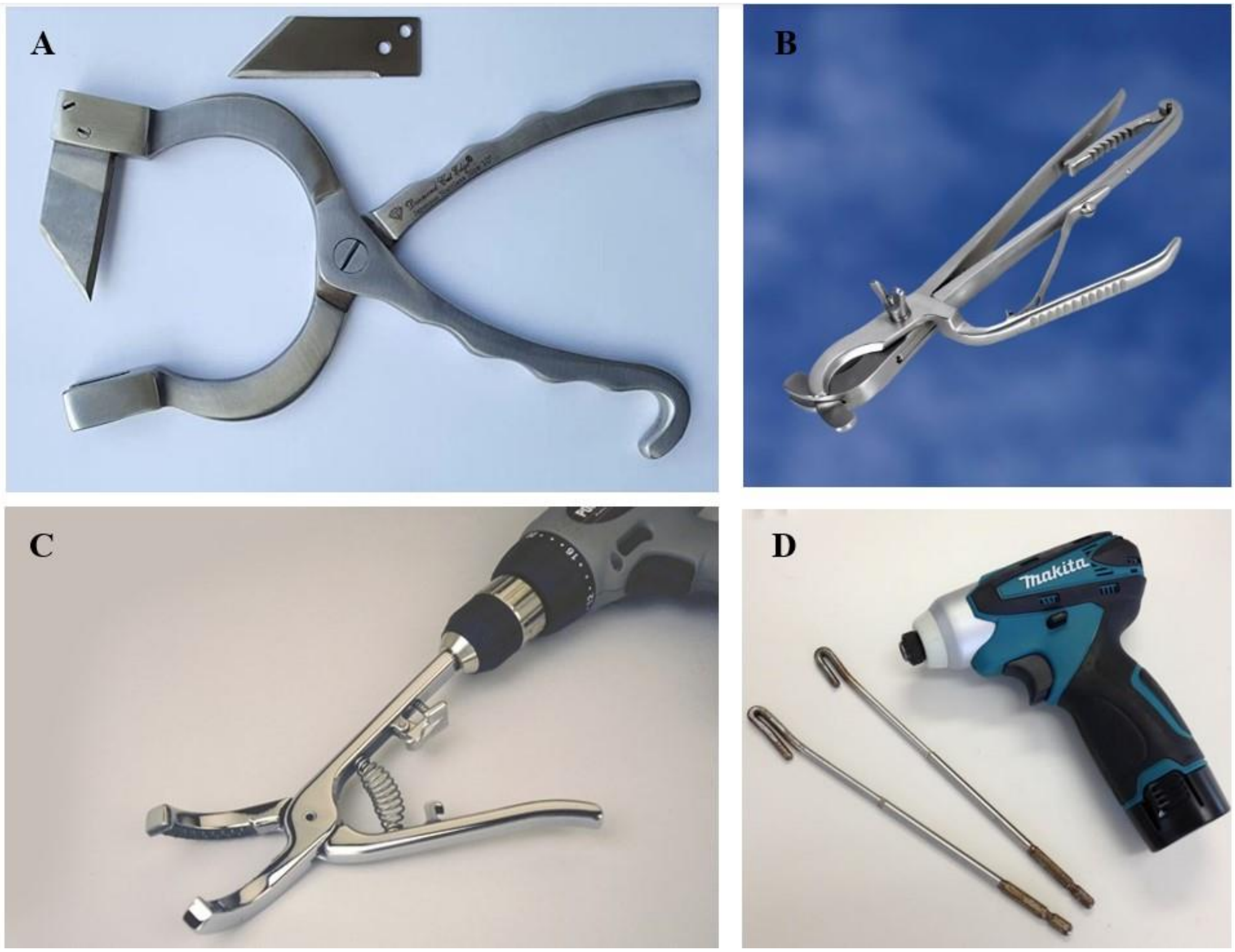

Figure 8: essential castration tools include A- the Newberry knife used to create an opening in the middle of the scrotum; B- the Reimer emasculator which can be used for both crushing and cutting the spermatic cord; C- and the Henderson castrator used to grasp the testis from its' apex and twisting it until the spermatic cord breaks; that same procedure can be achieved with the metal hook seen in figure D, which is placed over the spermatic cord and held close to the testis.

3.2.4. Closure of the surgical incision

As previously mentioned for boars, the closure of the surgical incision in cattle is not common practice and is only considered in settings where asepsis can be maintained, in which case, at least partial resection of the scrotum would be necessary to reduce dead space. For primary closure, a purse string-type suture is recommended for the subcutaneous tissue followed by a subcuticular suture, both of which are done with an absorbable suture (Gilbert, et al., 2017). None of the records included in our review described the use of primary closure. In fact, most records do not mention this subject, probably since the consensus is allowing for secondary intention healing.

4. Ovine and caprine castration

The factors pertaining to small ruminant castration are relatively similar to those mentioned previously for cattle in regard to the possibility of using bloodless castration methods, which are in fact significantly more common in these animals than surgical orchiectomy. Nevertheless, the latter is still practiced by veterinarians and therefore it would be interesting to review the different methods used in adult ovine and caprine orchiectomy and how the techniques and their results differ from those found in cattle.

4.1. Database Research

Following a complete database research on CABI, Web of Science, PubMed and Scopus using the keywords ((sheep) OR (goat) OR (buck) OR (ram) OR (kid) OR (lamb) OR (small ruminant)) AND ((surgical AND castration) OR (orchiectomy) OR (orchidectomy)), a total of 1134 records were found which were then screened for relevance by the title and abstract according to our inclusion criteria. The inclusion criteria consisted of 1) the animal species: ovine and caprine; 2) the age or weight of the animals: older than 1 month or weighing more than 20 kg; 3) text containing a description of surgical castration (whether brief or detailed) in healthy, non-cryptorchid animals. A total of 17 original articles were found to meet these criteria. These records were subjected to full text screening and relevant information was analyzed and extracted. Information regarding each surgical technique was divided into 4 categories; 1) approach to the tunica vaginalis; 2) site of incision; 3) hemostasis technique and 4) closure of surgical incision. This data was reported in a summary table (**Table 3**), allowing us to easily identify all the different techniques that have been used for orchiectomy in adult small ruminants. Farm management and surgery textbooks were used as a reference for common practices of orchiectomy in adult animals but were not included in the list of records meeting the criteria of the database research.

4.2. Surgical Techniques

4.2.1. Approach towards the tunica vaginalis

Both the open and closed techniques are valid options for the castration of small ruminants, and similarly as to boars and cattle, no studies have been conducted comparing the two approaches (Melches, et al., 2007; Scott, et al., 1996). Out of the 17 records included in our study, 5 followed the open technique, 2 followed the closed technique and the majority (7/17) did not mention whether the tunica vaginalis was opened or left intact. On the other hand, pinhole castration was studied more extensively in small ruminants, probably since this species is more suitable for such a procedure seeing the risk of unsuccessful or incomplete castration encountered in bulls (Okwee-Acai, et al., 2016) and donkeys (Abou-Ahmed, et al., 2012). In fact, with the exception of 1 kid with unilateral atrophy and another with no atrophy (Okwee-Acai, et al., 2016), the procedure was successful in all three studies, which was confirmed by the atrophy of the testis and histopathology (Abid & Al Baghdady, 2013; Fazili, et al., 2009).

4.2.2. Site of incision

The scrotum in small ruminants can be incised in the following two methods that were previously mentioned for bull castration: longitudinal incision (Kent & Molony, 1993) and the excision of the distal third of the scrotum (Harris, et al., 2021). The latter is in fact the method that was most employed in the records included in our study, with 10 publications mentioning it as the incision technique that was used. Scrotal ablation, wherein the incision is made at the level of the scrotal neck; and longitudinal scrotal incision have been compared to each other in 4-5 months old rams. Although no difference was noted between the 2 techniques at the level of weight 5 weeks after castration, cortisol level was higher at 180 min after the surgery in the group treated by a longitudinal incision. In addition, the subjects from this same group showed more severe swelling, which in one particular occasion developped into purulent discharge. Finally, the authors investigated the difference in the length of surgical procedure and it was found that scrotal ablation was the longer procedure, since it was also combined with primary closure of the incision (Bonelli, et al., 2008).

4.2.3. Hemostasis technique

The spermatic cord of small ruminants is considered relatively large, leading some authors to recommend the use of emasculation or ligation for better hemostasis (Tibary, et al., 2017). However, there were 2 records of castration by simply cutting the spermatic cord with a knife in 3-6 months old (Shutt, et al., 1988) and 6-12 weeks old lambs (Lomax, et al., 2010), and one record of using traction to sever the spermatic cord (Jongman, et al., 2000); all of which did not note the occurrence of excessive hemorrhage in the postsurgical period. The remaining records included in this review were divided as follows: 2 records where twisting was used for hemostasis; 3 using surgical suture ligature alone; 1 using crushing with an emasculator alone; 2 using both ligation and emasculation; and 2 using clamping combined with cauterization of the spermatic cord after severing. On a different note, a rarely described hemostasis technique consisting of tying vascular and spermatic portion of the cord upon each other, was used for the castration of 5-6 day old lambs (Thornton & Waterman-Pearson, 1999). A similar technique is used for the castration of tomcats, and was the inspiration for our subsequent study which employs this technique in adult boars.

4.2.4. Closure of the surgical incision

As mentioned for cattle, primary closure is not common practice in small ruminants, but is sometimes done when asepsis can be maintained. That was the case in 2 of the records included in this review, wherein the scrotum, which was excised, was later sutured at the end of the procedure by a simple continuous pattern for the subcutaneous tissue, followed by either an intradermal suture pattern (Malbrue & Arsuaga Zorilla, 2018) or the placement of surgical stainless steel staples (Straticò, et al., 2018). In a comparative study between two different surgical techniques, one consisting of a longitudinal scrotal incision that was left open and the other consisting of scrotal ablation with primary closure, the latter was found to be more time consuming, although it was associated with fewer complications in the postsurgical period (Bonelli, et al., 2008).

5. Conclusion

In conclusion, although surgical castration of adult farm animals is often practiced, this is not always well reflected in scientific publications, with records concerning surgical techniques in adult boars being relatively scarce. We were able to distinguish the different methods used for orchiectomy of adult boars, bulls, rams, and bucks in this review, although a significant amount of knowledge gap still needs to be filled regarding the advantages and disadvantages of each method.

Table 1: summary table of the different surgical techniques for boar orchiectomy identified through database research. (*For textbooks we reported the age/weight at which it is recommended using the technique described for "older boars")

Reference	Record type	Age/weight and number of boars*	Approach to the tunica vaginalis	Site of incision	Hemostasis technique	Closure of surgical opening
Anderson & Mulon, 2019	Swine medicine textbook	>2 weeks old	Closed castration	4-6 cm incision over the ventral aspect of the scrotum for each testis	-Twisting of the spermatic cord; -Double transfixation ligatures; -The use of an emasculator is possible but does not prevent inguinal hernia	Only to be considered if asepsis was maintained
Hughes & Berger, 2018	Original article	One 16-week-old boar	Open castration followed by suturing of the tunica vaginalis	Scrotal incision	-For the purpose of this study, the epididymis was preserved and only the vascular supply of the testis was crushed using a clamp for 5 min; -Testicular blood vessels ligated prior to excision of the testis	Subdermal skin was closed along with the tunica vaginalis; however this may have only been done for the purpose of the study which focused on apical blebbing of the epididymis
do Prado, et al., 2018	Original article	20 mixed race 3 months old boars	Open castration	Longitudinal ventral scrotal incision over each testis (3 cm or longer according to testicle size)	Spermatic cord was double ligated either with nylon clamps (commonly known as zip-ties) or with surgical nylon suture	Not closed
Lehman, et al., 2017b	Original article	8 mixed race 5 months old boars	Open castration followed by the suturing of the tunica vaginalis	Longitudinal scrotal incision parallel to the median raphe	Spermatic cord was ligated	Scrotal incision was closed to minimize the interference of littermates
Baars, et al., 2013	Original article	30 boars with a mean body weight of 50 kg	Open castration	Scrotal incision	Spermatic cord was clamped	Not closed
Umapathy, et al., 1997	Original article	5 mixed race 7 months old boars	Open castration followed by the suturing of the tunic	3 cm long median suprapubic incision	Spermatic cord was clamped	Surgical opening was sutured
Ames, 2014	Farm animal surgery textbook	Boars weighing more than 20 kg	Closed castration	Ventral scrotal incision; in boars >135 kg the median raphe should be excised at the end of the procedure	-Double ligature of the spermatic cord -Crushing with an emasculator for 30 seconds	Not specified

Holtgrew-Bohling, 2020	Veterinary technician textbook	Not specified	Not specified	Single or double scrotal incision	Ligation and crushing with an emasculator are recommended	Left open if a double scrotal incision was made but closed in case of a single incision
Callan, et al., 2017	Farm animal surgery textbook	Not specified	Closed castration	4-6 cm long ventral incision of the scrotum over each testis	-Twisting of the spermatic cord; -Double circumferential ligatures; -Crushing with an emasculator followed by rupture of the spermatic cord	Closure of the surgical opening is not recommended and should only be considered if asepsis was maintained

Table 2: summary table of the different surgical techniques for bull orchiectomy identified through database research; only studies conducted on animals older than 6 months or with an average weight of more than 200 kg, and thus considered as "older cattle" were included.

Reference	Site of incision	Hemostasis technique	Closure of surgical opening
OPEN CASTRATION			
Laurence, et al., 2018 Lehmann, et al., 2017a Musk, et al., 2017	Scalpel incision of the scrotum	Traction placed over the SpC until rupture and retraction of the vasculature into the abdomen	Left open for secondary intention healing
Yamada, et al., 2021a Yamada, et al., 2021b Ferreira, et al., 2015	Unspecific scrotal approach	Surgical suture ligation	Not specified
Coetzee, et al., 2012	Scrotum opened with a Newberry knife	SpC crushed with emasculator	Not specified
Van der Saag, et al., 2018b	Longitudinal scrotal incision	SpC cut with a scalpel	Not specified
Fernandes, et al., 2016 Ferreira, et al., 2015	Longitudinal scrotal incision (caudal side)	Plastic zip tie ligation	Left open for secondary intention healing
Silva, et al., 2009	-Longitudinal scrotal incision (lateral side) -Excision of the distal third of the scrotum	Plastic zip tie ligation	Not specified
Neves, et al., 2017	Scalpel incision of the scrotum	Not specified	Not specified
Fisher, et al., 2001	Not specified	SpC crushed with emasculator	Not specified
CLOSED CASTRATION			
Nordi, et al., 2019 Meléndez, et al., 2019 Meléndez, et al., 2018a	Scrotum opened with a Newberry knife	SpC crushed with emasculator	Not specified
Park, et al., 2018	Scrotum opened with a Newberry knife	Twisting (Henderson castrating tool)	Not specified
Roberts, et al., 2018	Excision of the distal third of the scrotum	SpC crushed with emasculator	Not specified
Kleinhenz, et al., 2018	Excision of the distal third of the scrotum	Twisting and traction	Not specified
Petherick, et al., 2014a Petherick, et al., 2014b	Scalpel incision of the scrotum	- SpC scraped until ruptured in younger bulls (7-10 months old)	Not specified

		- SpC crushed with emasculator in older bulls (22-25 months old)	
UNSPECIFIED APPROACH TOWARDS TUNICA VAGINALIS			
Machado, et al., 2018	Scalpel incision of the scrotum	Surgical suture ligation	Not specified
Anaruma, et al., 2020	Scalpel incision of the scrotum	Surgical suture ligation followed by removal of the testis by traction	Not specified
Gómez, et al., 2019 Moreira, et al., 2018 Amatayakul-Chantler, et al., 2013 Dockweiler, et al., 2013	Scalpel incision of the scrotum	Traction placed over the SpC until rupture and retraction of the vasculature into the abdomen	Not specified
Dockweiler, et al., 2013	Scalpel incision of the scrotum	SpC crushed with emasculator	Not specified
Devant, et al., 2012	Caudoventral scrotal incision	SpC crushed with emasculator	Not specified
Bertagnon, et al., 2017	Caudoventral scrotal incision	SpC crushed and ligated with surgical sutures	Not specified
Repenning, et al., 2013	Excision of the distal third of the scrotum	Twisting (Henderson castrating tool attached to a drill)	Not specified
Coetzee, et al., 2010 Gehring, et al., 2009 Rust, et al., 2007 Warnock, et al., 2012	Scrotum opened with a Newberry knife	Twisting (Henderson castrating tool attached to a drill)	Not specified
Warnock, et al., 2012	Scrotum opened with a Newberry knife	SpC crushed with emasculator	Not specified
Marti, et al., 2017	-Scrotum opened with a Newberry knife -Excision of the distal third of the scrotum	Not specified	Not specified
PINHOLE CASTRATION			
Okwee-Acai, et al., 2016	Needle entry at the level of the scrotal neck	Encircling ligature with a surgical suture	Non relevant

Table 3: summary table of the different surgical techniques for ovine and caprine orchiectomy identified through database research; only studies conducted on animals older than 1 month or with an average weight of more than 20 kg, and thus considered as "older sheep/goats" were included.

Reference	Site of incision	Hemostasis technique	Closure of surgical opening
OPEN CASTRATION			
Scott, et al., 1994 Scott, et al., 1996	Longitudinal scrotal incision (lateral side) in rams	Surgical suture ligation	Not specified
Molony & Kent, 1993 Kent & Molony, 1993	Longitudinal scrotal incision (caudolateral side)	SpC crushed with a clamp and cauterized with electrically heated cautery after severing	Not specified
Straticò, et al., 2018	Excision of the distal third of the scrotum	Not specified	Subcutis sutured by a simple continuous pattern and surgical wound closed with staples
CLOSED CASTRATION			
Melches, et al., 2007	Excision of the distal third of the scrotum	SpC crushed and ligated with surgical sutures	Not specified
Malbrue & Arsuaga Zorilla, 2018	Excision of the distal third of the scrotum	3 clamp technique with Rochester-Carmalt hemostatic forceps followed by SpC ligation with a modified miller knot	Subcutis sutured by a simple continuous pattern and surgical wound closed with an intradermal suture pattern
UNSPECIFIED APPROACH TOWARDS TUNICA VAGINALIS			
Karakus, et al., 2017	Excision of the distal third of the scrotum	SpC crushed with emasculator	Not specified
Harris, et al., 2020 Harris, et al., 2021	Excision of the distal third of the scrotum	Manual twisting followed by scraping of the SpC	Not specified
Bonelli, et al., 2008	-Longitudinal scrotal incision -Excision of the distal third of the scrotum	Surgical suture ligation	Not specified
Lomax, et al., 2010 Shutt, et al., 1988	Excision of the distal third of the scrotum	SpC cut with a knife	Not specified
Jongman, et al., 2000	Excision of the distal third of the scrotum	Traction	Not specified
PINHOLE CASTRATION			
Okwee-Acai, et al., 2016 Abid & Al-Baghdady, 2013 Fazili, et al., 2009	Needle entry at the level of the scrotal neck	Encircling ligature with a surgical suture	Non relevant

CHAPTER III: PILOT STUDY ON FEASIBILITY AND POWER ANALYSIS

1. Introduction

According to FAO statistics, pork meat was the second biggest product of animal origin produced in Italy in 2020 (FAOSTAT, 2022). In fact, pork meat is omnipresent in Italian culture and cuisine whether in the famous Italian sausages, prosciutto, pancetta, or any of their many other pork products. The significance of pork products in Italy is also reflected in the importance of their pig farming industry. In the rural areas of Italy, small pig farmers are found in large numbers. One such region is Sardinia, which is known for being one of the biggest agricultural areas in Italy. During our veterinary work in the north of Sardinia, we found that castration of mature boars is often required, since these farmers often rely on natural reproduction and therefore are constantly rearing boars for mating, which will need to be castrated at the end of their cycle to avoid the risk of boar taint.

Whereas surgical castration of young piglets is a standard routine procedure, that of adult boars is more complicated. The latter usually necessitates general anesthesia, since large boars are rather difficult to handle and restrain, but more importantly, orchiectomy of adult boars entails the use of hemostasis measures such as the ligation of the spermatic cord with surgical sutures or crushing the cord with an emasculator, both of which involve the introduction of foreign objects to the surgical site and excessive manipulation. This increases the risk of surgical contamination and therefore infection, which is one of the most common complications of orchiectomy.

For this purpose, we devised a novel technique of castration that does not require the use of foreign objects: the sutureless technique. This technique was inspired by the one used for the castration of tomcats and consists of an open castration where hemostasis is achieved by splitting the vas deferens from the testis and tying several square knots using the deferent duct and the testicular vasculature. In order to assess the validity of the sutureless technique, feasibility, and sample size necessary for a clinical study using this technique, we conducted the following pilot study and power analysis.

2. Materials and methods

The study was conducted in the North Sardinian region of Gallura in a period extending from October 2019 until September 2020 during the activities organized by the Mobile Clinic of the Veterinary Medicine Department of the University of Sassari.

2.1. Animals

A total of 101 entire mixed-race boars aged between 4 and 60 months were included in the study. The animals came from several small farms in the region of Gallura. They were presented to the Mobile Clinic for routine castration. Treatments were allocated randomly to the boars: 41 animals were subjected to classical surgical castration with ligation (group 1) and 60 were castrated with the sutureless technique (group 2). All animals were castrated in field conditions, within the farms in which they were being reared.

2.2. Anesthesia

The anesthetic protocol was the same for all the boars included in this study. Farmers were asked to keep the animals scheduled for castration fasted for 12h prior to the surgery and isolated from the rest of the pigs on the farm in order to facilitate capture and restraint at the time of the intervention. The boars were restrained using a snout snare passed caudally to the superior canine teeth and over the dorsal surface of the snout and tightened. Peripheral venal access was achieved through the auricular veins as seen in **Figure 9** in order to allow the intravenous (IV) administration of sedative and anesthetic drugs. Weight estimation was done at this point by an expert professional. For the purpose of avoiding errors that may arise due to interindividual variability, the same individual completed the weight estimation for all the animals.

Figure 9: IV access for the administration of anesthetic drugs was achieved through the auricular veins

Sedative and anesthetic drugs were administered in a single IV bolus. Sedation was achieved with Azaperone (1 mg/kg), a D_2 dopamine antagonist butyrophenone; and detomidine (0.1 mg/kg), an α_2 adrenergic antagonist; while general anesthesia was induced with a dose of 5 mg/kg of tiletamine/zolazepam sold under the commercial name ZOLETIL® (VIRBAC) combining a dissociative agent and a benzodiazepine sedative. The sedative and anesthetic medication administered was sufficient to maintain deep anesthesia for the entire duration of the procedure which did not exceed 5-10 min in all cases starting from the first incision of the parainguinal area and ending with the excision of the second testis.

2.3. Surgery

After sedation and anesthesia medication were administered, the animals were positioned in dorsal recumbency and the surgical site was prepared by first washing the parainguinal area with antibacterial soap to eliminate the large particles of contaminants, followed by scrubbing with povidone-iodine solution for a few minutes, thus allowing some time for deep anesthesia to initiate. The testis was pushed cranially into the inguinal area and a variable dose of 5 to 20 mL (according to testicular size) of 2% lidocaine solution was injected subcutaneously (SC) along the incisional line to achieve local analgesia. A 4 to 10 cm longitudinal incision was made with a scalpel over the skin covering the inguinally displaced testis. The incision was continued through the underlying tissue and the parietal tunica vaginalis was incised, thus all boars were castrated by open castration. The testis was freed from its' attachment to the tunica vaginalis by digitally breaking the ligament of the tail of the epididymis and the spermatic cord was manually stripped from the parietal tunic as proximally as possible to the inguinal ring. According to the treatment assigned to each animal, the surgeon performed either one of the following techniques to achieve hemostasis.

2.3.1. Classical castration (group 1)

Animals assigned to group 1 (n=41) were castrated using the previously described spermatic cord ligation technique. This was achieved by placing double ligatures around the vascular portion of the spermatic cord and the vas deferens using absorbable polyglycolic acid sutures. The suture USP size used was according to the estimated weight of the boars with USP 2 (Vicryl) being used for animals over 100 kg; USP 0 for animals between 50 and 100 kg and USP 2-0 for the ones under 50 kg. The first ligature was placed proximally to the spermatic cord and was followed by a second ligature a few centimeters lower on the testicular side of the spermatic cord. The spermatic cord was then transected distally to the ligatures and was inspected for the presence of hemorrhage before placing it back within the tunica vaginalis. Finally, the coagulated blood particles were removed, and the surgeon proceeded to castrate the contralateral testis using the same technique as the one described above.

2.3.2. Sutureless castration (group 2)

Animals assigned to group 2 (n=60) were castrated by the novel sutureless technique. Following the extraction of the testis from the vaginal process and stripping the spermatic cord of the parietal tunic up to the level of the inguinal ring, the deferent duct was manually detached from the tail of the epididymis and freed from the testis spermatic cord, thus splitting the latter into a spermatic portion consisting of the deferent duct and a vascular portion consisting of the testicular artery and vein. These two portions were tied to each other by making 3 to 5 square knots (6 to 10 throws). The first knot was made as proximally as possible to the internal inguinal ring while the following knots were made about 2 cm distal to the first one. The surgeon was careful not to apply excessive traction on the deferent duct when tying the knots in order to avoid accidental rupture. After a sufficient number of knots were tied, both portions of the spermatic cord were aligned and transected distally to the knots and the remnant part of the spermatic cord was inspected for hemorrhage before placing it back within the tunica vaginalis. If blood was observed to be continuously dripping from the remnant spermatic cord, a double ligature was placed and the sutureless technique was considered unsuccessful for this individual boar. Finally, the coagulated blood particles were removed, and the surgeon proceeded to castrate the contralateral testis using the same technique as the one described above. The different steps of the procedure can be seen in **Figure 10**.

In both groups, the surgical incision was left open to allow for second intention healing of the wound thus providing better drainage of the surgical site in the postsurgical period. The site of incision and its' surrounding area were then disinfected with povidone-iodine and sprayed with antibacterial chlortetracycline hydrochloride spray. Systemic antibiotic therapy was also given for prophylaxis in the form of an intramuscular (IM) injection of 30 mg/kg of long acting oxytetracycline (ENGEMICINA D.D.®, MSD).

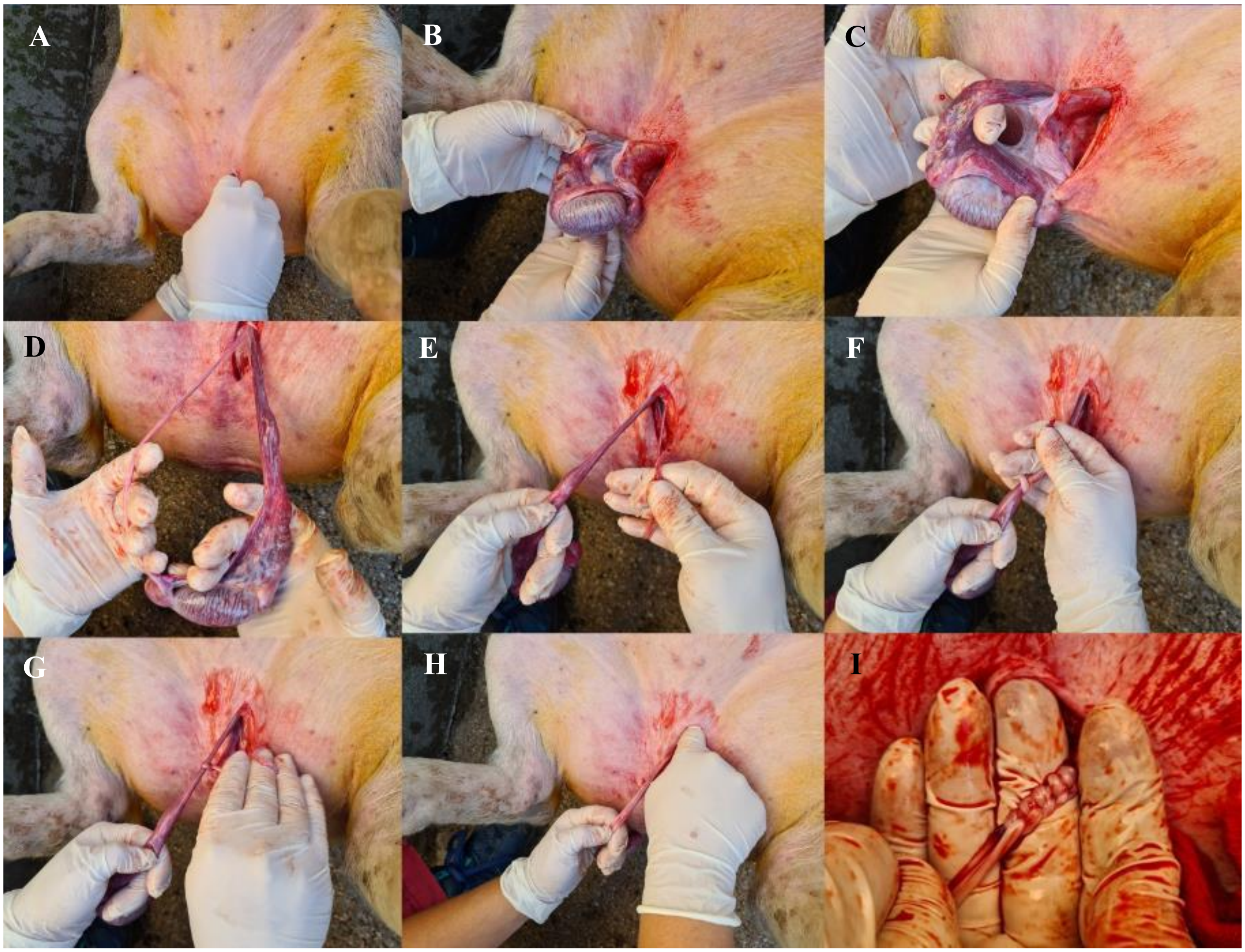

Figure 10: For orchiectomy using the sutureless technique, (A) the surgeon started with a skin incision in the parainguinal area using a scalpel. The incision was continued through the underlying tissue and parietal tunica vaginalis (B), and the testis was then exteriorized following digital rupture of the ligament of the tail of the epididymis. (C) The spermatic cord was then stripped from the tunica vaginalis up to the level of the inguinal canal and (D) the deferent duct was manually detached from the epididymis (E) thus splitting the spermatic cord into a spermatic and vascular portion. (F) The latter were tied together into 3 to 5 square knots. (G) The first knot was made as proximally as possible to the internal inguinal ring (H) whereas the following knots were made about 2 cm distally to the first. (I) Finally, the spermatic and vascular portions were aligned and transected.

2.4. Measurements and recorded data

Prior to the surgery, the age and weight of each animal were recorded. After the excision of the testis, the diameter of the deferent duct (in mm) was measured at the level of scalpel transection using grid paper and was recorded for the left and right testis for each animal of both groups. Tensile strength (in

Newton) of the left and right deferent ducts was also measured for the animals of both groups using a KOP 24387 (KennOptics, London) digital dynamometer. For that purpose, a portion of the excised deferent duct was taken following a standardized length of 4 cm for boars aged under 9 months and 5 cm for boars older than 8 months of age which was measured with grid paper. The sampled portion of the vas deferens was then grasped by each end with a straight Klemmer hemostatic forceps, attaching one to the dynamometer and pulling on the second with gradually increasing force until the deferent duct breaks (**Figure 11**). This procedure was performed by the same individual for the duration of the study in order to eliminate the risk of error arising from interindividual variability. Tensile strength was then recorded both in Newton and in kg for each deferent duct.

Figure 11: deferent duct tensile strength was measured by grasping with a hemostatic forceps each end of a 4-5 cm long sample of the cord and attaching the first hemostat to the digital dynamometer while pulling on the other until the deferent duct breaks

2.5. Statistical analysis

According to weight and age, a homogenous pool of 10 animals per group was picked from the sample using an Excel spreadsheet containing all previously collected data. The average and standard deviation of each variable was calculated for each group (entire sample) as well as for the each of the 2 pools. The correlation between age and tensile strength (CA-T) and weight and tensile strength (CW-T) was calculated for the pooled boars using the Pearson linear correlation coefficient R. A power analysis was conducted with the software G*Power 3.1.9.4 in order to accurately estimate the sample size necessary to find a statistically significant difference between the two groups.

3. Results

All 101 animals included in this pilot study were successfully castrated using either classical or sutureless castration. No complications were recorded during the surgery or in the immediate postsurgical period. The different variables recorded both for group 1 and group 2 are reported in **Table 4** and **Table 5** respectively.

Table 4: raw data of boars from group 1 (classical castration); R: right; L: left; DD: deferent duct

Ref. No.	Age (months)	Weight (kg)	DD length	Right DD diameter	Left DD diameter	Tensile strength R (kg)	Tensile strength L (kg)	Tensile strength R (N)	Tensile strength L (N)
1	4	35	4	1.5	1.5	0.80	0.90	7.85	8.83
2	7	80	4	1.8	2.0	1.23	1.33	12.07	13.05
3	5	80	4	1.6	1.5	1.40	1.20	13.73	11.77
4	5	90	4	1.5	1.6	0.75	0.87	7.36	8.53
5	5	100	4	1.5	1.5	1.30	1.40	12.75	13.73
6	5	100	4	1.6	1.7	2.10	2.30	20.60	22.56
7	6	100	4	1.6	1.6	2.30	2.60	22.56	25.51
8	10	120	5	1.5	1.6	1.98	1.76	19.42	17.27
9	7	140	4	1.9	1.7	1.30	1.70	12.75	16.68
10	7	140	4	1.6	1.6	1.50	1.80	14.72	17.66
11	12	150	5	2.1	2.1	1.55	1.20	15.21	11.77
12	10	150	5	2.0	2.2	1.40	1.58	13.73	15.50
13	9	150	5	1.7	1.6	1.05	1.64	10.30	16.09
14	12	170	5	3.0	3.1	2.33	2.45	22.86	24.03
15	12	180	5	2.2	2.1	1.43	1.98	14.03	19.42
16	15	200	5	3.0	2.6	2.65	2.77	26.00	27.17
17	12	200	5	3.2	3.0	1.77	1.82	17.36	17.85
18	18	200	5	3.7	3.7	2.60	2.10	25.51	20.60
19	12	200	5	3.1	3.1	1.60	2.10	15.70	20.60
20	12	200	5	3.0	3.2	1.60	2.14	15.70	20.99
21	18	200	5	3.6	3.8	1.90	2.30	18.64	22.56
22	12	210	5	4.1	3.9	2.30	2.80	22.56	27.47
23	24	220	5	4.0	4.1	2.30	1.70	22.56	16.68
24	18	230	5	4.4	4.2	1.80	1.72	17.66	16.87
25	12	230	5	4.6	4.5	1.32	1.78	12.95	17.46
26	18	230	5	2.7	2.8	2.98	2.91	29.23	28.55
27	18	240	5	4.8	4.6	1.51	2.28	14.81	22.37
28	24	250	5	5.0	4.9	2.19	1.69	21.48	16.58
29	30	250	5	5.3	5.1	2.34	2.20	22.96	21.58
30	36	250	5	4.9	5.1	2.80	2.68	27.47	26.29
31	20	260	5	2.4	2.2	3.55	3.50	34.83	34.34
32	24	280	5	5.2	5.0	1.93	1.80	18.93	17.66
33	24	280	5	5.7	5.9	2.10	1.90	20.60	18.64
34	24	300	5	6.0	6.3	4.12	4.30	40.42	42.18
35	36	300	5	6.0	6.2	2.94	2.97	28.84	29.14
36	36	300	5	6.1	6.1	3.47	3.60	34.04	35.32
37	36	320	5	6.2	6.0	4.20	3.80	41.20	37.28
38	30	330	5	5.1	4.9	4.80	5.13	47.09	50.33
39	36	330	5	6.3	6.4	2.70	3.05	26.49	29.92
40	36	350	5	6.5	6.3	5.20	5.70	25.13	25.77
41	36	360	5	6.2	6.0	5.78	5.84	56.70	57.29

Table 5: raw data of boars from group 2 (sutureless castration); R: right; L: left; DD: deferent duct

Ref. No.	Age (months)	Weight (kg)	DD length	Right DD diameter	Left DD diameter	Tensile strength R (kg)	Tensile strength L (kg)	Tensile strength R (N)	Tensile strength L (N)
1	4	35	4	1.6	1.8	0.92	0.83	9.03	8.14
2	4	35	4	1.7	1.6	1.10	1.30	10.79	12.75
3	4	40	4	1.8	1.9	1.37	1.65	13.44	16.19
4	4	50	4	2.0	1.8	1.98	1.48	19.42	14.51
5	5	70	4	2.1	1.8	0.90	0.70	8.83	6.87
6	5	70	4	2.2	2.0	1.10	0.80	10.79	7.85
7	5	80	4	2.0	1.9	0.75	0.76	7.36	7.45
8	7	80	4	2.5	2.3	1.34	1.18	13.15	11.57
9	7	100	4	2.4	2.2	1.30	1.00	12.75	9.81
10	7	110	4	2.5	2.3	0.75	0.90	7.36	8.83
11	6	120	4	2.1	2.1	2.10	2.20	20.60	21.58
12	7	130	4	2.7	2.1	2.30	2.10	22.56	20.60
13	8	130	4	2.9	2.6	1.30	0.90	12.75	8.83
14	10	130	5	2.8	2.5	2.03	1.88	19.91	18.44
15	9	140	5	2.8	2.4	1.50	0.90	14.72	8.83
16	12	170	5	3.0	2.6	2.24	2.54	21.97	24.92
17	16	170	5	3.2	3.4	1.71	2.28	16.78	22.37
18	18	170	5	3.6	3.7	1.50	1.80	14.72	17.66
19	18	170	5	3.7	3.9	1.90	1.50	18.64	14.71
20	12	180	5	3.1	2.7	1.40	1.60	13.73	15.70
21	18	180	5	3.5	3.6	1.48	1.72	14.52	16.87
22	18	180	5	3.4	3.6	1.60	1.90	15.70	18.64
23	18	180	5	3.8	3.7	1.77	1.69	17.36	16.58
24	18	180	5	4.0	4.0	1.87	1.48	18.34	14.52
25	15	190	5	3.5	3.8	2.70	2.73	26.49	26.78
26	12	200	5	3.3	2.8	1.60	2.10	15.70	20.60
27	12	200	5	3.2	2.9	1.70	1.30	16.68	12.75
28	12	200	5	3.4	3.2	2.40	2.00	23.54	19.62
29	12	200	5	3.1	3.1	2.67	1.13	26.19	11.08
30	18	200	5	4.0	4.1	1.90	2.20	18.64	21.58
31	24	200	5	4.1	4.3	1.80	2.97	17.66	29.13
32	24	200	5	4.0	4.1	1.80	3.05	17.66	29.92
33	18	210	5	3.5	3.6	1.09	2.57	10.69	25.21
34	18	210	5	5.5	5.6	1.82	1.94	17.85	19.03
35	18	210	5	5.0	5.1	2.34	2.10	22.96	20.60
36	18	210	5	3.7	3.8	2.57	2.20	25.21	21.58
37	24	210	5	4.1	4.2	1.51	2.34	14.81	22.95
38	18	220	5	4.8	4.8	2.20	2.60	21.58	25.51
39	18	230	5	5.8	5.8	1.40	1.80	13.73	17.66
40	18	230	5	4.2	4.6	2.19	2.91	21.48	28.55
41	18	230	5	5.0	5.4	3.01	2.93	29.53	28.74
42	18	240	5	5.6	5.6	2.40	2.10	23.54	20.60
43	18	240	5	5.5	5.6	3.57	4.01	35.02	39.34
44	18	250	5	4.4	4.2	1.93	2.37	18.93	23.25
45	18	250	5	4.7	4.6	2.10	2.10	20.60	20.60
46	18	250	5	4.5	4.4	2.10	2.60	20.60	25.51
47	18	250	5	5.2	5.5	2.40	2.10	23.54	20.60
48	20	270	5	11	10.6	3.47	3.61	34.04	35.41
49	18	280	5	5.6	5.7	3.19	2.97	31.29	29.13
50	18	300	5	6.0	6.0	2.06	3.10	20.21	30.41
51	18	300	5	5.6	5.8	2.68	2.06	26.29	20.21
52	18	300	5	5.8	5.7	3.30	3.60	32.37	35.32
53	18	300	5	6.0	6.0	3.34	4.20	32.77	41.20
54	18	300	5	6.0	5.9	3.80	4.30	37.28	42.18
55	18	300	5	6.1	5.9	4.20	4.60	41.20	45.13
56	24	300	5	5.1	5.3	4.05	4.27	39.73	41.89
57	18	320	5	5.8	5.9	5.20	7.05	51.01	69.16
58	30	320	5	5.8	5.9	4.76	5.20	46.70	51.01
59	18	350	5	6.0	5.8	4.10	3.90	40.22	38.26
60	36	380	5	3.0	3.0	6.02	6.00	59.06	58.86

The age of boars from group 1 ranged between 5 and 36 months with an average of 18.22 months and a standard deviation of 10.65 while the range of age of group 2 was between 4 and 60 months with an average of 17.97 months and a standard deviation of 12.80. The statistical results of the different variables for the main two groups and their pools are shown in **Table 6** and

Table 7 respectively.

Table 6: average and standard deviation of the different variables recorded for each of the 2 groups; R: right; L: left; DD: deferent duct

	Age (months)	Weight (kg)	Tensile strength R (kg)	Tensile strength L (kg)	Right DD diameter	Left DD diameter	DD tensile strength R (N)	DD tensile strength L (N)
	GROUP 1							
Average	18.22	211.75	2.35	2.46	3.67	3.64	22.42	23.76
Standard deviation	10.65	78.90	1.16	1.16	1.72	1.71	10.42	10.20
	GROUP 2							
Average	17.97	192.72	2.22	2.40	4.05	4.02	22.17	23.56
Standard deviation	12.80	93.85	1.14	1.30	1.62	1.67	10.83	12.75

Table 7: average and standard deviation of some of the variables recorded for the pools drawn from groups 1 and 2; R: right; L: left; DD: deferent duct

	Age (months)	Weight (kg)	Tensile strength R (kg)	Tensile strength L (kg)	DD tensile strength R (N)	DD tensile strength L (N)
	GROUP 1 POOL					
Average	17.60	208.50	2.89	2.86	28.32	28.02
Standard deviation	10.18	108.12	1.25	1.24	12.25	12.14
	GROUP 2 POOL					
Average	17.60	208.50	3.45	3.43	33.82	33.63
Standard deviation	10.18	105.51	1.92	1.97	18.85	19.29

3.1. Correlation

For the study of correlation between tensile strength and age/weight, castration technique used was disregarded and therefore all the pooled animals were considered as a single group. In fact, in this regard, since the variable of interest was tensile strength of the vas deferens, the observation unit became the vas deferens itself instead of the boar, and therefore each deferent duct (*i.e.* left or right) was considered as a single observation. Accordingly, the tensile strength's average, median, standard deviation, and confidence interval were calculated for the totality of the pool (**Table 8**)

Table 8: statistical values calculated for the tensile strength of all the deferent ducts of the pool

Average	Median	Standard deviation	Confidence Interval
33.73	28.94	18.57	8.14

Pearson's linear correlation was used to determine the presence of a correlation between deferent duct tensile strength and age and weight in the sampled pool. The correlation coefficient R for tensile strength and age was very close to 1. In fact, with R = 0.99 while $p<0.05$ a significantly strong correlation is to be predicted between age and tensile strength (**Figure 12**).

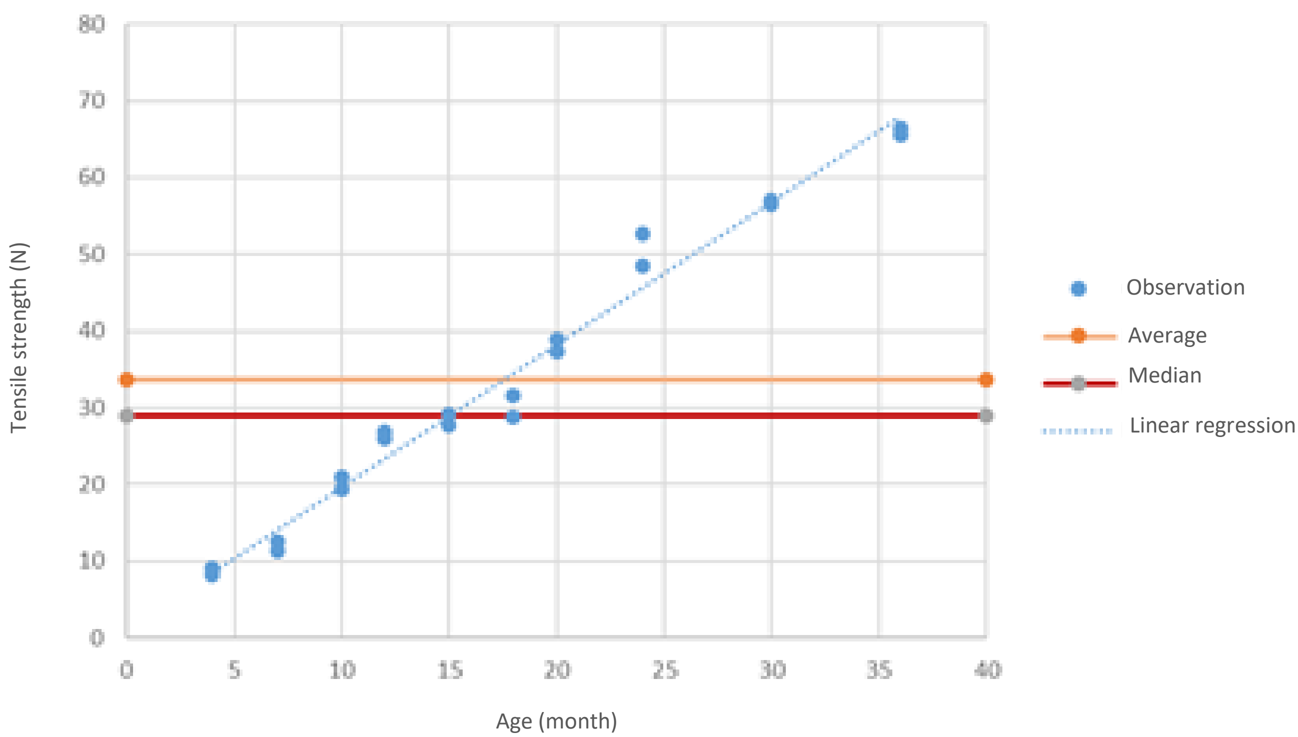

Figure 12: graph showing the correlation between age and tensile strength in the pooled sample; a significantly strong correlation was found with R = 0.99 ($p<0.05$)

Correlation between tensile strength and weight was found to be slightly lower than that of tensile strength-age. However, with a correlation coefficient r = 0.96 and p<0.05 the correlation was still considered to be a significantly strong one (**Figure 13**).

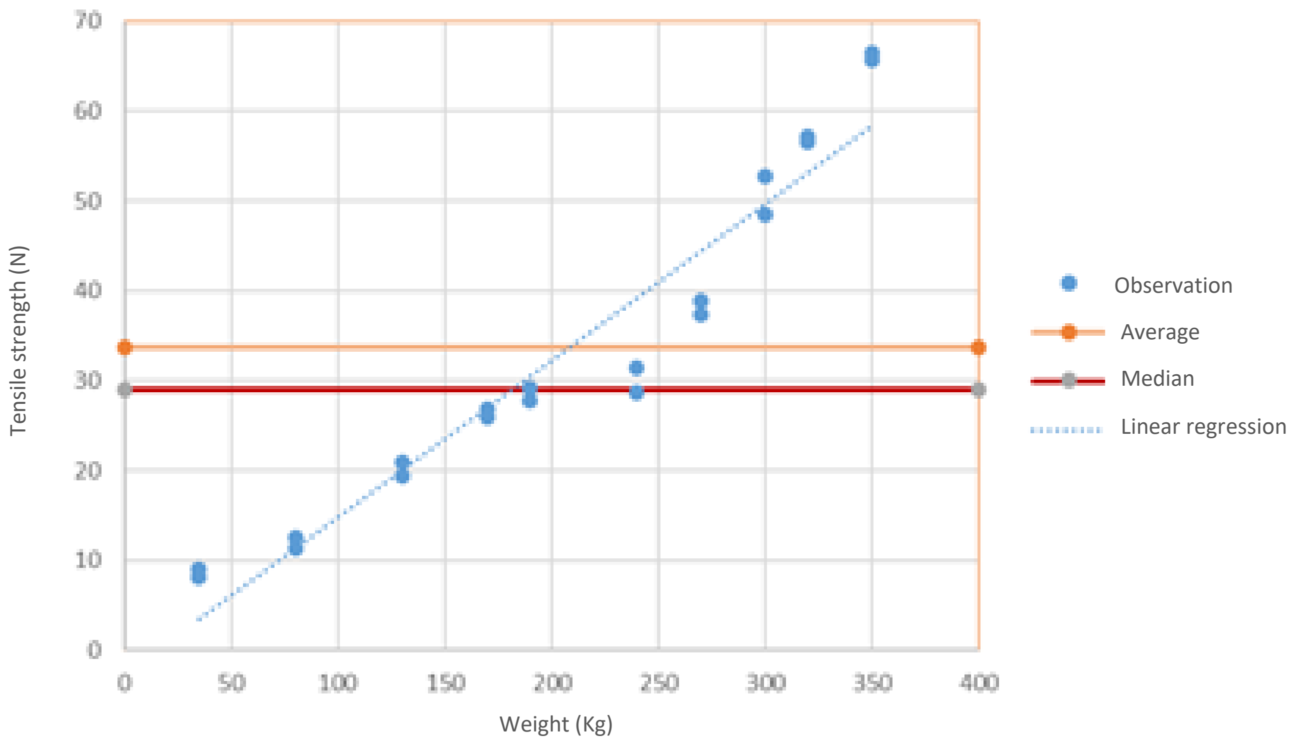

Figure 13: graph showing the correlation between weight and tensile strength in the pooled sample; a significantly strong correlation was found with R = 0.96 (p<0.05)

3.2. Power analysis

Power analysis was conducted to learn the number of samples necessary for futures studies in order to be able to accurately find a significant difference between the 2 groups with a level of significance α of 0.05. This showed that a power of 95% is reached with a total sample size of 388 deferent ducts (194 per group) or 194 animals in total (97 per group). The power of the study according to sample size can be seen in **Figure 14**.

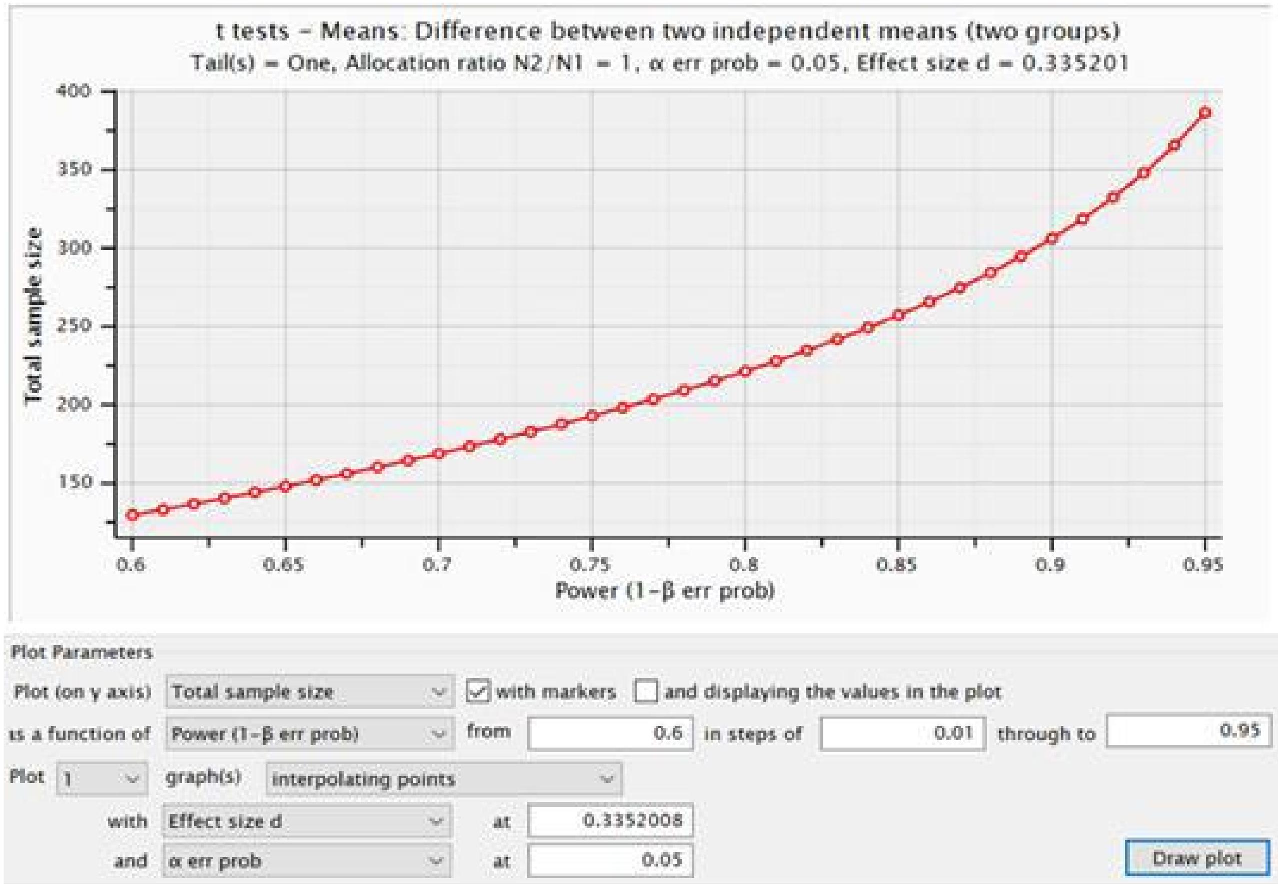

Figure 14: graph showing the increase of the power of the study according to the size of the sample

4. Discussion

After profound bibliography research, we found that there was a lack of knowledge about how different surgical castration techniques compare to each other in adult boars. This study was conducted in order to determine the feasibility and validity of a novel technique for orchiectomy in adult boars that we named the sutureless castration technique. This technique was based on the one used for the castration of tomcats and was also reported to be used successfully in young lambs of 4 to 6 days old (Thornton & Waterman-Pearson, 1999). The sutureless technique has the potential to be less expensive than classical orchiectomy and may even exhibit less postsurgical complications such as infection. Other advantages of the sutureless technique include its' surgical time, which although not included in our study, is evidently lower than that required for any other castration technique whether by ligation or emasculation. Teachability of the sutureless technique is also an important aspect of it. In fact, this study was conducted during the activities of the Mobile Clinic of the Department of Veterinary Medicine at the University of Sassari, where students were often attending the surgery, sometimes even participating, or assisting the surgeon, and the ease with which they were able to grasp this technique was noticeable.

Our main objective was to attempt sutureless castration successfully and compare its’ success rate to that of classical castration which was considered as the control group in this study. This objective was achieved with all 60 animals that were included in the study group being successfully castrated without recording any postsurgical complications or eventual death, same as for the control group. However, seeing that this was only a pilot study, a sufficient description of immediate postsurgical complications such as hemorrhage is not to be expected. Indeed, in a similar comparative study conducted on 3 months old boars and aiming to establish the validity of orchiectomy using nylon clamps for the ligation of the spermatic cord, only 20 boars were included and divided into a study group and a control group castrated by classical orchiectomy and no immediate postsurgical complications were noted (do Prado, et al., 2018). An accurate investigation into the occurrence of postsurgical complications was left to the next stage of our research which consisted of a large-scale clinical study based on the findings of this pilot study.

Regarding complications that are hypothesized to be potentially linked to our novel technique, a reasonable concern would be the increased risk of inguinal hernia since this procedure is done using the open castration approach where the vaginal tunic is not closed with the spermatic cord. The higher risk of inguinal hernia with the open technique as opposed to the closed technique in swine has been frequently discussed in veterinary surgery textbooks and is mostly linked to the large size of the inguinal ring through which visceral organs can get incarcerated if the vaginal process is left open following orchiectomy (Anderson & Mulon, 2019). Some surgeons even go as far as suturing the vaginal tunic after the excision of the testis in order to reduce the risk of inguinal hernia (Lehmann, et al., 2017b) or even closing the external inguinal ring when the boar has a preexisting inguinal hernia (Salcedo-Jiménez, et. al., 2020). In this study however, no such measures were taken and there was no record of the occurrence of inguinal hernia in any of the castrated boars regardless of surgical technique.

The parainguinal approach employed in this pilot study was later found to be poorly described for adult boars in published data. In fact, the most adopted incisional site in boars is the scrotal one with the incision being made ventrally in order to allow for better drainage (Ames, 2014; Anderson & Mulon, 2019; do Prado, et al., 2018). Parainguinal incision has only been described once in adult boars undergoing orchiectomy (Umapathy, et al., 1997) and this approach is thought to allow an even more proper drainage given the ventral positioning of the incision. Possible complications that may arise from a parainguinal incision include the higher risk of evisceration in the case of inguinal hernia and the risk of surgical wound infection to develop into peritonitis given the proximity of the surgical site to the inguinal canal which can act as a route for ascending infections.

The most critical part of the sutureless technique is the tightening of the square knots made with the spermatic and vascular portions of the spermatic cord, as the vas deferens at this stage may break, thus rendering the completion of the surgery impossible. For this purpose, we decided to direct our attention to this factor by measuring and studying several variables related to the deferent ducts, most notably their tensile strength. Therefore, important parameters such as deferent duct diameter and tensile strength were measured, and the latter was correlated with age and weight in order to investigate the possibility of estimating the traction force to be applied for future procedures. Therefore, animals from the control group were castrated during the first phase of the study, and the correlation was established using these samples to allow for an early estimation of deferent duct tensile strength during the castration of animals from the study group.

Although it is hypothesized that deferent duct tensile strength is directly proportional to age and weight, to our knowledge, no studies have been conducted in any species correlating these variables, let alone measuring the tensile strength of the vas deferens in swine. This was another reason for our interest in that parameter and in clarifying how it relates to age and weight. In this study with a relatively small number of samples and a largely variable range of age and weight, we thought it would be more accurate to draw more homogeneous pools from the initial sample and conduct the correlation analysis on the pooled sample. As expected, a strong correlation was established between age-tensile strength ($R = 0.99$ with $p<0.05$), as well as weight-tensile strength ($R = 0.96$ with $p<0.05$). According to our results, tensile strength is minimal in young boars and increases progressively with age and weight. These promising results were used as the basis for the work conducted in the following part of our research project. However, given the small number of samples, we were reluctant to extract a linear equation from the Pearson correlation that could be used to predict tensile strength based on age or weight. In fact, we deemed this analysis to be more suitable for the following large-scale study. Finally, power analysis was conducted in order to determine the number of samples necessary to confirm these correlations. The target sample size was found to be a manageable number of 194 animals in total which we aimed for in our subsequent study.

5. Conclusion

Sutureless castration seems to be an acceptable alternative to classical castration. It is less expensive, time consuming and potentially even less associated with postsurgical castration, in addition to having a faster learning curve for students. This pilot study laid the cornerstone that was needed to proceed with large-scale clinical studies on the sutureless castration technique. It would be interesting for future studies to explore other essential aspects of this technique such as wound healing, postsurgical recovery, and the occurrence of short and long-term complications.

CHAPTER IV: CLINICAL STUDY ON EFFICACY, REPEATABILITY AND POSTSURGICAL COMPLICATIONS

1. Introduction

Surgeons performing orchiectomy on adult boars have a wide range of options regarding the technique to be adopted. Indeed, between open and closed castration; scrotal or parainguinal approach; torsion, ligation, or emasculation; primary closure or second intention wound healing; one may be confused while deciding which technique to choose for every step of the surgery. The fact that all these different elements are poorly described in adult boars and a significant lack of comparative studies was found during our bibliography research, only makes this decision even more difficult. Nevertheless, some common knowledge extrapolated from other animal species and piglet castration can help establish a few hypotheses regarding these techniques, and while some have better prospects than others, they all have disadvantages. In this spirit, we proposed the use of the sutureless technique as an alternative method of surgical castration. This technique consists of an open castration during which the spermatic cord is separated into a vascular and spermatic portion after rupturing the deferent duct from its' attachment to the epididymis followed by tying several square knots with these two structures, thus achieving hemostasis without the need to use any ligature or emasculator.

The feasibility of this technique was established in the previous pilot study where 60 adult boars were castrated using the sutureless technique and 41 were castrated by classical orchiectomy with spermatic cord ligation. Preliminary results showed a perfect success rate with all 60 boars being successfully castrated with the sutureless technique, which was similar to that of the control group. Additional findings of importance included the establishment of a strong correlation between deferent duct tensile strength and age or weight. This is an important variable to take into consideration with the sutureless technique since deferent duct rupture during the tightening of the square knots can result in the failure of the surgery and therefore the surgeon needs to have a preestablished knowledge of the traction strength to be applied during surgery. A power analysis was also conducted in the previous study in order to determine the sample size needed to accurately draw the linear model resulting from tensile strength-age and tensile strength-weight correlations, which was one of the main purposes of this study; and the sample size was determined at a total of 388 deferent ducts in total, or 194 boars. In addition, the following work was conducted as a clinical study on the sutureless castration technique allowing the assessment of the evolution of the surgical wound and the occurrence of postsurgical complications by means of a short and long-term follow up.

2. Materials and methods

2.1. Animals

The study was conducted in the North Sardinian region of Gallura in a period extending from September 2019 until October 2021 during the activities organized by the Mobile Clinic of the Veterinary Medicine Department of the University of Sassari A total of 403 entire mixed-race boars aged between 2 and 91 months were included in the study. The animals came from 378 different small farms in the region of Gallura. They were presented to the Mobile Clinic for routine castration. All animals were castrated by the sutureless technique in field conditions, within the farms in which they were being reared.

2.2. Anesthesia

The anesthetic protocol was the same for all the boars included in this study. Farmers were asked to keep the animals scheduled for castration fasted for 12h prior to the surgery and isolated from the rest of the pigs on the farm in order to facilitate capture and restraint at the time of the intervention. The boars were restrained using a snout snare passed caudally to the superior canine teeth and over the dorsal surface of the snout and tightened. Peripheral venal access was achieved through the auricular veins in order to allow the intravenous administration of sedative and anesthetic drugs. Weight estimation was done at this point by an expert professional. For the purpose of avoiding errors that may arise due to interindividual variability, the same individual completed the weight estimation for all the animals.

Sedative and anesthetic drugs were administered in a single IV bolus. Sedation was achieved with Azaperone (1 mg/kg), a D_2 dopamine antagonist butyrophenone; and detomidine (0.1 mg/kg), an α_2 adrenergic antagonist; while general anesthesia was induced with a dose of 5 mg/kg of tiletamine/zolazepam sold under the commercial name ZOLETIL® (VIRBAC) combining a dissociative agent and a benzodiazepine sedative. The sedative and anesthetic medication administered was sufficient to maintain deep anesthesia for the entire duration of the procedure which did not exceed 5-10 min in all cases starting from the first incision of the parainguinal area and ending with the excision of the second testis.

Before the beginning of the surgery, a dose of 3 mg/kg IM of ketoprofen (KETOSOL®, VIRBAC) was administered to reduce inflammation in the postsurgical period. A dose of 30 mg/kg of long acting oxytetracycline (ENGEMICINA D.D.®, MSD) and 0.3 mg/kg ivermectin (TOLOMEC®, ATI/FATRO) were also administered by intramuscular injection for a prophylactic antibacterial and antiparasitic effect.

2.3. Surgery

After sedation and anesthesia medication were administered, the animals were positioned in dorsal recumbency and the surgical site was prepared by first washing the parainguinal area with antibacterial soap to eliminate the large particles of contaminants, followed by scrubbing with povidone-iodine solution for a few minutes, thus allowing some time for the onset of deep anesthesia. The testis was pushed cranially into the inguinal area and a variable dose of 5 to 20 mL (according to testicular size) 2% lidocaine solution was injected subcutaneously along the incisional line to achieve local analgesia. A 4 to 10 cm longitudinal incision was made with a scalpel over the skin covering the inguinally displaced testis. The incision was continued through the underlying tissue and the parietal tunica vaginalis was incised, thus all boars were castrated by open castration. The testis was freed from its' attachment to the tunica vaginalis by digitally breaking the ligament of the tail of the epididymis and the spermatic cord was manually stripped from the parietal tunic as proximally as possible to the inguinal ring.

Following the extraction of the testis from the vaginal process and stripping the spermatic cord of the parietal tunic up to the level of the inguinal ring, the deferent duct was manually detached from the tail of the epididymis and freed from the testis spermatic cord, thus splitting the latter into a spermatic portion consisting of the deferent duct and a vascular portion consisting of the testicular artery and vein. These two portions were tied to each other by making 3 to 5 square knots (6 to 10 throws). The first knot was made as proximally possible to the internal inguinal ring while the following knots were made about 2 cm distal to the first one. The surgeon was careful not to apply excessive traction on the deferent duct when tying the knots in order to avoid accidental rupture. After a sufficient number of knots were tied, both portions of the spermatic cord were aligned and transected distally to the knots and the remnant part of the spermatic cord was inspected for hemorrhage before placing it back within the tunica vaginalis. If blood was observed to be continuously dripping from the remnant spermatic cord, a double ligature was placed and the sutureless technique was considered unsuccessful for this individual boar. Finally, the coagulated blood particles were removed, and the surgeon proceeded to castrate the contralateral testis using the same technique as the one described above.

The surgical incision was left open allowing for second intention healing of the wound thus providing better drainage of the surgical site in the postsurgical period. The site of incision and its' surrounding area were then disinfected with povidone-iodine and sprayed with antibacterial chlortetracycline hydrochloride spray.

2.4. Measurements and recorded data

Prior to the surgery, the age and weight of each animal were recorded. After the excision of the testis, the diameter of the deferent duct (in mm) was measured at the level of scalpel transection using grid paper and was recorded for the left and right testis for each animal of both groups. Tensile strength (in Newton) of the left and right deferent ducts was also measured for the animals of both groups using a KOP 24387 (KennOptics, London) digital dynamometer. For that purpose, a portion of the excised deferent duct was taken following a standardized length of 4 cm for boars aged under 9 months and 5 cm for boars older than 8 months of age which was measured with grid paper. The sampled portion of the vas deferens was then grasped by each end with a straight Klemmer hemostatic forceps, attaching one to the dynamometer and pulling on the second with gradually increasing force until the deferent duct breaks. This procedure was performed by the same individual for the duration of the study in order to eliminate the risk of error arising from interindividual variability. Tensile strength was then recorded both in Newton and in kg for each deferent duct.

2.5. Clinical follow up

A sample of 50 animals was followed clinically in order to study the occurrence of complications on the short and long term. Boars that were included in the postsurgical follow up were chosen randomly using a randomization software. The clinical examination was conducted by two individuals (a student and a veterinarian) in order to allow for objective assessment and reduce the risk of bias. Regardless of whether the animals were included in the clinical follow up, all owners were told to contact us in case they noticed any alarming complications, odd behavior or in the case of death.

2.5.1. Short-term

Animals included in the follow up study were subjected to clinical examination during the first 4 days following the surgery and then on day 7 and 14. A comprehensive checklist was completed evaluating the general condition of boars and the evolution of surgical wound healing. The elements of the checklist can be seen in **Table 9** and included evaluation of the aspect and quality of wound healing, gait stiffness, feeding behavior, additional complaints stated by the farmer and the occurrence of death as well as its' cause.

Table 9: variables recorded during short-term follow up, the unite/category of answers are found between brackets

Aspect and quality of wound healing

- Presence of edema was noted (Y/N)
- Duration of swelling (days) was recorded as the cumulative sum of days during which edema was noted
- Degree of swelling was recorded following a graded scale (1 = mild; 2 = mild to moderate; 3 = moderate; 4 = moderate to severe; 5 = severe)
- Presence of pus in the surgical wound or the formation of an abscess in the adjacent area (Y/N)
- The presence of parasites in the surgical wound or myiasis was recorded (Y/N)

Gait stiffness

- Presence of gait stiffness was noted (Y/N)
- Duration of gait stiffness (days) was recorded as the cumulative sum of days during which gait stiffness was noted

Feeding behavior

- Inappetance was recorded (Y/N)
- Time needed until return to feeding (day) was recorded as the cumulative sum of days during which inappetance was noted

Other

- Any symptoms or behavior noted by the farmer that may be related to the surgery

Death

- Time and cause of death were noted

2.5.2. Long-term

Long-term follow up was done at the time of slaughter by inspecting the carcass of boars included in the follow up. The variables recorded consisted of the presence of scar tissue formation; presence of abscess and/or granuloma around the surgical site that may be related to castration, or the surgical technique used; and the presence of adherences at the level of the inguinal area as well as their nature.

2.6. Statistical analysis

The results of the measurements taken during the surgery for all 403 boars were reported in an Excel sheet and the correlation between deferent duct tensile strength and age and weight was analyzed by the

Pearson linear correlation coefficient R allowing the determination of the correlation coefficient R indicating the existence of correlation between the two variables. The closer the correlation coefficient is to 1, the stronger the correlation and the significance of those results were determined by the probability of error p which was considered to be acceptable at a value of $p<0.05$.

Data collected from the 50 boars that were randomly selected for the follow up study was also reported in a separate Excel sheet and descriptive statistical analysis was conducted in addition to one way ANOVA using the software STATISTICA 10® (Stat Soft Inc., 2011). Results were considered significantly different when p-value was below 0.05.

3. Results

3.1. General results

A total of 403 boars were castrated with the sutureless technique. The age of the animals varied within a range between 2 and 91 months with an average of 25 months and a standard deviation of 32.52. on the other hand, weight ranged between 20 and 350 kg while having an average of 140 kg and a standard deviation of 169.70. Average and standard variation of all the variables recorded during the surgery are reported in **Table 10**. The sutureless technique was considered successful in all the animals, meaning that at the end of the surgery all the spermatic cords were properly closed with no visible hemorrhage that would have necessitated the addition of ligatures. A single case of vas deferens rupture occurred when the surgeon was tying one of the square knots. However, the rupture was distal to the knot and there was still sufficient vas deferens length left to complete the surgery with the sutureless technique. In addition, one case of mortality was recorded and was attributed to an unrelated clostridial infection following an outbreak of clostridiosis infection in the farm therefore bringing the mortality rate to a value of 0.25%.

Table 10: average and standard deviation of the different variables recorded during sutureless castration; R: right; L: left; DD: deferent duct

	Age	Weight	DD tensile strength R (kg)	DD tensile strength L (kg)	DD diameter R (mm)	DD diameter L (mm)	DD length R (cm)	DD length L (cm)	DD tensile strength R (N)	DD tensile strength L (N)
Average	25	140	2.28	2.62	3.13	3.35	4.5	4.5	22.41	25.70
Standard deviation	32.53	169.70	2.71	2.23	3.91	3.46	0.71	0.71	26.57	21.92

3.2. Tensile strength – age/weight correlation

Pearson's linear correlation was used to determine the presence of a correlation between deferent duct tensile strength and age and weight. The correlation coefficient R for tensile strength and age was 0.91 with $p<0.05$ and therefore a significantly strong correlation was found between these two variables. We proceeded drawing the linear model (**Figure 15**) allowing for the prediction of tensile strength according to age and the linear equation y = 0.84 x + 3.06 was extracted from the resulting graph.

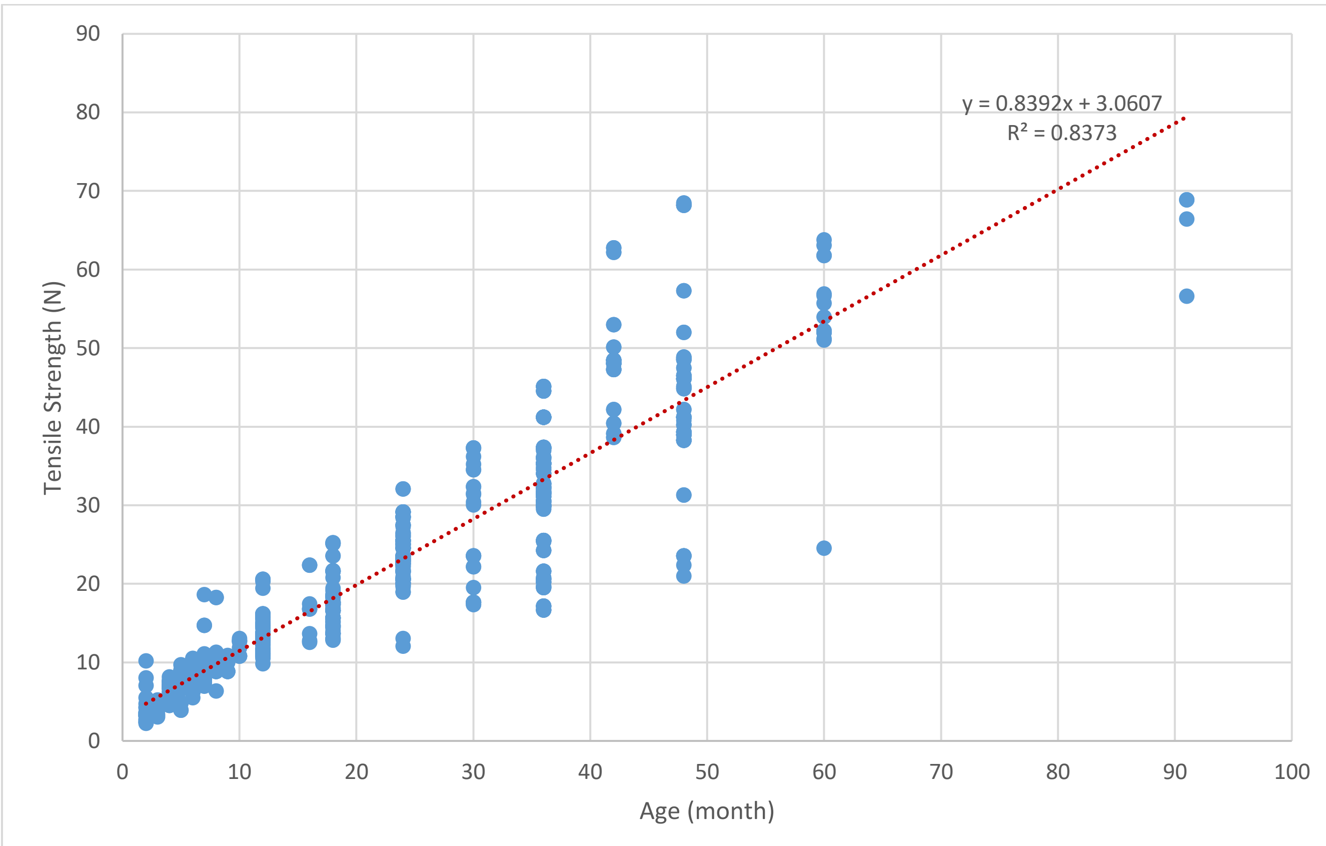

Figure 15: graph showing the correlation between age and tensile strength; the linear equation y = 0.84x + 3.06 is the expression of the model allowing the prediction of tensile strength according to age with significant accuracy ($p<0.05$)

Correlation between tensile strength and weight was found to be slightly lower than that of tensile strength-age. However, with a correlation coefficient R = 0.87 and $p<0.05$ a significantly strong correlation was still established between these two variables as well. We proceeded to drawing the linear model (**Figure 16**) allowing for the prediction of tensile strength according to weight and the linear equation y = 0.12 x – 1.16 was extracted from the resulting graph.

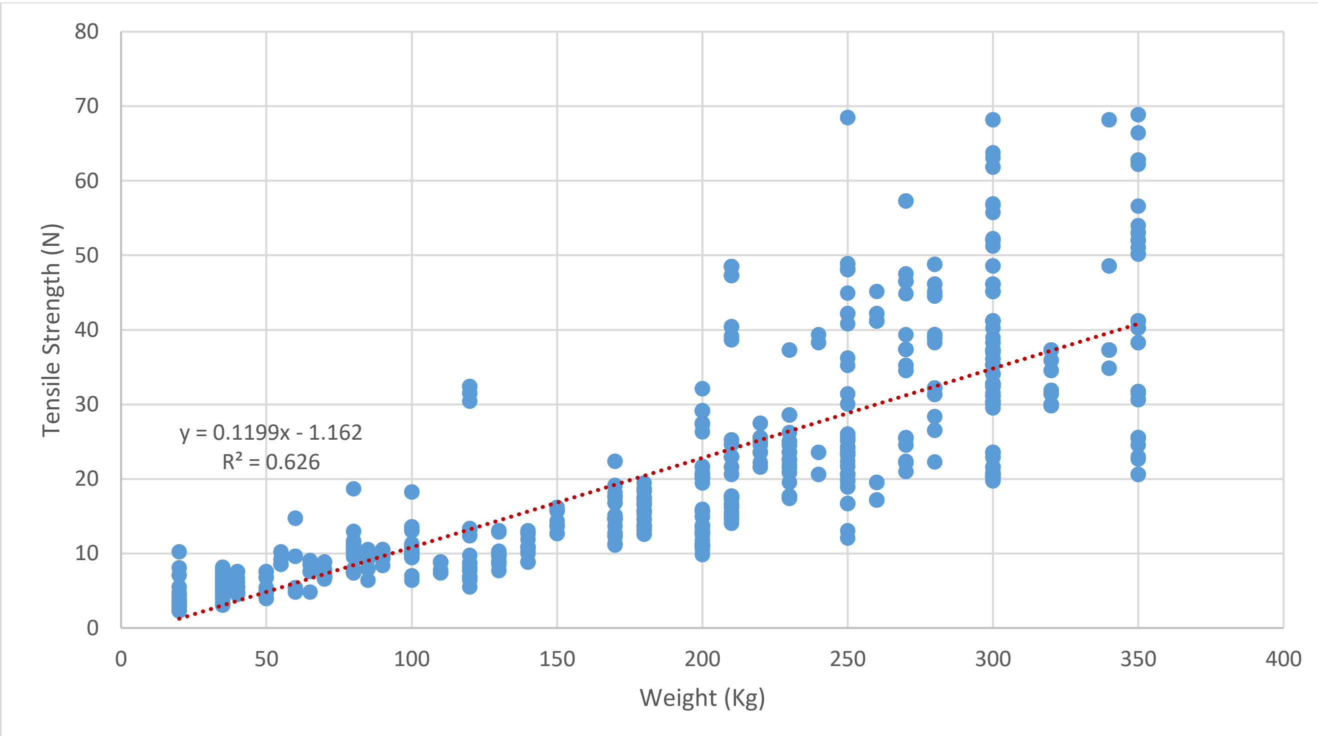

Figure 16: graph showing the correlation between weight and tensile strength; the linear equation y = 0.12 x – 1.16 is the expression of the model allowing the prediction of tensile strength according to weight with significant accuracy ($p<0.05$)

3.3. Clinical follow up

50 boars were randomly chosen to be included in the clinical follow up study and several parameters relating to short and long-term onset of complications were recorded as described previously in section **2.5**. Some of the important data recorded for these animals are reported in **Table 11**.

Table 11: some of the important parameters recorded during clinical examination in the short-term follow up are reported below; [1] the degree of swelling reported in the table below consists of the maximal level of swelling noted during clinical examination and swelling was assessed following a 5 grade scale (1 = mild; 2 = mild to moderate; 3 = moderate; 4 = moderate to severe; 5 = severe); [2] 0 = Absence; 1 = presence; continued in Table 12

Boar ref. no.	Duration of swelling (days)	Degree of swelling[1]	Duration of inappetence (days)	Myiasis[2]	Gait stiffness[2]	Duration of gait stiffness (days)
1	1	1	1	0	0	0
2	1	1	1	0	0	0
3	5	4	2	1	1	3
4	3	1	1	0	1	1
5	1	1	1	0	0	0
6	1	1	1	0	0	0
7	1	1	1	0	0	0
8	2	1	1	0	0	0
9	2	1	1	0	0	0
10	6	5	4	1	1	4
11	4	2	1	0	1	1
12	4	2	1	0	1	1
13	3	1	1	0	1	1
14	1	1	1	0	0	0
15	1	1	1	0	0	0
16	1	1	1	0	0	0
17	4	2	1	0	1	3
18	3	1	1	0	1	1
19	1	1	1	0	0	0
20	5	2	1	1	1	3
21	4	2	1	0	1	2
22	1	1	1	0	0	0
23	1	1	1	0	0	0
24	3	1	1	0	1	1
25	1	1	1	0	0	0
26	4	2	1	0	1	3
27	1	1	1	0	0	0
28	1	1	1	0	0	0
29	1	1	1	0	0	0
30	4	2	1	0	1	2
31	5	3	2	1	1	3
32	3	1	1	0	0	0
33	1	1	1	0	0	0
34	1	1	1	0	0	0
35	1	1	1	0	0	0

Table 12: continuation of Table 11; some of the important parameters recorded during clinical examination in the short-term follow up are reported below; 1 the degree of swelling reported in the table below consists of the maximal level of swelling noted during clinical examination and swelling was assessed following a 5 grade scale (1 = mild; 2 = mild to moderate; 3 = moderate; 4 = moderate to severe; 5 = severe); 2 0 = Absence; 1 = presence

Boar ref. no.	Duration of swelling (days)	Degree of swelling[1]	Duration of inappetence (days)	Myiasis[2]	Gait stiffness[2]	Duration of gait stiffness (days)
36	1	1	1	0	0	0
37	2	1	1	0	0	0
38	1	1	1	0	0	0
39	1	1	1	0	0	0
40	1	1	1	0	0	0
41	1	1	1	0	0	0
42	4	2	1	0	1	2
43	1	1	1	0	0	0
44	1	1	1	0	0	0
45	5	3	2	0	1	3
46	1	1	1	0	0	0
47	1	1	1	0	0	0
48	5	2	1	1	1	5
49	4	2	1	1	1	3
50	1	1	1	0	0	0

3.3.1. *Short-term clinical follow up*

The variables investigated during short-term follow up are recorded in **Table 11** and **Table 12**. Descriptive statistics were conducted on this data and are summarized in **Table 13**.

Table 13: summary of descriptive statistics conducted for the boars followed on short term; [1] refers to the maximal degree of swelling according to the 5-grade scale recorded for the boar during the 14-day follow-up was considered

	Duration of swelling (days)	Degree of swelling[1]	Duration of inappetence (days)	Myiasis	Gait stiffness	Duration of gait stiffness (days)
Average	2.24	1.42	1.12	-	-	0.84
Sum	-	-	-	6	18	-
Perc.	[56% - 2%]	[72% - 2%]	[92% - 2%]	12%	36%	[64% - 2%]
Range	[1-6]	[1-5]	[1-4]	-	-	[0-5]
Std. dv.	1.585	0.835	0.480	-	-	0.480

Swelling of the surgical wound was recorded in all of the castrated boars and ranged between mild swelling which was present in 36 animals (72%) and severe swelling which was noted in a single case (2%). The average of the maximum degrees of swelling that were recorded for each animal was 1.42 with a standard deviation of 0.835. The duration of swelling in turn ranged between 1 and 6 days with most animals exhibiting swelling for only a single day (56%) and the only boar having surgical wound swelling being the one that had a "grade 5" swelling. All 28 boars in which edema was recorded on a single day had a grade 1 tumefaction. The average duration of tumefaction in our sample was 2.24 days with a standard deviation of 1.585. Using a one-way ANOVA, we discovered a significant relationship between the degree of swelling and duration of swelling. In fact, the duration of mild swelling in animals was substantially shorter (p0.05) than that of mild-moderate, moderate, moderate-severe, and severe swelling. As shown in **Figure 17**, we also observed that the average duration of swelling tended to rise as the degree of edema increased.

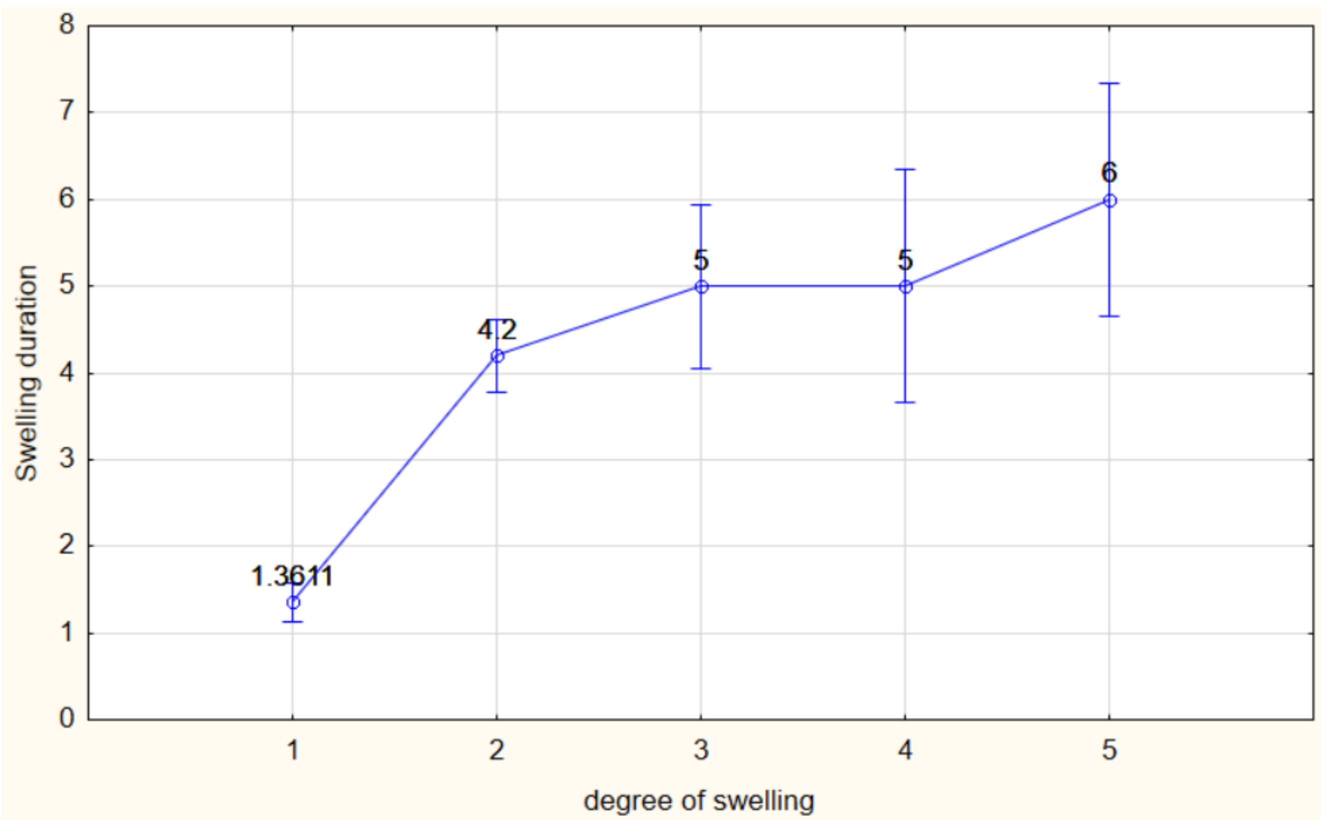

Figure 17: average duration of swelling and CI is shown following the degree of swelling; mild swelling had a significantly lower average duration than all the other grades ($p<0.05$); no other significant effects were found

Postsurgical inappetence was recorded in all 50 boars with this symptom lasting for 1 day in most of the animals (46 pigs or 92%). As for the remaining boars, 3 experienced inappetence for 2 days (6%) and a single case (2%) of 4-day long inappetence was recorded. The latter was in fact the same boar mentioned above that was showing the highest grade of swelling and in which the edema lasted for 6 days. The average time needed for the boar to return to normal feeding behavior following the surgery was 1.12 days with a standard deviation of 0.48. The correlation between inappetence duration and degree of swelling was calculated and we found a significant correlation ($p<0.05$) between the two variables with $R = 0.84$.

Myiasis was recorded in a total of 6 pigs (12%) all of which showed at least a grade 2 surgical wound swelling with the latter lasting for a minimum of 4 days after the surgery. In fact, of these 6 animals, 4 (66.7%) exhibited tumefaction until day 5 while the remaining 2 were exhibited swelling for 4 and 6 days with the latter being none other than the boar previously mentioned. The effect of myiasis on the average duration of swelling, degree of swelling, duration of inappetence, and duration of gait stiffness was then analyzed using a one-way ANOVA and we found that myiasis was associated with a significant increase in the average of all these variables as seen in **Figure 18** to **Figure 21**.

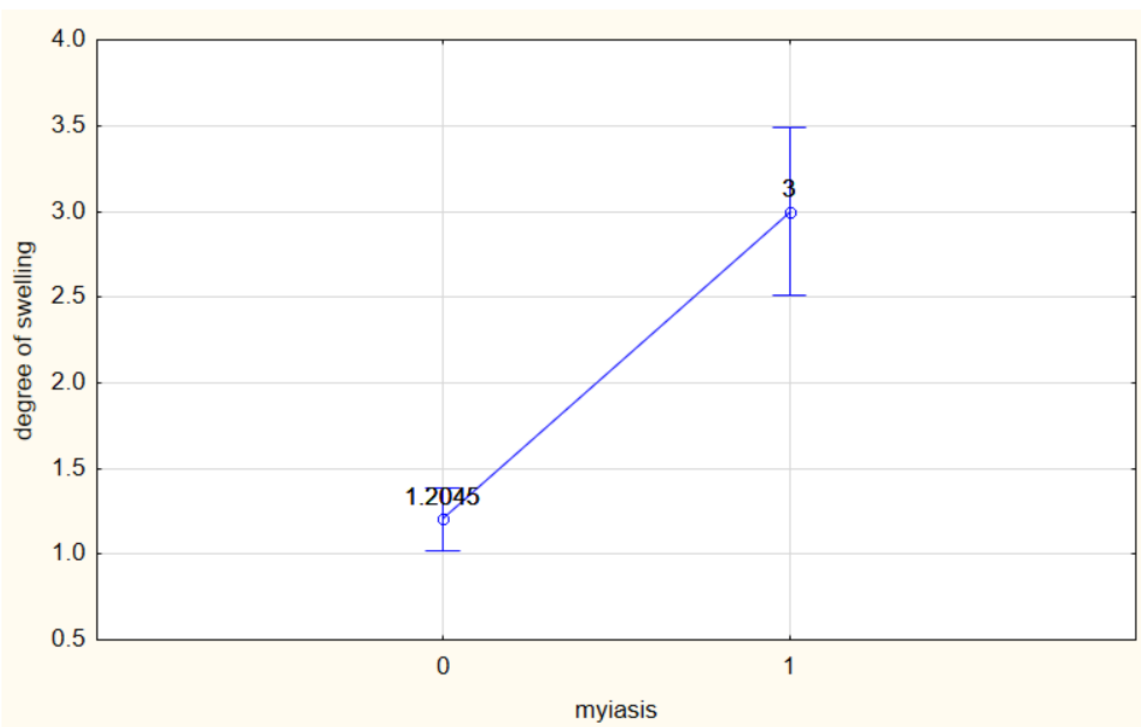

Figure 18: association between myiasis and the degree of swelling; the average degree of swelling was significantly higher ($p<0.05$) in animals with myiasis with the average grade being 1.20 for non-infested animals and 3 for animals with myiasis

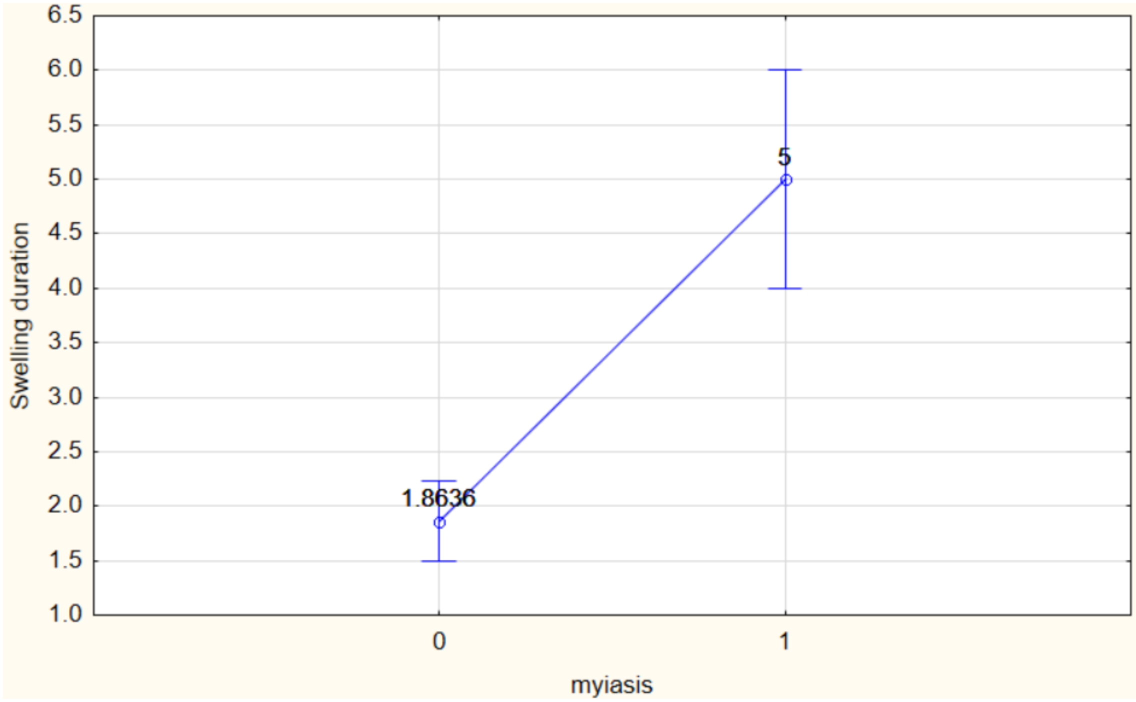

Figure 19: association between myiasis and the duration of swelling; the average duration of swelling was significantly higher ($p<0.05$) in animals with myiasis with the average duration being 1.86 days for non-infested animals and 5 days for animals with myiasis

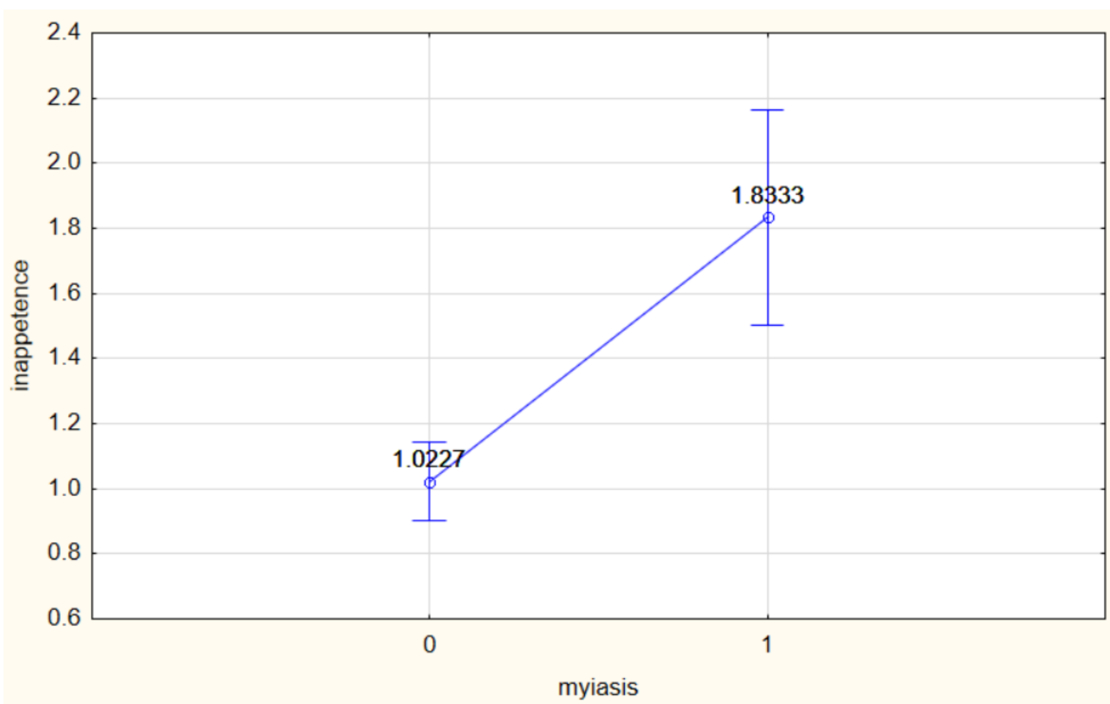

Figure 20: association between myiasis and the duration of inappetence; the average duration of inappetence was significantly higher ($p<0.05$) in animals with myiasis with the average duration being 1.02 days for non-infested animals and 1.83 days for animals with myiasis

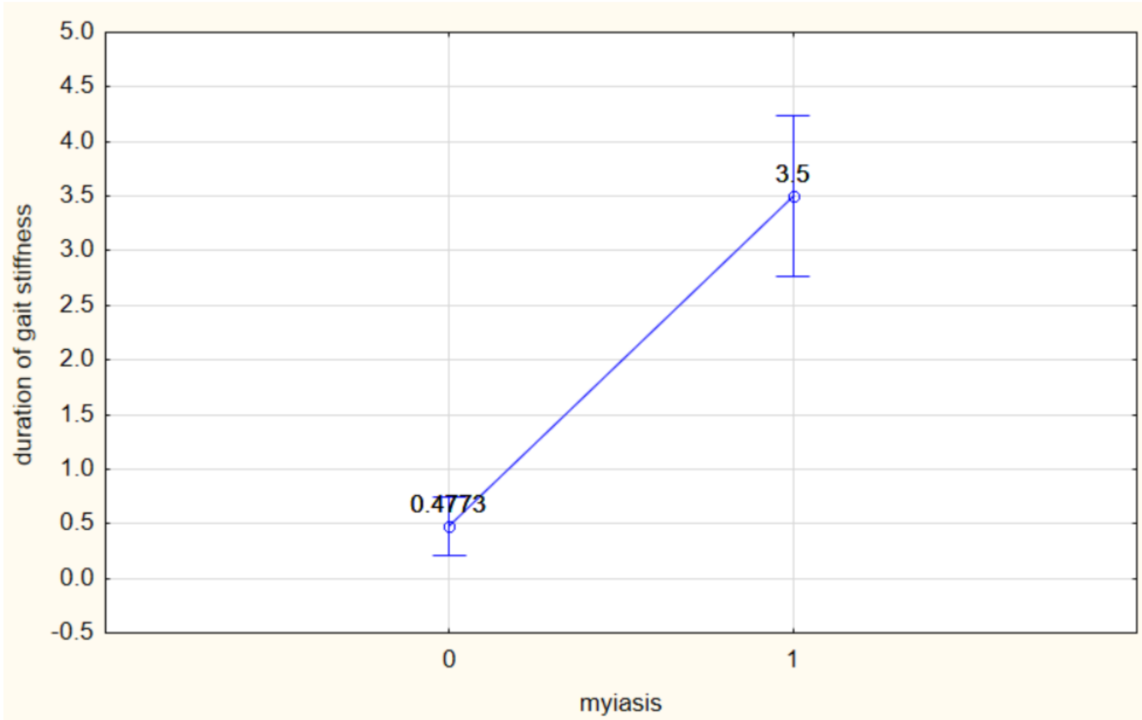

Figure 21: association between myiasis and the duration of gait stiffness; the average duration of gait stiffness was significantly higher ($p<0.05$) in animals with myiasis with the average duration being 0.48 days for non-infested animals and 3.5 days for animals with myiasis

Gait stiffness was recorded in 18 pigs (36%), most of which (14/18 or 77.8%) were showing a degree of swelling of 2 or higher and all of the 18 animals had a swelling duration of more than 3 days. In fact, the duration of swelling was of 3 days in 4/18 animals (22.2%), 4 days in 8/18 animals (44.4%), 5 days in 5/18 animals (27.8%) and 6 days in a single case (5.6%). Duration of stiffness ranged between 0 and 5 days with most animals not showing gait stiffness at all (64%) and all of the latter only showing mild swelling of the surgical wound (grade 1). Gait stiffness lasted for 1 day in 6 pigs (12%); 2 days in 3 pigs (6%); 3 days in 7 pigs (14%); 4 days in 1 pig (2%) and finally 5 days in the pig showing the highest degree and duration of swelling. Mean duration of gait stiffness was 0.84 days with a standard deviation of 0.480. The correlation between the duration of gait stiffness and degree and duration of swelling was calculated using Pearson's linear correlation coefficient. R was determined at 0.815 and 0.919 for duration of gait stiffness - degree of swelling and duration of gait stiffness – duration of swelling respectively, thus proving the presence of a significant correlation with both variables ($p<0.05$).

Finally, a case of small-sized abscess formation was recorded in one of the 50 pigs selected for the clinical study on day 4 during physical examination. Thus the rate of occurrence of postsurgical abscess formation was considered to be 2% (1/50). In that particular case, weight loss was also recorded, and intervention was deemed preferable. The surgical wound was opened to allow for drainage and a supplementary dose of ketoprofen and oxytetracycline was administered. The farmers had no additional complaints related to castration that were worth noting.

3.3.2. Long-term clinical follow up

At the time of slaughter, all animals included in the long-term follow up study had adequate wound healing and scar tissue formation was already completed. No adherences around the surgical site and inguinal area were recorded. However, subcutaneous scrotal granulomas were found in a total of 13 cases thus giving an incidence of 26%. 9 of these were between 0.5 and 1 cm in size; 3 were between 1 and 2 cm; and only one was larger than 2 cm in size.

4. Discussion

After proving the feasibility of the sutureless technique in our previous study; and establishing the correlation between the tensile strength of the deferent duct and age and weight, we proceeded to a large-scale study aiming to determine the repeatability of this technique and monitoring the incidence of postsurgical complications on the short and long term. The number of samples necessary for accurate evaluation of these elements was already calculated in the previous study by the means of a power analysis based on the results of the pilot study and the number of samples required was found to be 388 deferent ducts or 194 animals in total. This number was far exceeded in our clinical study where 403 animals were included, and therefore 806 deferent ducts were castrated by the sutureless technique.

4.1. Tensile strength – age/weight correlation

As estimated previously, the sutureless castration procedure was considered to be successful for all the animals that were subjected to it. This was based on the fact that all the surgeries performed were successfully completed using the sutureless technique and the surgeon was not forced to halt sutureless castration in any given situation and substitute it for the classical technique (*i.e.* spermatic cord ligation with surgical sutures). Nevertheless, a single case of deferent duct rupture was recorded when the surgeon was tightening one of the square knots. This incident was however irrelevant to the success rate of the sutureless technique since the vas deferens was ruptured at its' distal end, leaving enough length to allow for the continuation of the procedure. This occurrence only reinforces our hypothesis regarding the relevance of deferent duct tensile strength and confirms the importance of attention we have accorded to the correlation between tensile strength and age and weight. Indeed, the strong correlation that was found in the previous study between tensile strength – age and tensile strength – weight was confirmed in this study where the correlation coefficient R was determined at a value of 0.91 and 0.87 respectively which implies significant linear correlation ($p<0.05$). Subsequently, a linear model was drawn, and the equation was deduced for each one of the 2 correlations. This equation will serve as a reference for future studies, allowing the surgeon to have a preestablished knowledge of the amount of traction that can be practiced while pulling on the deferent duct. The absence of studies attempting the correlation of deferent duct tensile strength with any other variable makes the model we designed all the more convenient and beneficial. The correlation coefficient of both tensile strength – age and tensile strength - weight calculated in the first study (R = 0.99 and 0.96 respectively) was higher than the one found in the clinical study (R = 0.91 and 0.87 respectively); however the latter was conducted with a significantly large sample size allowing to accurately determine the value of R which was still considered to be significantly high even with such a large sample. Despite both age and weight correlating strongly with deferent duct tensile strength, the slightly higher value of R found for the correlation with age leads us to recommend the use of the latter for the estimation of tensile strength when the surgeon has both information readily available.

4.2. Clinical follow up

This study aimed to assess the incidence of short- and long-term postsurgical complications in addition to evaluating the repeatability of the sutureless technique. This was achieved via the follow up designed for a sample of 50 animals selected randomly from our original sample. The number of animals included in this part of the study was limited since it is labor-intensive, and it requires constant visits to the farms where the animals are reared which sometimes are located in very remote areas. However, all farmers were well informed regarding the main postsurgical complications to monitor (*i.e.* inguinal hernia, excessive hemorrhage, and wound site infection) and were asked to contact us directly in case they suspected the presence of any of these conditions. A single case of mortality was recorded in the entire sample of 403 boars. It was the result of a clostridial infection following an outbreak on the farm and was thus considered to be unrelated to the surgical technique employed.

The sample size necessary for the accurate evaluation of postsurgical complication incidence is dependent on the estimated incidence of these complications. The main issue that may cause concern with the sutureless technique is the achievement of adequate hemostasis to avoid hemorrhage. This common complication was not observed in any of the animals subjugated to sutureless castration in either one of our two studies, therefore we could not estimate its' incidence. Other than hemorrhage, inguinal hernia is a complication that may potentially be associated with the sutureless technique since the latter requires the opening of the tunica vaginalis, thus completing an open castration without the possibility of closing the vaginal process which renders inguinal hernia more probable. This complication was not recorded in our study either and again, estimation of its' incidence was not possible. The extrapolation of the incidence of excessive postsurgical hemorrhage and inguinal hernia from other similar castration techniques would have been possible and would have helped us form a better idea of the sample size necessary for the detection of these complications and recording their rate of occurrence. However, to our knowledge, there are no published records describing the incidence of postsurgical complications in adult pigs undergoing elective orchiectomy.

According to our hypothesis, postsurgical infection should have been the least probable complication since the sutureless technique does not require the application of ligatures or an emasculator thus reducing the risk of surgical wound contamination and perioperative manipulation. However, a case of small-sized abscess formation arose in one of the 50 pigs included in the clinical study during physical examination conducted on day 4 thus the incidence of postsurgical abscess formation was considered to be 2% (1/50). Nonetheless, this value does not account for the entire sample of 403 animals, and despite this note being included in the follow-up sample, the remaining 353 boars should be considered when calculating the incidence rate of complications because the owners of these animals were asked to contact us if they noticed the occurrence of these specific issues. Therefore, the correct value of the incidence of postsurgical abscess for the sutureless technique should be 0.25% (1/403).

4.2.1. Short-term follow up

The short-term follow up consisted of physical examination of the castrated animals on a period extending from the first day after orchiectomy until the 14th day. Several parameters relating to surgical wound healing and the animals' general condition were recorded and subsequently analyzed. This type of follow up is commonly used in similar studies assessing postsurgical recovery in animals of different species undergoing orchiectomy (Abou-Ahmed, et al., 2012; Mintline, et al., 2014; Saifzadeh, et al., 2008).

For the purpose of evaluating wound healing, boars subjected to orchiectomy were examined and a score of 1 to 5 was assigned according to the degree of swelling, with 1 indicating the lowest amount of swelling (*i.e.* mild swelling) and 5 the highest (*i.e.* severe swelling) and the total duration of swelling was then deduced depending on the total amount of days during which swelling was still obvious to the clinician. Alternatives to this method for wound healing assessment have been used in similar studies conducted in other species and these include the measurement of scrotal and preputial dimensions

(**Figure 23**) which has been used in stallions undergoing castration by the "section-ligation-release" technique (Saifzadeh, et al., 2008); and the 5-grade wound healing scale (**Figure 22**) which was used for calves undergoing orchiectomy (Mintline, et al., 2014). Regarding scrotal and preputial dimension measurement, despite this method being accurate, it is inadequate for animals being castrated by the parainguinal approach, not to mention its inefficacy for boars.

Example	Score	Description
	1	The incision runs the length of the scrotum and tissue is exposed in this area. The incision may have exudate, either wet or dry. Scabbing is uncommon but may be present in isolated locations at the edges or across the center of the wound. There are few wrinkles/folds and swelling extends to the area around the scrotum.
	2	The incision is greater than or approximately ¾ the length of the scrotum and scabbing is present. The incision may also have exudate, either wet or dry. There are some wrinkles/folds and swelling extends to the area around the scrotum.
	3	The incision is scabbed or open and is less than ¾ of the scrotum. The incision site may also have exudate, either wet or dry. There are many wrinkles/folds, but swelling still extends to the area around the scrotum.
	4	The wound/incision site is less than ¼ of the scrotum, and is smaller than or equal to the size of a teat visible in the photo. A small scab or discoloration is present at the center of the scrotum/wound site. This wound site may have exudate, either wet or dry. There are many folds/wrinkles present. The scrotum may have slight swelling, but it should be almost flush with the body of the animal.
	5	The incision site is no longer visible. There is no tissue exposed anywhere on the scrotum. There is no scabbing and/or dried exudate. The scrotum has folding/wrinkling, is not inflamed and is flush with the body of the animal.

Figure 22: photographical representation of the 5-grade scale adopted for wound healing assessment following orchiectomy in calves (Mintline, et al., 2014)

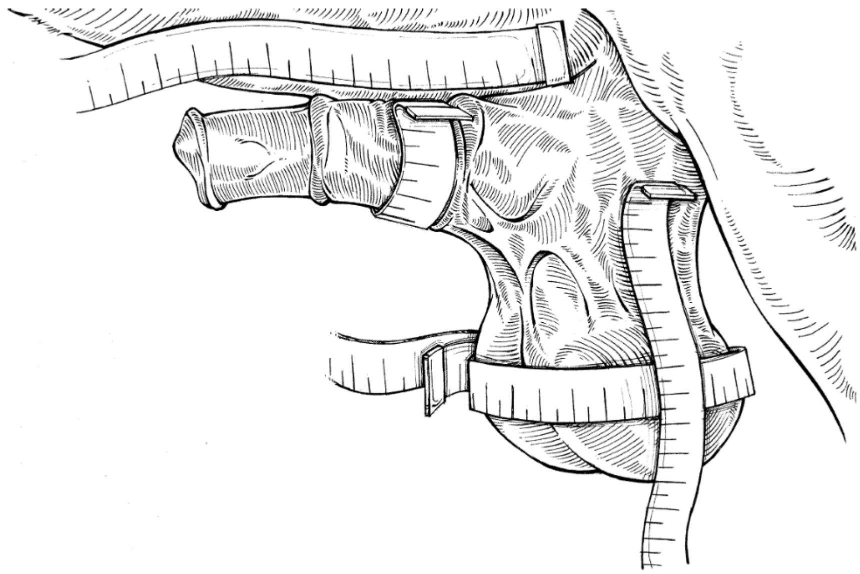

Figure 23: scrotal and preputial dimensions are measured at 4 different levels as seen in the scheme above in order to assess postoperative swelling in horses following orchiectomy (Saifzadeh, et al., 2008)

As for the 5-grade wound healing scale, it would have been interesting to use in addition to the parameters followed in our study since it allows a better assessment of scar tissue formation for the duration of the study as opposed to only recording it at slaughter, as was done by our method.

Some degree of surgical wound edema is to be expected after any given procedure and is usually no particular cause for alarm but is rather simply monitored to ensure it does not develop into more serious complications. In the case of the 50 boars that were followed in a short postoperative period, a healthy amount of 72% of the animals was found to have only grade 1 postsurgical edema. The remaining animals had a degree of swelling that ranged between mild-moderate and moderate-severe edema, with the exception of a single outlier case in which the boar exhibited severe postsurgical edema that lasted for a record duration of 6 days. This boar was also found to have myiasis and had several concomitant symptoms including inappetence that lasted for 4 days and a stiff gait that lasted for 5 days. Excessive edema can be attributed to the hygienic conditions in which the procedure was undertaken and in which these animals are housed. In a similar study, 20 boars were castrated either by classical castration technique or using nylon clamps to establish hemostasis and several animals were found to have edema, however no significant effect was established with either castration technique or both techniques (do Prado, et al., 2018).

The main purpose of castration is an economical one: farmers require the veterinarian to do this procedure in order to be able to eventually slaughter the boar and profit from selling its' meat, which would not be possible in adult boars unless it is castrated. One of the few advantages of performing castration in adult boars is that it works around the reduced feed conversion and weight gain avoiding these two well established disadvantages of piglet castration (Hegerová & Juhás, 2021). This explains the importance of taking a factor such as inappetence into consideration in our study. In addition, inappetence is an indicator of pain and possible underlying complications therefore it is important to take such a variable into consideration. In our sample, most of the animals returned to normal feeding behavior after 1 day of the procedure while only 3 animals showed inappetence also on day 2, of course in addition to the boar with severe edema previously mentioned and which did not return to normal feeding behavior until day 4.

Myiasis was recorded in 12% of the animals selected for follow up and statistical analysis established the presence of a significant effect of myiasis on the degree of swelling, duration of swelling, duration of inappetence and duration of gait stiffness. In fact, surgical wounds are a common site for bot-fly infestation and edema only increases the chances of infestation. In this case, especially when swelling was persistent for several days; which was the case for all 6 animals that exhibited myiasis, the risk of bot-fly infestation increases accordingly.

Gait stiffness after castration is a common occurrence in orchiectomy that results from edema and pain originating from the surgical site. Stiffness was recorded in 36% of the animals in our study. Our findings indicated the presence of a correlation between the duration of gait stiffness and degree/duration of swelling which is consistent with common knowledge regarding this variable (Van der Saag, et al., 2018a).

4.2.2. Long-term follow up

The long-term follow up revealed successful scar tissue formation for all the animals and the absence of adherences related to the surgical site. The remnant spermatic cord was inspected, and no granulomas or abscesses were found at this level. The only findings recorded on carcass inspection were the presence of small subcutaneous scrotal granulomas in 13 animals. However, given their localization which was rather far from the surgical site, they were deemed irrelevant to the sutureless technique.

5. Conclusion

In conclusion, this large-scale clinical study successfully demonstrated the efficacy of the sutureless technique and its' safety. The linear model correlating deferent duct tensile strength to age, and weight was established and is hence presented as a tool for future sutureless castration. Contrary to our initial estimation, inguinal hernia which should have been the more probable complication to occur, was not recorded but instead a single case of abscess formation, which we thought would be less probable with the sutureless technique; was noted. Some elements still require additional investigation such as the time of surgery, time required for wound healing, and maybe even the teachability of this technique since one of its' advantages is the ease with which it can be performed. Finally, the question of the possibility of using this technique in other farm animals such as small ruminants should be raised.

GENERAL CONCLUSION AND PERSPECTIVES

Surgical castration may be less practiced in adult boars than in piglets, however it remains a rather common occurrence in veterinary practice, and one that is poorly described. Quite a few options exist for orchiectomy in boars, and these were highlighted in the critical review section of this thesis. This allowed for a broad spectrum of understanding of common knowledge regarding adult boar castration and allowed for the learning of the gaps found in this regard in order to present a work that can be used to fill these gaps.

The sutureless technique which we devised as a fast, easy to learn and cost-efficient method for orchiectomy; was validated in the pilot study, establishing essential values necessary for proceeding with the second phase of our study. These included a strong correlation between deferent duct tensile strength and age/weight and the sample size necessary to accurately conduct the following large-scale studies. In addition, the sutureless technique proved to be comparable in success rate to the classical technique of orchiectomy using ligatures.

Based on the findings of the pilot study, the large-scale study proved to be successful in establishing the repeatability of the sutureless technique. The linear model allowing the deduction of deferent duct tensile strength based on age/weight was produced with a strong level of correlation between the variables. This can serve as a reference for future studies necessitating an estimation of tensile strength. The lack of preestablished knowledge of essential values such as the incidence of complications in adult swine undergoing orchiectomy made our study more difficult to complete. However, we used this factor to our advantage by profiting from this gap of knowledge to describe this value in our own study. The incidence of abscess formation around the surgical wound in the postoperative period was found at 0.25% while other complications such as hemorrhage and inguinal hernia were not recorded (0/403), and the mortality rate was 0.25%. These findings can be used as a base of reference for future studies that may be conducted to determine different variables relating to sutureless castration such as surgical time, wound healing time or teachability. It would also be interesting to conduct studies using sutureless castration in other farm animals such as rams and bucks. It is our belief, that with the appropriate amount of accurate and well directed research, this technique has the potential to compete with classical techniques of surgical castration.

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