ALI AL-BAYATI

DEVELOPMENT OF ABDOMINAL ADHESIONS AFTER LAPAROSCOPIC ABOMASOPEXY
–
AN ULTRASONOGRAPHIC STUDY

INAUGURAL-DISSERTATION zur Erlangung des Grades eines **Dr. med. vet.**
beim Fachbereich Veterinärmedizin der Justus-Liebig-Universität Gießen

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Betreuer: **Prof. Dr. K. Doll**

Development of abdominal adhesions after laparoscopic abomasopexy – an ultrasonographic study

INAUGURAL-DISSERTATION
zur Erlangung des Grades eines
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eingereicht von
Ali Fouad Ibrahim Al-Bayati
Tierarzt aus Bagdad (Irak)

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To My Father

Dr. Fouad Ibrahim Mohammed Al-Bayati (1944-2005)

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Abbreviations

#	Number
1^{st}	First
2^{nd}	Second
3^{rd}	Third
4^{th}	Fourth
Ca	Caudal
Ce	Central
Cm	Centimetres
Cr	Cranial
D	Dropped out animal
Kg	Kilogram
L c-c	Length cranial-caudal
La	Lateral
LDA	Left-side displacement of abomasum
Me	Medial
MHz	Megahertz
Mm	Millimetres
nD	No data
nV	Not visible
PAA	Plasminogen activator activity
RBH	Rotbunte Holstein
RDA	Right-side displacement of abomasum
SBH	Schwarzbunte Holstein
SD	Standard deviation
W l-m	Width lateral-medial
$\bar{x}$	Mean

1 Introduction

1.1 General considerations

“Like hitting an empty steel drum or tin can”, “like a tin can kicked down a long alley in the still of the night”, and “like a penny dropping into a well”. These are some descriptions of sounds which, using the auscultation-percussion method will appear if either the left- or right-side of the abomasum is displaced (HUNGERFORD 1990).

Left displacement of the abomasum was first described in 1950 by BEGG (STEINER 2006).

Diseases of bovine abomasum comprise an interesting group of conditions only really appreciated in comparatively recent years (EDDY 2004).

Abomasal displacement is an economically important disease in dairy cattle. Factors such as a parturition, concurrent diseases, season, breed, sex, age, diet, housing, management, and weather may influence the incidence of abomasal displacement. Abomasal hypo-motility is considered to be an important factor in the etiology of abomasal displacement; however, mechanical factors, such as abomasal position and volume, and the volume of the rumen and uterus, may also be important (WITTEK et al. 2005).

There are three important conditions that probably have a similar etiology and epidemiology, yet produce widely differing clinical syndromes. These are: left displacement of the abomasum (LDA), also called fourth stomach displacement; dilatation, distension, and torsion in the right flank (right-side displacement of abomasum or RDA); and ulceration. LDA is almost entirely restricted to dairy cattle and rarely found in suckler beef cows. Diet undoubtedly has an important role in the etiology of these diseases, with the use of concentrated cereal-based feeds and low-fiber diets generally being blamed. In some countries the feeding of root crops that are heavily contaminated with soil, sand, and gravel has also been deemed responsible (HUNGERFORD 1990; EDDY 2004). Certainly it has been observed that in normal cows in late pregnancy the presence of the gravid uterus displaces the abomasum forwards and to the left, and after calving the organ returns to normal position; to remain displaced after calving, the abomasum must have developed atony and subsequent accumulation of gas. Atony of the abomasum is likely to be caused by one of the following factors: feeding of rapidly fermentable concentrated feeds, which have a tendency to produce acidosis; stress conditions or metabolic diseases that frequently occur around the time of parturition; hypocalcaemia; the occurrence of systemic diseases that produce toxemia, such as acute metritis; and the lack of a suitable quantity of high-fiber roughage (WOLF 2001; EDDY 2004; DOLL 2007).

It is likely therefore that atony of the abomasum and accumulation of gas within the organ is the prime factor in the pathogenesis of the condition (EDDY 2004).

In a left-side displacement the abomasum becomes greatly distended with gas and fluid and assumes a position between the rumen and the left abdominal wall (HOFMEYER 1988; MESARIC and ZADNIK 2002; ZADNIK 2003), thus restricting the abomasal outflow and reducing the cow's appetite.

A simple repositioning of the abomasum by rolling the cow or deflating the organ by surgical procedure is usually connected with a very high rate of short-term relapses. For this reason several techniques have been developed to fix the repositioned abomasum into its physiological position.

Techniques with blind percutaneous fixation (blind stitch, toggle-pin method) have increasingly replaced methods used successfully up until now with a laparotomy (Utrecht method; Hannover method). The invasive technique to correct the displaced abomasum depends on omentopexy between the omentum and the abdominal wall, and the laparoscopic abomasopexy depends on adhesion formation between the abomasum and the site of fixation on the abdominal wall.

In 1980 Grymer and Sterner suggested the use of the "roll and suture" technique for the treatment of left displacement of the abomasum. For the fixation of the repointed abomasum, they used a "bar suture". In 1982 they suggested the trocar-cannula and toggle suture for correction of a displaced abomasum, recommending two toggles (GRYMER and STERNER 2002).

The latest development for surgical correction of LDA is the minimally invasive laparoscopic technique (Janowitz method) (JANOWITZ 1998; KEHLER and STARK 2002; SEEGER et al. 2002). The abomasum is subsequently fixed to the abdominal wall percutaneously; this is also known as the two-step abomasopexy (JANOWITZ 1998; STEINER 2006).

The Janowitz method depends on developing inflammatory adhesions in the abomasal fixation region, and these adhesions prevent relapses during the current and the subsequent lactation (KEHLER and STARK 2002).

Janowitz suggested in 1998 that using one toggle was sufficient and the fixation bandage should be not removed until two weeks after the fixation; in fact, he recommended that a better result could be achieved if the bandage remained in place for more than three and up to four weeks.

Kehler and Stark in 2002 recommended some modifications to improve this method, such as fixing the abomasum with a second toggle pin and maintaining the suture for longer to provoke a longer duration of inflammation. Another modification of the two-step abomasopexy is the administration of the abomasopexy in a simple procedure known as the one-step abomasopexy, sometimes named the Christiansen or the Barisani method (CHRISTIANSEN 2004; STEINER 2006).

In 2006 the Canadian veterinary journal published research describing a new method in fixation of the abomasum by endoscopic technique plus suturing of the abomasum’s serosal-muscular layer to the abdominal wall; but the downside was that this technique required two experienced veterinary surgeons to perform the operation (BABKINE et al., 2006).

Actually, the weak point of all percutaneous fixation techniques seems to be reliability of inflammation adhesions after removal of the threads (gauze bandage) that keep the abomasum in its physiological position. It is supposed that after a certain time the adhesions and the tissue fixation will fade away, just as happens with fibrinous adhesions during the healing process of traumatic reticulo peritonitis; or it may be that there are just not enough adhesions to maintain the abomasum in its physiological position (KOCH 2003).

So, upon these ideas’ dissimilarities about maintaining the fixation between the abomasum and the abdominal wall, this study been based.

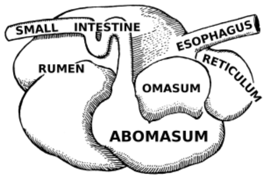
1.2 Aims of the thesis

The aim of the study is to find answers to the following questions:

- How do the size and the quality of inflammation adhesions develop after a one-toggle laparoscopic abomasopexy?
- How big will this area be when the toggle-pin is removed after four weeks?
- How long will a reliable adhesion that protects the animal from a relapse of displacement persist in the fixation area after removal of threads and toggle?

2 Literature review

2.1 General considerations



Source: www.wikipedia.org

2.1.1. The digestive system

The digestive system consists of a muscular-membranous tube extending from the mouth to the anus. Its functions are ingestion, grinding, digestion and absorption of food, and elimination of solid wastes. The digestive system reduces the nutrients in the food to compounds that are simple enough to be absorbed and used for energy and building other compounds for metabolic use.

The digestive tract consists of a tube lined with mucous membrane, which is continuous with the external skin at the mouth and at the anus. The four layers making up the wall of digestive tract are:

1- The *epithelium* (stratified squamous to the glandular part of the stomach and simple columnar from there on in)
2- The *lamina propria* (including the muscularis mucosa and submucosa)
3- The muscles (striated into or through the esophagus, smooth the rest of the way)
4- Caudal to the diaphragm, and covering most of the digestive tract, an outer serous covering, the *visceral peritoneum*

Figures 1 and 2 show the microanatomy and the layers of the digestive tube; portions of the digestive tract are the mouth, pharynx, esophagus (forestomachs in ruminants), glandular stomach, small intestine, large intestine, and the accessory glands, which are the salivary glands, the liver, and the pancreas (SISSON and GROSSMAN 1948; FRANDSON 1974).

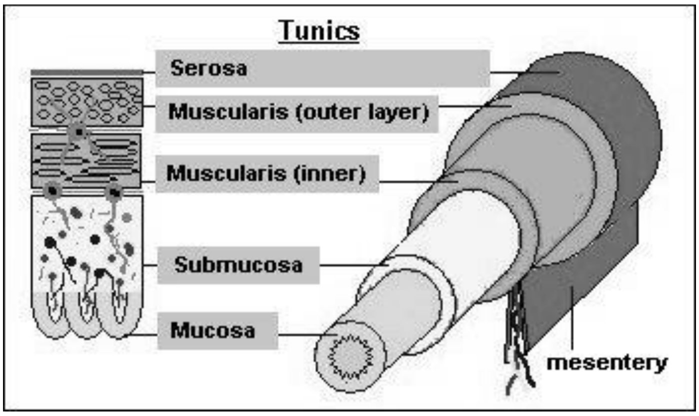


Figure 1: Microanatomy of the digestive tube (the four basic layers or tunics) (Bowen 2006).

2.1.2 Four-compartment stomach (rumen, reticulum, omasum, and abomasum)

The stomach of the cow is very large, and occupies nearly three quarters of the abdominal cavity. It fills the left half of the cavity (except the small space occupied by the spleen and a few coils of small intestine) and extends considerably over the median plane into the right half. It is compound, and consists of four parts, the rumen, reticulum, omasum and abomasum (UMPHERY and STAPLES 1992).

The first three parts are often regarded as proventriculi or esophageal sacculations, since they are lined with a mucous membrane which is covered with squamous stratified epithelium and is non-glandular. The abomasum, on the other hand, has a glandular mucous membrane and hence is popularly termed the “true stomach” (SISSON and GROSSMAN 1948).

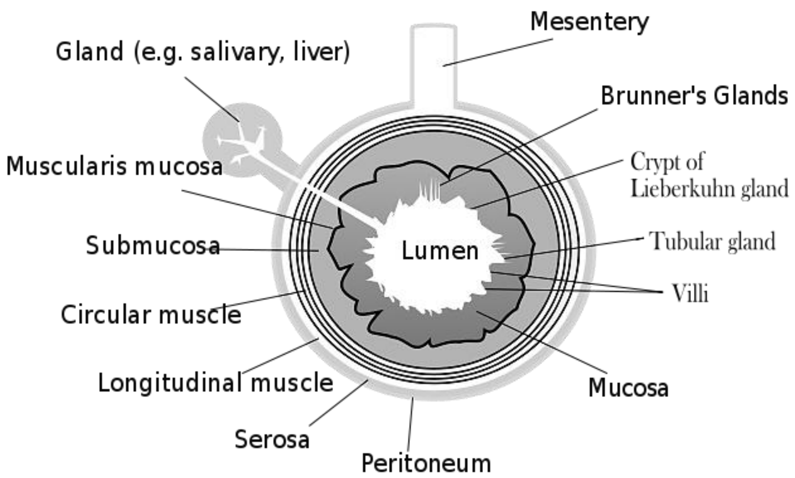


Figure 2: General structure of the gut wall (Kierszenbaum 2002).

2.1.3 Capacity of stomach

The capacity of the stomach varies greatly, depending on the age and size of the animal. In cattle of medium size it holds 110 to 150 liters; in large animals, 150 to 220; and in small, 90 to 130. The relative sizes of the four parts vary with age, in correlation with the nature of the food. In new-born calves the rumen and reticulum together are about half as large as the abomasum; in ten or twelve weeks this ratio is reversed. During this period the omasum appears to be contracted and functionless (SISSON and GROSSMAN 1948).

At four months the rumen and reticulum are about four times as large as the omasum and abomasum together. At about one or one and a half years, the omasum equals or closely approaches the abomasum in capacity. The four parts have now reached their definitive relative capacities, with the rumen constituting about 80%, the reticulum 5%, the omasum 7 or 8%, and the abomasum 8 or 7% of the total amount (FRANDSON 1974; HOFMEYR 1988; DIRKSEN 1990).

2.2 The abomasum

2.2.1 Generals

The abomasum, also known as the maw and the rennet bag, is the fourth and final stomach compartment in ruminants. It secretes rennet, an ingredient used in cheese creation. The word 'abomasum' is from New Latin and it was first used in English in 1706. It comes from the late 17th century modern Latin *ab-* "away, from" + *omasum* "intestine of an ox" (CHAMBERS DICTIONARY 2003; OXFORD DICTIONARY 2011).

It is an elongated sac which lies chiefly on the abdominal floor. The anterior blind end, the fundus, is in the xyphoid region in relation to the reticulum to which it is in part attached. In a healthy non-pregnant cow, the abomasum is positioned below the rumen in the ventral part of the abdomen and is oriented towards the left side of the animal (VAN WINDEN et al. 2002). The body of the sac extends backward between the ventral sac of the rumen and the omasum, and turns to the right behind the latter. The terminal, smaller pyloric part inclines dorsally and joins the duodenum at the pylorus, which is usually situated at or near the ventral part of the ninth or tenth rib. The parietal surface is in contact mainly with the abdominal floor, while the visceral surface is for the most part related to the rumen and omasum. Figure 3 shows the normal topographic anatomy of the digestive system of the cow.

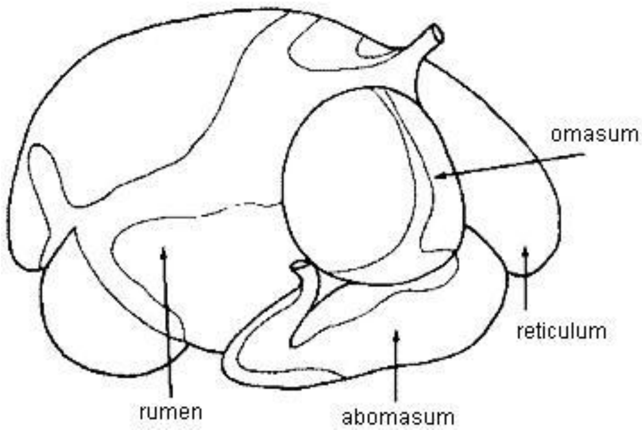


Figure 3: Divisions of stomach in the bovine, left view (Weaver and Moseley1993).

The greater curvature gives attachment to the superficial part of the greater omentum, except at the fundus, which is adherent to the rumen. The lesser curvature is related to the omasum to which it is attached by peritoneum and connective tissue with more or less fat, except at the pyloric part which is attached to the liver by the lesser omentum (SISSON and GROSSMAN 1948).

The cavity of the abomasum is divided by a constriction into two areas. The first of these (the fundus gland region) is lined with a soft glandular mucous membrane, which forms a dozen or more extensive spiral folds (*plicae spirales*). The second part (pyloric region) is much narrower. A small cardiac gland zone surrounds the omaso-abomasal orifice. The pyloric orifice is small and round (SISSON and GROSSMAN 1948; FRANDSON 1974).

The muscular coat of the abomasum consists of longitudinal and circular layers; the latter forms a well-developed pyloric sphincter (FRANDSON 1974).

The mucous membrane of the first three divisions is destitute of glands, and covered with a thick, stratified, squamous epithelium; the superficial part of the latter is horny, and is shed in large patches in the rumen and omasum. The tunica propria is papillated. The mucous membrane of the abomasum is glandular. The fundus glands (which are relatively short) occur in that part which presents the large folds, while the long pyloric glands are found in the remainder, except about the omaso-abomasal orifice, where cardiac glands occur. The mucosa of the fundus gland region is very thin; toward the pyloric glands there is an increase in thickness. There is a round prominence (*torus pyloricu*s) on the upper part of the pyloric valve (SISSON and GROSSMAN 1948).

The abomasum produces hydrochloric acid and digestive enzymes, such as pepsin (breaks down proteins), and receives digestive enzymes secreted from the pancreas, such as pancreatic lipase (breaks down fats). These secretions help prepare proteins for absorption in the intestines. The pH in the abomasum generally ranges from 3.5 to 4.0. The chief cells in the abomasum secrete mucous to protect the abomasal wall from acid damage (PARISH et al., 2009).

The chief digestive function of the abomasum is the partial breakdown of proteins. The enzyme pepsin is responsible for this. Proteins from the feed and the microorganisms coming from the rumen are broken down to smaller units called peptides before leaving (UMPHERY and STAPLES 1992).

2.2.2 Vessels and nerves

The blood supply is derived from the celiac artery, and the veins go the portal vein. The nerves come from the vagus and are sympathetic. Numerous ganglia are present in the submucous and intermuscular tissue (SISSON and GROSSMAN 1948).

2.2.3 Gastric movement of the abomasum

Activity in the area of the fundus is quite limited. Contractions of the body are more marked, and peristaltic waves are seen in the pyloric portion. Activity of the abomasum depends to some extent on the contents of the duodenum. Abomasal activity is inhibited by weak hydrochloric acid and fat emulsion, but activity is stimulated by hypertonic saline solutions and emptying of the duodenum (FRANDSON 1974).

2.3 Displacement of the abomasum (DA)

This is also known as fourth stomach displacement when to the left (LDA), or distension, dilatation or torsion when displaced to the right (RDA). Normally, the abomasum lies slightly to the right of the midline.

This condition was described by Begg in 1950 and by Ford in 1950; a detailed description was also made by Mather and Dedrick in 1966. The displaced abomasum was first diagnosed after digestive upsets when exploratory laparotomies were carried out. Since then, the condition has become readily recognizable and satisfactory treatments have worked out (HUNGERFORD 1990). Figure 4 shows the normal position of the abomasum and surrounding organs.

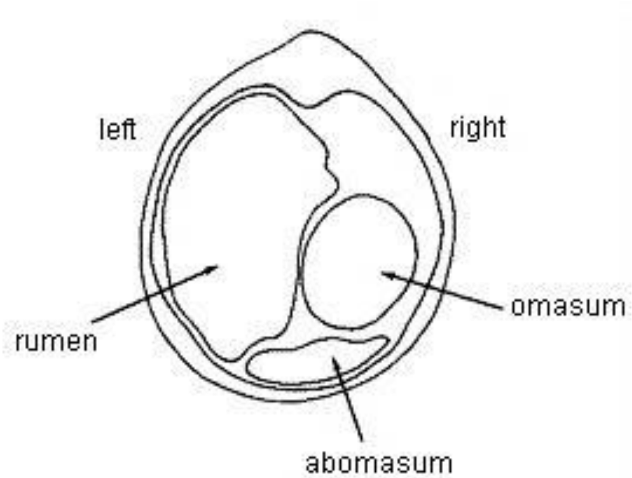


Figure 4: Normal position of abomasum on right side (Weaver and Moseley 1993).

2.3.1 Right-side displacement of abomasum (RDA) or abomasal dilatation

2.3.1.1 Etiology

Usually 2–4 weeks postpartum the cause of the dilatation is thought to be that primary distension of the abomasum occurs because of either obstruction of the pylorus or primary atony of the abomasal musculature. It seems to be that atony is caused by interplay of grain feeding, relative inactivity during winter housing, and the stress of parturition (BLOOD et al. 1989).

2.3.1.2 Clinical findings

These include anorexia, scant feces, poor milk production, moderate dehydration, sluggish rumen, fluid-filled viscous under right costal arch, ping commonly audible, palpable per rectum, alkalosis, hypochloremia, and hypokalemia (BLOOD et al., 1989).

2.3.1.3 Treatment

Some cases recover spontaneously with medical therapy, by being given calcium borogluconate and a hay diet. Surgery may be required; right-flank omentopexy may be used in the treatment of left-sided displacement of the abomasum (LDA) and right-sided dilatation of the abomasum (TURNER and MCILWRAITH 1989).

2.3.1.4 Operative procedure

Laparotomy in the standing animal is performed in the right flank. The abdominal cavity is explored, the abomasum decompressed and then manually returned to its normal position. The pyloric antrum of the abomasum is pulled to the ventral aspect of the abdominal incision and identified, and the omentum is sutured to the right flank at 10 cm caudal to the pylorus. Originally, perlon buttons were used for secure fixation of the abomasum to the right body wall (DIRKSEN 2006, STEINER 2006).

2.3.2 Left-side displacement of the abomasum (LDA)

2.3.2.1 Etiology

The abomasum has a natural tendency to migrate to the left at parturition but a healthy abomasum is not trapped to the left (HUNGERFORD 1990). Factors such as parturition, especially during the first six weeks of lactation, concurrent disease, season, breed, gender, age, diet, housing management, high-producing dairy cows, and weather may influence the incidence of abomasal displacement (SVENDSON 1969; DOLL 2007).

Recent studies suggest that genetically determined neuronal imbalance in German Holstein in comparison with German Fleckvieh breeds could be predisposing factors for the displacement of the abomasum (DOLL 2007; SICKINGER 2008).

Abomasum (fourth stomach) motility is known to be reduced when cattle are fed a diet with high fat and/or high protein content and once the abomasum is hypotonic it will become distended with food material and gas. It has been suggested that the abomasum moves forward and to the left, to under the rumen, during late pregnancy when the gravid uterus causes the rumen to lift slightly from the abdominal floor, and then becomes trapped when the rumen re-expands after calving. Epidemiological studies have shown that cows affected with LDA were higher producers, heavier, and older than their herd mates (BLOOD et al., 1989).

2.3.2.2 Clinical findings

These can be defined as acetonemia in the cow within days of parturition, inappetence or complete anorexia, soft feces variable in amount (usually reduced) although periods of profuse diarrhea may occur, ketonuria, marked drop in milk production, rumen sounds present but faint, and pinging sound on percussion and auscultation of the left side (BLOOD et al., 1989). Figure 5 shows the position of the abomasum when it has displaced to the left side.

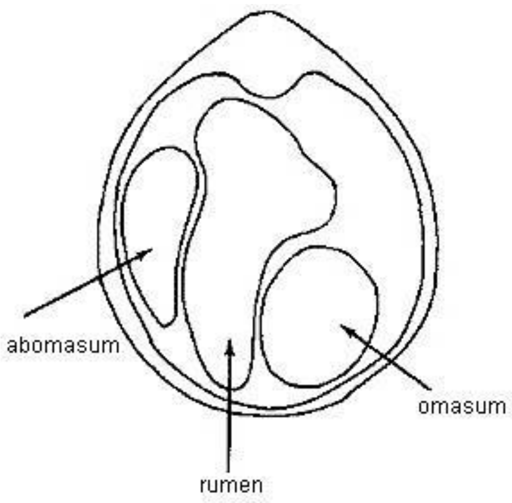


Figure 5: Abomasum displaced to the left of rumen (Weaver and Moseley 1993).

2.3.2.3 Treatment

Laparoscopy has become the preferred approach to a number of different diseases because it allows a correct diagnosis and treatment at the same time (AGRESTA et al., 2006).

Surgical corrections include percutaneous fixation (blind stitch, toggle pin method) and the Utrecht and Hannover methods. The latest development for surgical correction of the LDA is the minimally invasive laparoscopic technique (JANOWITZ 1998).

The visual control makes this safer than the percutaneous fixation technique (JANOWITZ 1998; KEHLER and STARK 2002).

2.3.2.3.1 Laparoscopic abomasopexy methods

2.3.2.3.1.1 Two-step laparoscopic abomasopexy

This technique was originally described by Janowitz in 1998. It includes decompression of the abomasum and introduction of one toggle into the abomasal body under laparoscopic control in the standing animal (step 1), followed by exteriorization of the toggle suture through and fixation of the abomasum at the ventral abdominal wall with the cow in dorsal recumbency (step 2) (JANOWITZ 1998; VAN LEEUWEN and MÜLLER 2002; DIRKSEN 2006). The technique depends on the repositioning and fixation of the left-displaced abomasum (KEHLER and STARK 2002; RAIZMAN and SANTOS 2002).

The inflammation and the irritation due to the fixation of the abomasum produces the adhesion in the fixating area and these adhesions prevent the abomasum from relapsing during the current or the subsequent lactation (KEHLER and STARK 2002).

2.3.2.3.1.2 One-step laparoscopic abomasopexy

This technique was described in 2004 by Christiansen and also by Barisani 2004. The first part of the intervention is similar to step 1 of the Janowitz technique. Thereafter, the toggle suture is guided to the site of perforation at the ventral aspect of the abdominal wall with a special instrument (“Spieker” according to Christiansen and “Barisani applicator” according to Barisani). Perforation of the abdominal wall and fixation of the abomasum is performed in the standing animal. As compared to the Janowitz method, this technique carries the advantage that the complete procedure is performed in the standing animal (CHRISTIANSEN 2004).

2.3.2.3.1.3 One-step laparoscopic abomasopexy in dorsal recumbency

This technique has recently been developed in North America. Surgery is performed with the cow in dorsal recumbency. Fixation of the abomasal body to the right paramedian ventral abdominal wall is achieved by placement of four simple interrupted sutures using a USP2 polydioxanone suture material on the seromuscular abomasal wall layer, which are tied and knotted subcutaneously (BABKINE et al., 2006).

2.4 Inflammation

2.4.1 General information

A pathological phenomenon known and studied from ancient times, inflammation has long been defined as the reaction of the tissue to an irritant. When the body is injured, a sequence of events is initiated that leads to the eventual repair of the site. The first stage in this process is inflammation which is followed by tissue healing and repair.

The inflammatory response is the body's natural response that occurs immediately following tissue damage. Its main functions are to defend the body against harmful substances, dispose of dead or dying tissue and to promote the renewal of normal tissue (RINGLER 1997; SERHAN and SAVILL 2005).

2.4.1.1 Clinical signs of inflammation

As stated by Celsus (30 BC–38 AD) in the first century and by every medical writer since that time, *rubor et tumor cum calore et dolore* which, translated, means “redness and swelling with heat and pain” (RINGLER 1997).

The inflammatory reaction is normally characterized by five distinct signs, each of which is due to a physiological response to tissue injury.

- Pain (due to chemicals released by damaged cells)
- Swelling or edema (due to an influx of fluid into the damaged region)
- Redness (due to vasodilatation – the widening of blood vessels)
- Heat (due to an increase in blood flow to the area)
- Loss of function (due to increased swelling and pain) (THOMSON 1984; RINGLER 1997; SERHAN and SAVILL 2005).

2.4.1.2 Stages of the inflammatory reaction

The inflammatory reaction is the combination of a number of overlapping reactions within the body. Although a lot of these occur simultaneously a certain order of events may be seen as detailed in the points below.

2.4.1.2.1 Tissue injury

Tissue damage may occur from such traumas as a tackle, collision, or from an awkward fall. However, quite commonly tissue injury is as a result of overuse, commonly known as micro trauma (SERHAN and SAVILL 2005).

2.4.1.2.2 Release of chemicals

When tissue cells become injured they release a number of chemicals that initiate the inflammatory response. Examples of these are kinins, prostaglandins and histamine. These chemicals work collectively to cause increased vasodilation (widening of blood capillaries) and permeability of the capillaries. This leads to increased blood flow to the injured site. These substances also act as chemical messengers that attract some of the body's natural defense cells – a mechanism known as chemotaxis.

Although highly beneficial to the body's defense strategies, some chemicals also increase the sensitivity of the pain fibers in the area and so the area becomes painful (SERHAN and SAVILL 2005; ZOGRAFOS et al., 2005).

2.4.1.2.3 Leukocyte migration

This includes a great number of lymphocytes, especially T cells which stimulate certain enzymes, and are a group of proteases that regulate the metabolic pathways by cleaving protein bonds (ZOGRAFOS et al., 2005). Figure 6 shows the migration process.

Chemotaxis leads to the migration of certain white blood cells (leukocytes) to the damaged area. Two types of leukocyte are predominant in the inflammatory response – macrophages and neutrophils. Neutrophils are first to the injured site and function by neutralizing harmful bacteria. Macrophages aid the healing process by engulfing bacteria and dead cells and ingesting them so that the area is clear for new cells to grow. They arrive at the injured site within 72 hours of the injury and may remain in the area for weeks after the injury (CHANDRASOMA and TAYLOR 1998; ZOGRAFOS et al., 2005).

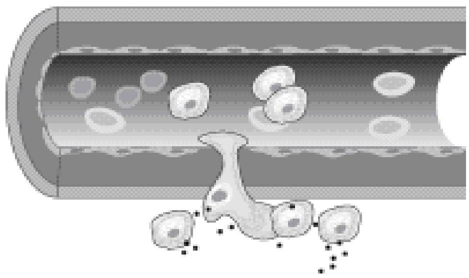


Figure 6: Migration of white blood cells out of blood vessel and release of white blood cell chemicals (The Cleveland Clinic Foundation 2008)

2.4.1.3 Tissue healing

2.4.1.3.1 Collagenation

Wound healing occurs towards the end of the inflammatory process; however, the two processes overlap considerably. Macrophages work tirelessly to clear the damaged area and make space for the regeneration of new tissue. After a number of days fibroblasts (collagen producing cells) begin to construct a new collagen matrix which will act as the framework for new tissue cells (RINGLER 1997).

2.4.1.3.2 Angiogenesis

Once sufficient cleansing of the area has been achieved the damaged area begins to sprout new capillaries to bring blood to the region – this is known as angiogenesis or revascularization. When blood flow has been re-introduced to the area specific tissue cells begin to re-grow, for example in a muscle tear muscle cells will repopulate the area (VARNER 2006). Figure 7 shows the process taking place in angiogenesis.

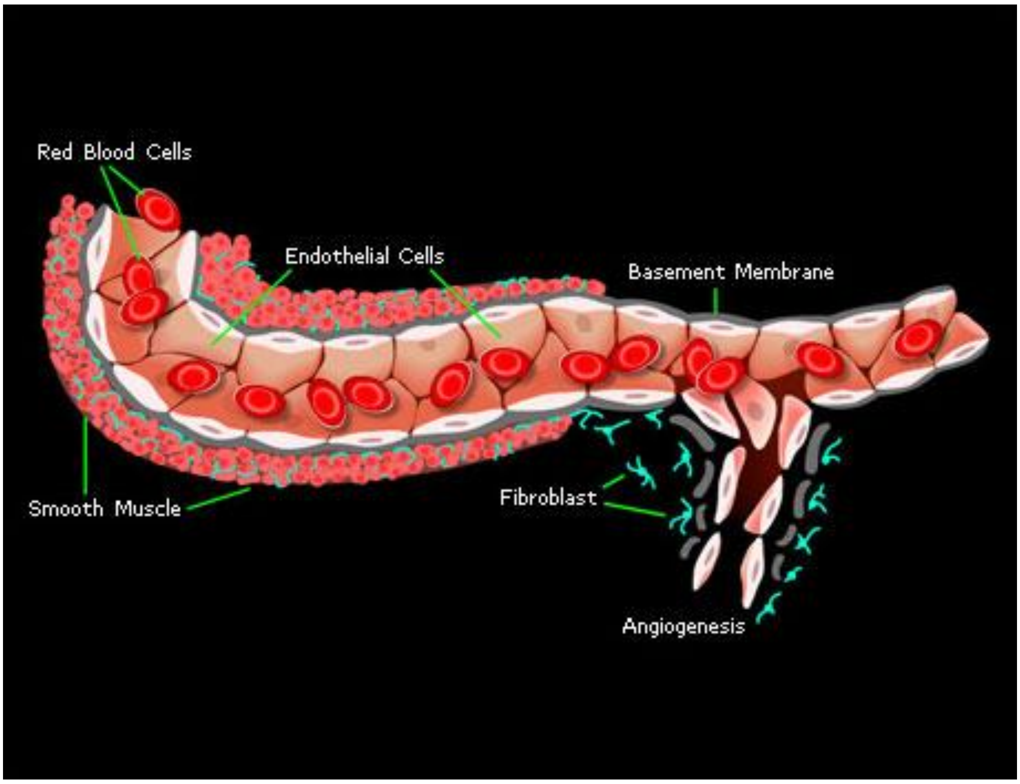


Figure 7: The angiogenesis process (Ferrara 2009).

2.4.1.3.3 Proliferation

The proliferation phase lasts up to four weeks. In cases where the injury sustained has been more severe the affected area may be composed of a mixture between specific tissue cells (such as muscle cells) and other tissue known as granulation tissue. If this granulation tissue is not removed it will remain and form scar tissue, which can lead to a decreased functional ability of the tissue.

2.4.1.3.4 Remodeling

The stage of remodeling now occurs whereby the new cells mold (interact) into their surroundings to once again produce a functioning tissue. This process of remodeling can take months or even years, altering the new tissue slowly. The new cells and protein fibers become arranged in a way that is best suited to the stresses imposed on the tissue. Hence when a tissue is healing it is important to stretch it in the correct direction so as to optimize the strength of the new tissue (CHANDRASOMA and TAYLOR 1998; SERHAN and SAVILL 2005; GURELIAN 2006; KINDT et al., 2006).

2.4.2 Inflammation and wound healing in bovines

Cattle are known for a well-developed immune system that minimizes the likelihood of an infection. Cows with a high titer against a specific antigen are able to deliver these antibodies to the site of infection via a leaky endothelium (arising from an inflammatory response) (FORSBERG 2004).

Another difference between the immune system of ruminants and another animals, humans included, is the presence of different types of γδ T cells; these cells revealed an unusual feature in combination with immunoglobulin’s; however, the functional studies of ruminants’ γδ T are limited and inconclusive (HEIN and MACKAY 1991).

FU et al. (2000) discovered that burns and chronic dermal ulcer wounds in humans, when treated with the bovine basic fibroblast growth factor, had an accelerated rate of granulation tissue formation and epidermal regeneration as compared to those in controls.

2.5 Adhesions

2.5.1 Generals

The adhesions could be defined as the layers of fibrin on each of two opposing surfaces tending to become organized by immigrating fibroblasts, and two surfaces often becoming tied together by strands of fibrous connective tissue (JONES et al., 1997). Another definition of adhesions is bands of tissue that connect anatomic sites at locations where there should not be connections (DIAMOND and MOGHISSI 2001).

Adhesions form as a result of defective healing processes related to blood coagulation; an inadequate fibrinolysis process leads to persistent fibrin structures that subsequently mature into fibrous tissue, followed by organization into rigid, persistent fibrous adhesions, possibly containing blood vessels and nerve fibers (SCHNEIDER et al., 2006).

Causes of adhesions: inflammation, surgery, or injury can cause tissues to bond to other tissue or organs, much like the process of forming scar tissue. Sometimes, fibrous bands (adhesions) can form between the two surfaces. Abdominal surgery, endometriosis, attacks of appendicitis, or pelvic inflammatory disease can also cause intra peritoneal adhesions (DEBERNARDO 2004; KOSAKA 2008).

Depending on the tissues involved, adhesions can cause various disorders. In the eye, adhesion of the iris to the lens can lead to glaucoma. In the intestines, adhesions can cause partial or complete bowel obstruction (DEBERNARDO 2004).

Tissues that cannot regenerate are repaired with fibrous tissue or scar tissue, and whether the tissue replaces itself or is repaired with scar tissue often determines the extent of recovery (BRUMBAUGH 2007).

OPUKO et al. (2007) discovered that bovine fibrinogen-enriched plasma samples show pseudo-plastic characteristics when stimulated by different kinds of temperature and stress.

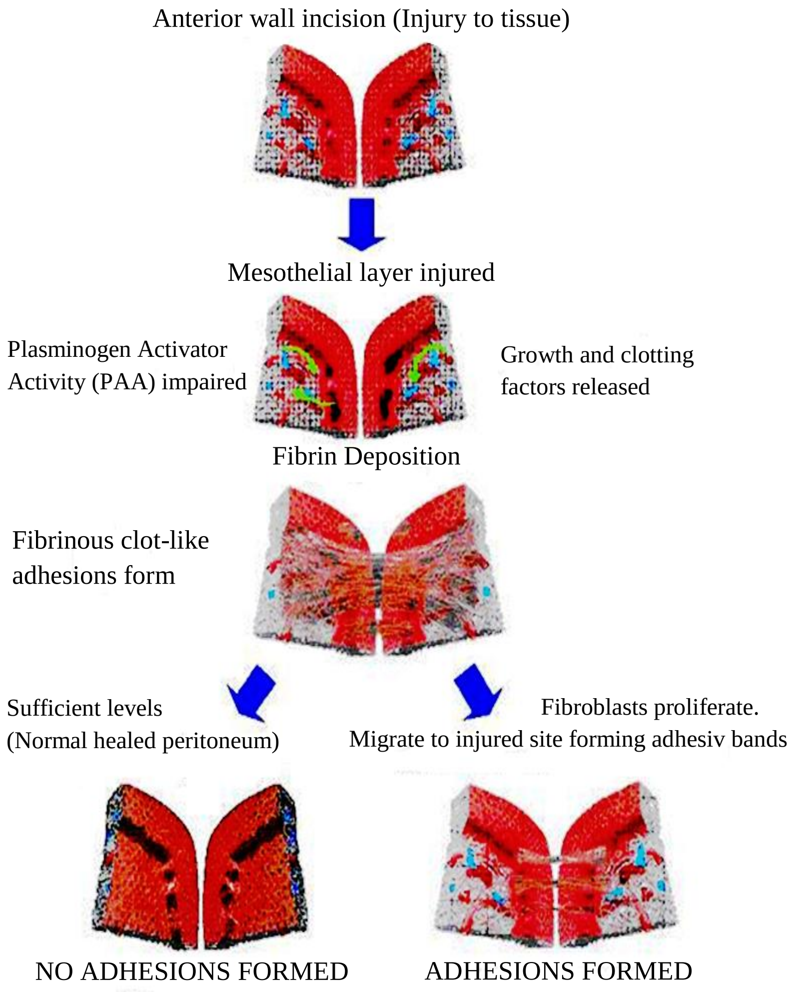


Figure 8: Pathogenesis (formation) of adhesions

(Diamond and Moghissi 2001).

Fibrin deposit, the primary step in the formation of post-surgical adhesions, is the result of an imbalance between the fibrin-forming and the fibrin-dissolving capacity of the peritoneum; a transient reduction in local plasminogen activator activity after peritoneal trauma results in reduction of fibrinolysis and can therefore permit deposited fibrin to become organized into fibrous adhesions (KAYAOGLU et al. ,2005).

RAWLINGS et al. (2001) discovered after performing a laparoscopic gastropexy in dogs that, depending on the maturity of the connective tissue and the amount of collagen at the adhesion site, the adhesion site had abundant fibroplasia extending from the submucosa of the stomach through the abdominal wall and infiltrating into the musculature of the stomach and abdominal wall.

2.5.2 Formation of adhesions

Post-operative surgical adhesions are formed as a result of trauma or injury to tissue such as the surgical incision made into the abdominal wall. The mechanism of peritoneal repair following tissue injury is currently being established. Fluid from an injury is collected into vessels, lymphatics, and tissues. This fluid includes proteins such as the factors involved in clotting, inflammatory cells, cytokines, and growth factors. Histamine released from these injured sites induces further increases in exudation of proteinaceous material. These products collect at sites of injury of the mesothelium which lines the abdominal cavity and underlying tissue. Following completion of the procedure, one or two general pathways can be followed. If plasminogen activator activity (PAA) which resides in mesothelial cells and underlying fibroblasts is sufficient the (PAA) proteinaceous mass will be degraded such that no connection exists between opposing tissue surfaces. In this situation, fibroblasts which proliferate migrate to the tissue edge but no further, and mesothelial cells regenerate to form an uninterrupted surface. If plasminogen activator activity (PAA) is impaired, the proteinaceous mass (clot) will persist and congeal to form a connection between adjacent surfaces. Subsequently, fibroblasts which proliferate and migrate towards the site of injury will then migrate into the clot where collagen and other forms of extra-cellular matrix will be deposited resulting in a band of tissue connecting adjacent sites, that is, an adhesion. Figure 8 depicts the two general pathways – the normal no-adhesion and the adhesion-formation pathway. Concurrently, mesothelial cells on the surface of the injured tissue (clot) develop and subsequently form an uninterrupted surface. If tissue is relatively hypoxic, signals initiating angiogenesis will be elaborated resulting in a vascularized adhesion (DIAMOND and MOGHISSI 2001).

So, the intra-abdominal adhesions after laparotomy represent the fibrinous resolution of an acute mesothelial inflammatory response provoked by peritoneal injury or irritation. The immediate peritoneal cell reaction that initiates this process is followed by a sustained period of increased local vascular permeability with inflammatory cell infiltration that facilitates subsequent tissue repair and remodeling. Implicit to this concept is the interruption of normal peritoneal and anti-fibrinolytic capabilities that allows deposition of a fibrin matrix between adjacent serosal surfaces; this then acts as a framework for a reparative activity but also often persists as a mature fibrous band or adhesion (CAHILL et al., 2006).

Histological examination of tissues harvested from an adhered area revealed fibroblasts, collagen, macrophages, and lymphocytes (GOLDENBERG et al., 2005).

2.5.3 Abdominal adhesions by cattle

Post-operative adhesion formation after laparoscopic abomasopexy is the key to ensuring adequate abomasum fixation (BABKINE et al. 2006).

Adhesions are assumed to be almost inevitable consequences of abdominal surgeries. The most powerful stimuli are ischemic, abscesses, and foreign bodies, all of which attract macrophages which are activated to generate a fibrotic process; from another point of view, the peritoneum normally produces the plasminogen activator factor which leads to fibrinolysis and decreases the occurrence of adhesion (BRAZ et al., 2009).

BROWN et al. (2000) studied the crystal structure bovine fibrinogen which reveals a high clottability.

The high molecular weight of the bovine plasma fibrinogen may play a role in the clotting mechanism in comparison with human fibrinogen (CULASSO et al., 1974).

2.6 Ultrasound

2.6.1 Generals

Use of diagnostic ultrasound in medicine is a non-invasive method of imaging soft tissues. A transducer sends low-intensity, high-frequency sound waves into the soft tissues, where the waves interact with the tissue interface. Some of the sound waves are reflected back to the transducer, and some are transmitted into deeper tissues. The sound waves that are reflected back to the transducer (echoes) are then analyzed by the computer to produce a gray-scale image (HOFER 2005; SIEMS 2005).

2.6.2 Ultrasonographic examination

Ultrasonography provides an alternative, non-invasive method to describe the anatomic dimensions and associations of organs within the abdomen in cattle. The first cross-sectional study that used ultrasonography to characterize the abomasal position in cattle appears to have been performed by Kurosawa et al. in 1991 (WITTEK et al., 2005).

A large cross-sectional ultrasonographic study of the abomasal position in 50 non-pregnant Swiss Braunvieh and Simmental cows was made by BRAUN et al. (1997).

The normal abdominal wall consists of several layers. Picture 1 shows in ultrasound the normal abdominal wall of the cow. The hyper-echoic lines represent facial planes while the innermost line is the peritoneum. The most superficial hyper-echoic band represents subcutaneous fat and the iso-echoic bands represent abdominal muscle layers (LANG 2006).

2.6.3 Ultrasound fibrous tissue manifestation

The appearance of the fibrinous lesions can be seen as echogenic deposits and often have a honeycomb-like appearance and fluid accumulation (BRAUN et al., 1998). The fibrinous adhesions and abscesses can be accessed via ultrasonography (BRAUN 2005).
Adhesion-fibrous tissue can also be distinguished as echogenic-constricting bands or high-level echoes (SCHMIDT 2007).

In 1998 Bischoff explained and described the adhesions in cows suffering from traumatic reticulo peritonitis (TRP). The ultrasound picture of the adhesions could be defined by three categories: structure, size, and echogenicity. Structure could be subdivided into seven more groups (on a scale of 1 to 7): compact homogeneous and heterogeneous, cancellous (spongy), lamellar, and three cavity-like appearances (simple, septated, and simple capsulated). Size consists of three subgroups: less than 2 cm, described as (a); between 2 and 4 cm, described as (b); and more than 4 cm, described as (c). Echogenicity can be subdivided into four types: echo-free, scant-echo, moderate-echo and strong-echo, scaled as I, II, III and IV respectively (BISCHOFF 1998).

2.6.4 Ultrasound manifestation of the abomasopexy adhesions

The abomasopexy area in ultrasound pictures has not been so far examined, so there are no references that explain the appearance of the abomasopexy; however, the appearances in ultrasound pictures are the same as the appearance of the gastropexy in dogs (KRAMER 2009; personal contact). This also been confirmed in this study.

3 Materials and methods

3.1 Study animals

3.1.1 Animal patients

Thirty-five cows with left displaced abomasum (LDA) were admitted to the Clinic of Ruminants and Swine (Internal Medicine and Surgery), Justus Liebig University, Giessen, and have been included in this study. The cows originated from different parts of the Federal Republic of Germany: 11 patients were from Hessen forming 31% of the sample; 19 from Nordrhein-Westfalen forming 54%; and 5 from Rheinland-Pfalz forming 14%.

The age of the animals was between 2 and 7.4 years, (4.8 ± 1.6) ($\bar{x}$ ± SD). Figure 9 shows the age distribution between the animals. The estimated weight of the cows varied between 450 and 650 kilograms (kg).

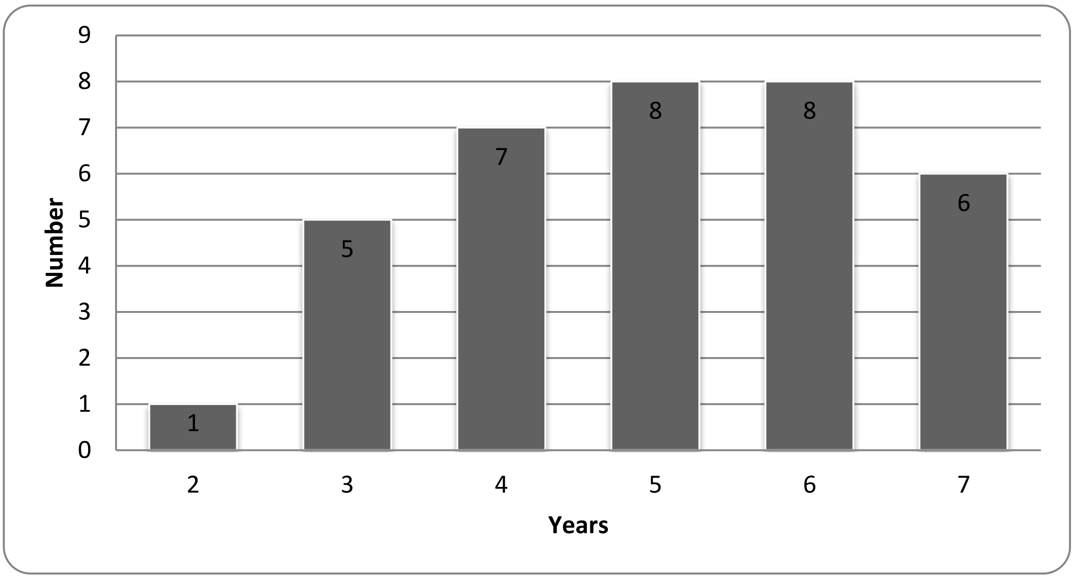


Figure 9: Age distribution in the cow patients.

3.1.2 Breed of the animals

German Black Holsteins, known as "SHB" (*Schwarzbunte Holstein*), and German Red Holsteins, known as "RBH" (*Rotbunte Holstein*) are the only two races that were included in this study. There were 25 SBHs and 10 RBHs in total, as is shown in Figure 10.

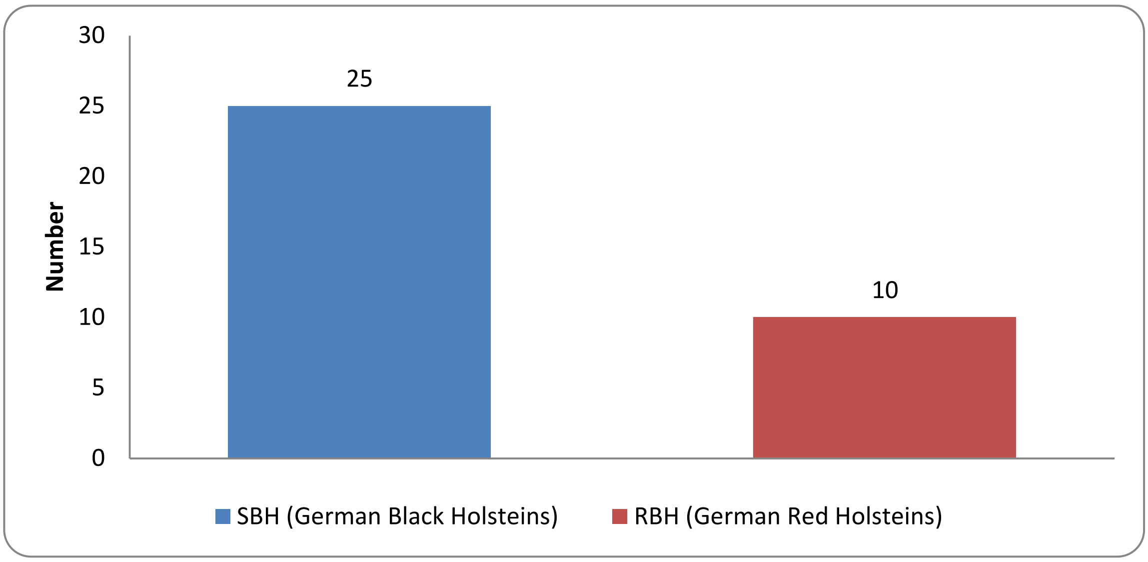


Figure 10: Animal breed distribution.

3.1.3 Animals' case histories

The case history of the cow patients varied between two forms; these were as follows:

- 33 cows had arrived at the clinic between the fourth and the thirty-fifth day after calving.
- 2 cows were pregnant (4.4–5 months).

Table 1 shows in detail the case history of each animal admitted to the clinic and also include the operating date (treatment day).

Table 1 Animal patient's case history with the operation date.

Animal number	Date of operation	Case history (remarks)
1	08.05.2007	Calved 4 days before
2	16.05.2007	Calved 5 days before
3	31.05.2007	Calved 5 days before
4	21.05.2007	Calved 14 days before
5	22.05.2007	Calved 30 days before
6	25.05.2007	Calved 16 days before (twins)
7	30.05.2007	Pregnant: 4.5 months
8	31.05.2007	Calved 7 days before
9	30.05.2007	Calved 8 days before
10	29.05.2007	Calved 23 days before
11	06.06.2007	Calved 10 days before
12	18.06.2007	Calved 16 days before
13	22.06.2007	Calved 30 days before
14	22.06.2007	Calved 25 days before
15	28.06.2007	Calved 28 days before
16	29.06.2007	Calved 14 days before
17	21.06.2007	Calved 14 days before (retained placenta)
18	22.06.2007	Calved 35 days before (heavy calf)
19	02.07.2007	Calved 10 days before
20	09.07.2007	Calved 18 days before
21	10.07.2007	Calved 14 days before
22	15.07.2007	Calved 7 days before
23	15.07.2007	Calved 4 days before
24	19.07.2007	Calved 14 days before
25	24.07.2007	Calved 12 days before (twins)
26	30.07.2007	Calved 14 days before
27	08.08.2007	Calved 12 days before
28	10.08.2007	Calved 4 days before
29	18.08.2007	Pregnant: 5 months
30	26.08.2007	Calved 10 days before
31	26.08.2007	Calved 7 days before
32	29.08.2007	Calved 11 days before
33	21.09.2007	Calved 10 days before
34	10.10.2007	Calved 14 days before
35	25.10.2007	Calved 14 days before

3.2 Operation

3.2.1 Treatment

Animals were treated with laparoscopic reposition by abomasopexy (Janowitz method). This technique was originally described by Janowitz in 1998.

3.2.2 Procedure

The laparoscopic abomasopexy technique, which takes place in the clinic, is the two-step toggle technique. The left paralumbar area is clipped, disinfected and locally anesthetized[1] and prepared for aseptic surgery, and a laparoscope is placed into the abdomen through the left paralumbar fossa. The abdomen is insufflated with air to allow identification of the displaced abomasum to the left of the rumen. The toggle trocar is inserted through the 11th intercostal space and thrust into the abomasum. A purpose-designed toggle is placed down the trocar and into the abomasum.

The abomasum is allowed to decompress before the trocar is removed, and the suture attached to the toggle is pulled into the abdomen when it is released outside the abdomen. At this point, the cow is positioned in dorsal recumbency (originally it was described that the laparoscopic incision is closed with a skin suture and the cow is sedated and then positioned in dorsal recumbency).

The ventral abdomen area is clipped, disinfected, and locally anesthetized[1] and prepared for aseptic surgery. The laparoscope is inserted 20 cm cranial to the navel and 10 cm to the left of midline. Figure 11 shows the location related to the left laparoscopic abomasopexy area.

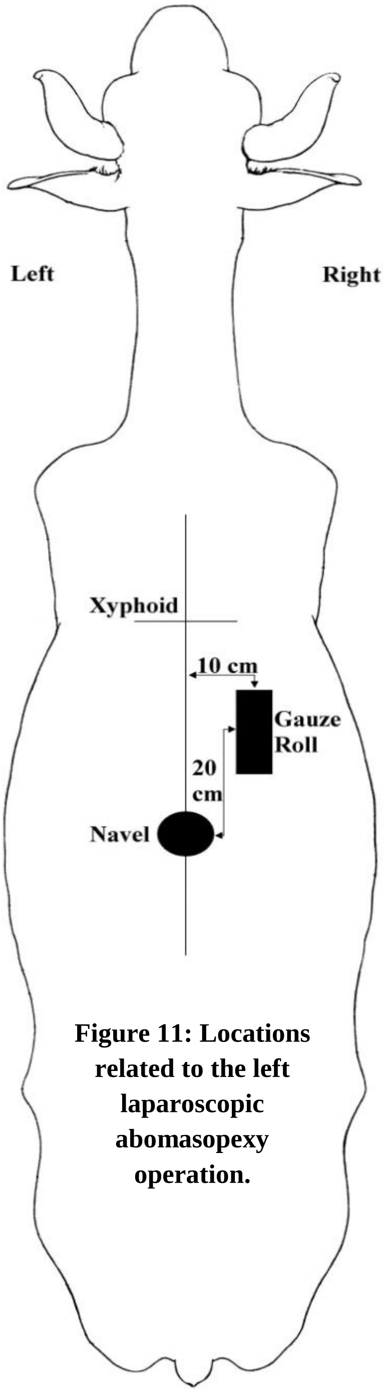


Figure 11: Locations related to the left laparoscopic abomasopexy operation.

[1] Procaine 2 % (Procasel ® 2%, Selectavet, Dr. Otto Fischer GmbH, D-83629 Weyarn-Holzolling)

An instrument portal is placed 20 cm cranial to the navel and 10 cm right to the midline and the toggle suture is located and exteriorized through the cannula instrument. The abdomen is deflated and the two strands of the toggle suture are tied over a gauze roll. The toggle suture is cut close to the skin four weeks after surgery (JANOWITZ 1998; STEINER 2006; WILSON 2008).

3.3 Ultrasonography

3.3.1 Sonographic device

The digital ultrasonic diagnostic imaging system DP-3300 Vet – MINDRAY®[2] allows the calculation of distance, area, and circumference between two points for the selected region. Ultrasonic (75L50EAV linear array 5.0/ 7.5/ 10 MHz) and (35c50EB convex array 2.5/ 3.5/ 5.0 MHz) transducers are used for this study.

The frequencies of the devices depend upon the best echo image which the device can obtain and these are determined as 5.0, 7.5 and 10.0 MHz. The optimal values are between 3 and 9 MHz as suggested by Wild (WILD 1995).

3.3.2 Sonographic investigation

3.3.2.1 Ultrasound examination timetable

The first examination was carried out directly after the laparoscopic abomasopexy to determine the position and thickness of the abomasal wall layers. The second examination was appointed four weeks (28 days) later than the laparoscopic abomasopexy (Janowitz operation) in order to establish the location of the abomasopexy site and the baseline values for the length and depth of the fixation area directly after removal of the gauze roll (fixating bandage). This exam was followed by a third ultrasound examination, which was carried out 90 days post-operation and again subsequently at 180, 270, 360 and 450 days (with three-month intervals between each examination). The ultrasound examination is performed with ultrasonic transducers 75L50EAV and 35c50EB on standing non-sedated animals. After a successful laparoscopic abomasopexy, the animals were discharged from the clinic and visited on-site at the farm according to the time periods that are mentioned above.

The site of sonographic examination is clipped, and the skin washed, degreased with ethanol, and coated with transmission gel (HERZOG et al., 2004; BRAUN 2005).

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[2] MINDRAY® Mindray Building, Keji 12th Road South, Shenzhen 518057, P.R. China.

3.3.2.2 Position and patterns of examination

The site is directly in the region of the fixation of the suture which is 20 cm in the right lateral paramedian region of the animal cranial to the navel (JANOWITZ 1998) (as already shown in Figure 9). After the gauze roll is situated in its position the cow is rolled over into the normal standing position.

At this point begins the ultrasonic assessment. The wound area is considered to be ground zero for the ultrasound transducer maneuvers; these maneuvers are made from two aspects: sagittal and transversal to ground zero.

The transducer maneuvers are performed as follows. First a plane is maneuvered cranially and then caudally in the transverse direction; whereas the second plane is maneuvered laterally and then medially in the longitudinal (sagittal) direction. Figure 12 shows the planes of examinations.

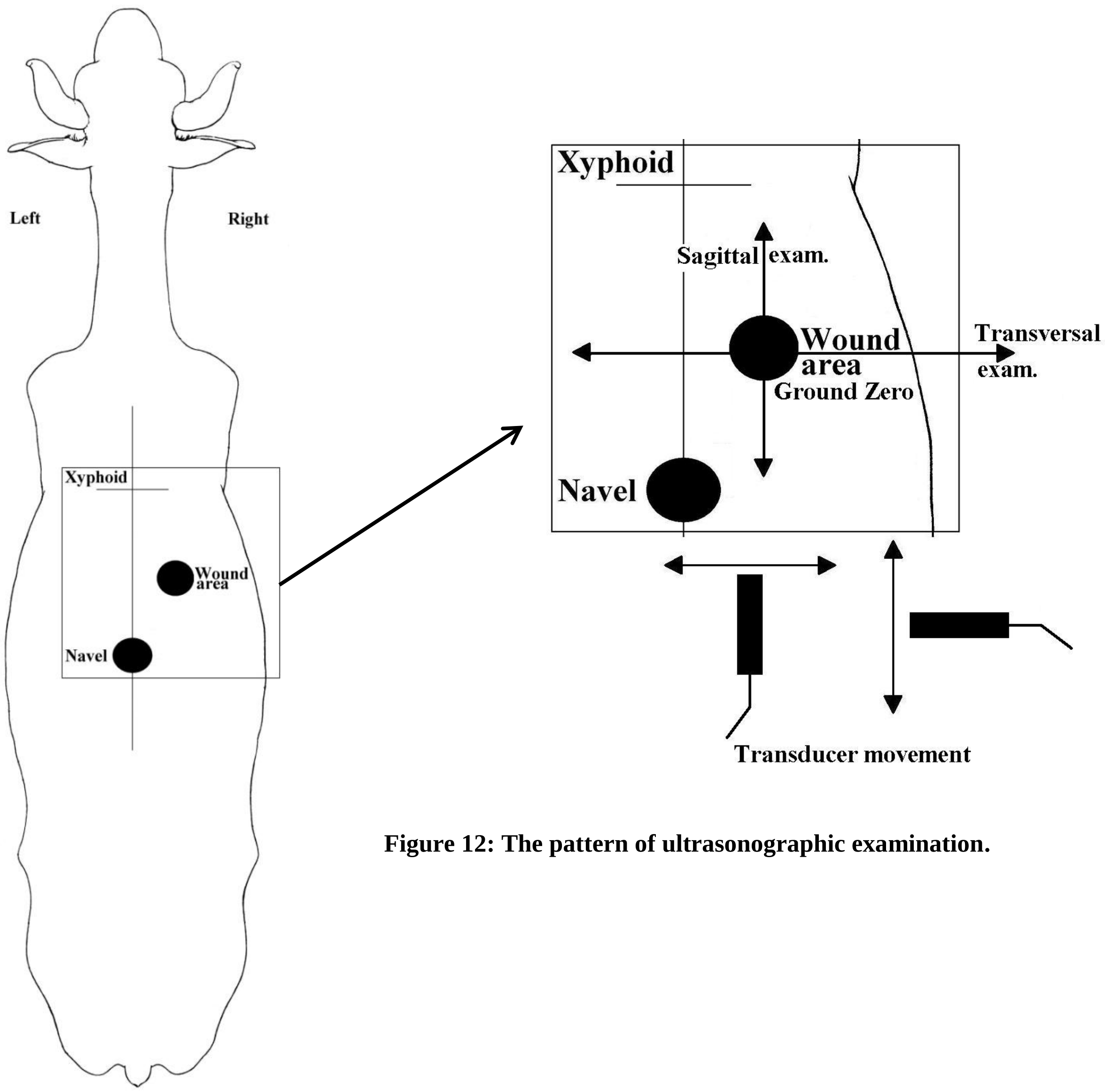

Figure 12: The pattern of ultrasonographic examination.

3.3.3. Assessment criteria

In this study the following categories were measured in the same manner after one month, three months, nine months, and one year until the adhesion disappeared for each patient.

3.3.3.1 Abomasopexy (adhesion)

The area between the abdominal wall and the abomasum is calculated. This is accomplished by calculating the following proceedings (the ultrasonic examination pattern is as follows).

3.3.3.1.1 The length (cranial-caudal) and the width (lateral-medial)

In order to mass the length of the adhesion area, the transducer is moved mainly in two directions. Beginning from the point “ground zero” which actually depicts the wound area, a sagittal line is made in cranial and caudal directions, and another transversal line in lateral and medial directions (the width) from this point. The length of the lines is assumed to be the length of the adhesions cranially.

3.3.3.1.2 The depth (lateral-medial)

The deepness of the adhesion is measured between the abdominal wall and the abomasum.

3.3.3.1.3 The position

The position corresponds to the wound insertion area.

3.3.3.2 Toggle visibility

Ultrasound is performed to confirm the existence of the toggle in the abomasum until it fades away in the ultrasonographic examination.

3.3.3.3 Toggle position

The toggle location is found within the abomasum in relation to the fixation area.

3.3.3.4 Insertion inflammation of the wound area

In calculating the gap made from laparoscopic abomasopexy, this wound area should cover the space made from the surgical suture from skin that holds the bandage of fixation to the wall of the abomasum.

3.4 The ultrasound pictures measurements program

The program SYNEDRA®[3] was used to calibrate the ultrasound pictures that were taken during the study period.

3.5 Duration of the study

The first animal patient underwent its ultrasonography examination in February 2007 and the last ultrasonography assessment was in January 2009.

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[3] SYNEDRA®
synedra information technologies GmbH
Feldstraße 1/13
A-6020 Innsbruck

4 Results

4.1 Toggle visibility

The visibility of the toggle can be detected via ultrasound as a hyper-echoic (very white and shiny) object, and beneath it a shadowing artifact can also be seen. Figure 13 and 14 shows two different aspects of the appearance of the toggle-pin after a successful laparoscopic abomasopexy.

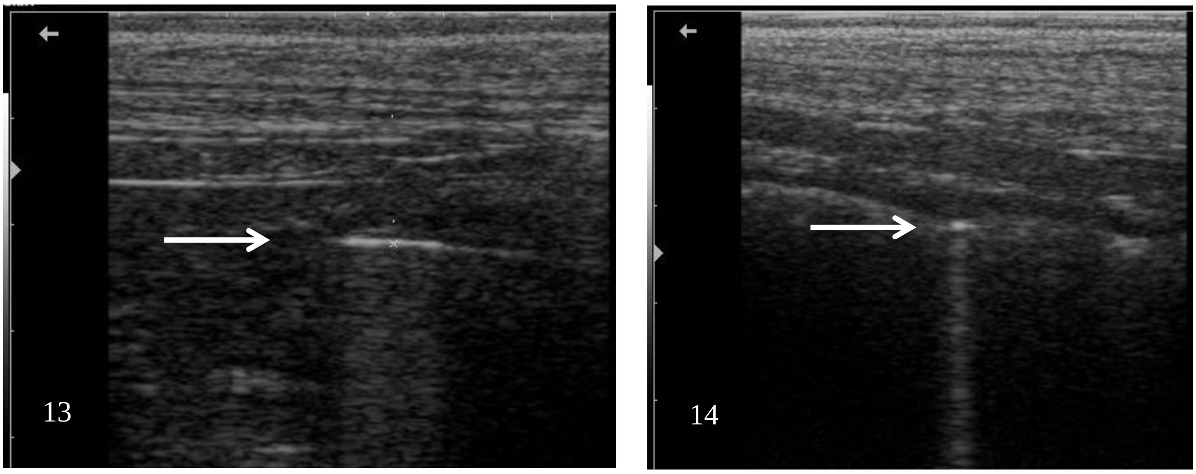


Figure 13 and 14: Toggle-pin situated in the abomasum wall after a laparoscopic abomasopexy. 13 show a transversal aspect and 14 a sagittal aspect for the toggle; the arrow shows the toggle-pin position. A linear transducer 5 MHz was used.

The optimal transducer frequency for detecting the toggle-pin is 5 MHz; the toggle visibility is characterized by the hyper-echoic area with comet-tail artifact, which is the typical picture of metal objects in sonographic examination.

The toggle-pin manifestation was successful on the day of the operation; the position and the distance between toggle-pin and the abdominal wall were also calculated to give a basis for further examinations. Figure 15 shows the visibility of the pin at various times of examination corresponding to the animal patients.

The study shows that, on the first day (day 0), the toggle was visible in all 35 animals. By the time of the second examination the toggle was visible in 33 cows. This examination was made before the removal of the gauze roll (fixating bandage); after removal of the gauze a second examination was made to control for whether the toggle-pin directly disappeared. The result of this inspection showed that all the toggle-pins stayed in their designated fixed area and did not fade. The third assessment, which took place 90 days post-operatively, showed that only 2 animals had a persistent toggle-pin presence. As for the fourth and fifth assessments (180 and 270 days after the operation) only in 1 animal could the toggle be detected. Finally, at the sixth examination (360 days post-operation) no toggle presence was noticed.

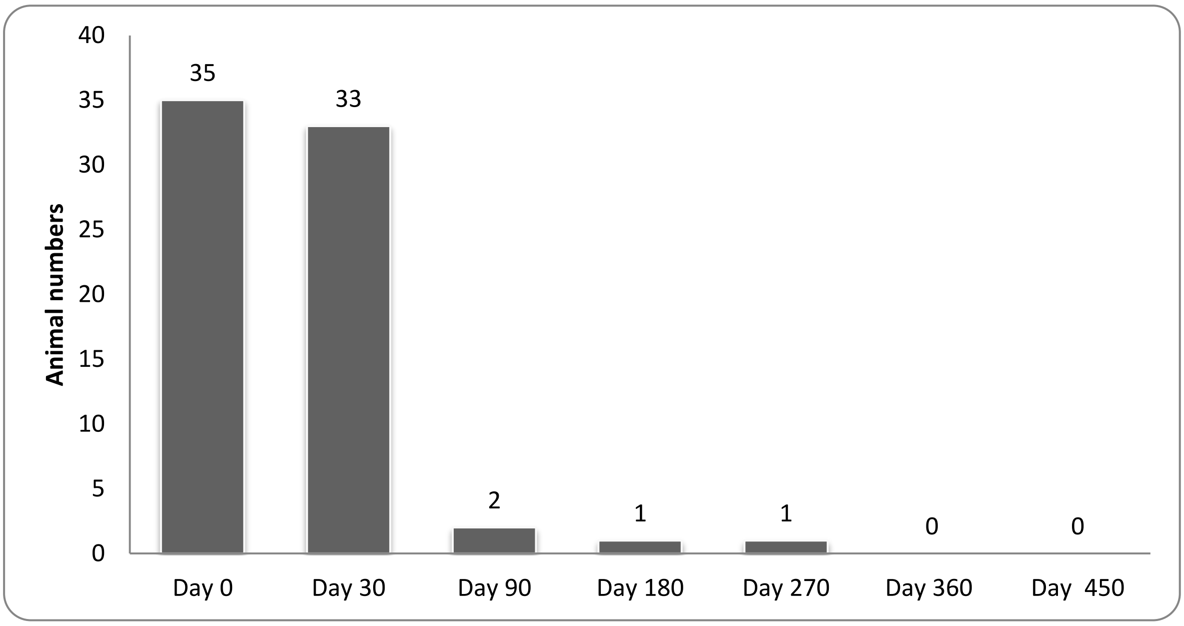


Figure 15: Toggle-pin presence in the 35 animals within the ultrasound examination periods.

A detailed dataset of each animal shows the individual data records of the animals in different time periods with their corresponding numbers (Table 2).

Table 2: The toggle visibility in each individual animal during the study period.

Animal	Day 0	Day 28	Day 90	Day 180	Day 270	Day 360	Day 450
1	V	V	nV	nV	nV	nV	nV
2	V	V	nV	nV	nV	nV	nV
3	V	V	nV	nV	nV	nV	nV
4	V	V	D	D	D	D	D
5	V	V	nV	nV	nV	nV	nV
6	V	V	nV	nV	nV	nV	nV
7	V	V	V	nV	nV	nV	nV
8	V	V	nV	D	D	D	D
9	V	V	nV	nV	D	D	D
10	V	V	nV	nV	nV	nV	nV
11	V	V	nV	nV	nV	nV	nV
12	V	V	nV	nV	nV	nV	nV
13	V	V	D	D	D	D	D
14	V	V	nV	nV	nV	nV	nV
15	V	V	nV	nV	nV	nV	nV
16	V	V	nV	nV	nV	nV	nV
17	V	V	nV	nV	nV	nV	nV
18	V	V	nV	nV	nV	nV	nV
19	V	D	D	D	D	D	D
20	V	V	nV	nV	nV	nV	nV
21	V	V	V	V	V	nV	nV
22	V	V	nV	nV	nV	nV	nV
23	V	V	nV	nV	nV	nV	nV
24	V	D	D	D	D	D	D
25	V	V	nV	nV	nV	nV	nV
26	V	V	nV	nV	nV	nV	nV
27	V	V	nV	nV	D	D	D
28	V	D	D	D	D	D	D
29	V	V	nV	nV	nV	nV	nV
30	V	V	nV	nV	nV	nV	nV
31	V	V	nV	nV	nV	D	D
32	V	V	nV	D	D	D	D
33	V	V	nV	nV	nV	nV	nV
34	V	V	nV	nV	nV	nV	nV
35	V	V	nV	nV	nV	nV	nV

V = Visible in ultrasound picture
nV = not Visible
D = Dropped out of animal

4.2 Toggle position

In this study the toggle-pin position has also been examined; on the point of origin (ground zero), which is the adhesion area, the ultrasound transducer is positioned parallel to the location of the toggle-pin and then its position can be determined.

The toggle-pin position varies centrally, cranially, caudally, medially, and laterally in a 10 cm radius. On the day of the operation the toggle can be positioned in the center, then repositioned and moved into an adjacent area. Figure 16 shows the positions that the toggle-pin takes within the study's time periods.

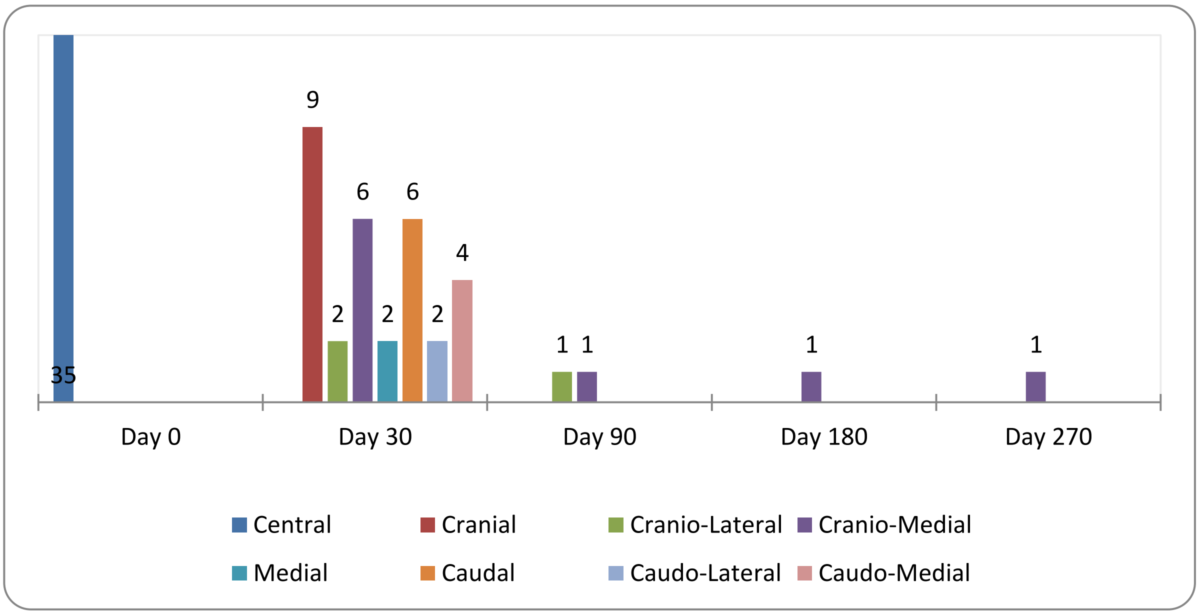


Figure 16: Toggle-pin positions in animals within the ultrasound examination periods.

On the day of the operation the toggle was situated in the center of the presumed adhesion area, and afterward the site differed as follows.

After four weeks post-operation, in 17 cows the toggle was situated cranially and cranio-medially to the adhesion; in 2 cows it was situated in the medial aspect in relation with the adhesion area; and in 12 cows the toggle-pin was seen in caudal areas.

Three months after administration of the toggle-pin, in 2 cows the pin showed in the cranial areas.

Six months post-operation, only 1 cow showed the toggle-pin in the cranial area of the adhesion, specifically, in the cranio-medial area.

After this date the toggle-pin cannot be seen in ultrasound pictures taken of the area of the operation.

4.3 Insertion wound area

This measurement is made to determine the dimensions of the wound in the toggled area that includes the length of both the cranial-caudal and the lateral-medial aspects and also the depth of the wound.

The following picture shows the wound insertion area. Depending on the examination time periods of the study,

Figure 17: Cow patient number 11 after 6 months post abomasopexy, the wound of the fixation area was still visible: swelling and redness were to be seen, no exudate and formation of scar tissue been noticed.

The area of insertion on day 0 after a successful endoscopic abomasopexy can be identified by the presenting of a gauze bandage under macroscopic examination and, in a sonographic picture, a mild distorted subcutaneous tissue with the presence of a comet-tail artifact locates the toggle-pin. Cow number 34 showed after operation on the same day a slight loosening of the fixation of the gauze bandage when arriving at the farm. To remedy this, the fixation area was examined with ultrasound and then the knots of the fixation materials were tightened.

Four weeks post-operation, the wound area is inflamed with purulent exudates. The sonographic characteristics at this stage are: in the middle, shadowing artifacts, indicating the presence of suture materials, and hyper-echoic spots, indicating inflammation at the site. In patients 3, 12 and 17 the macroscopic appearance of the wound area was extremely inflamed and there were signs of necrosis on the edges of the wound due to the pressure of the gauze bandage on the wound area.

Ninety days after the operation, the open wound area on the skin decreased radically in size, and the purulent exudates had also reduced. In the sonographic image, the length, width and depth shown in this examination’s picture are all decreased in comparison with the earlier period; and no sign of the suture materials can be seen, only a hypo-echoic area with small hyper-echoic spots indicates an inflammatory tissue reaction. Patient number 27 showed on the wound area the formation of an abscess which withdrew and had disappeared by the next examination.

The wound area, 180 days post-operation, showed no sign of purulent exudates and the inflammatory reaction had disappeared. The insertion wound was covered by scar tissue formation. The ultrasound examination revealed that the length, width, and depth of this examination’s picture was decreased in comparison with the earlier period; and no sign of the suture material can be seen; only a hypo-echoic area with small hyper-echoic spots indicates an inflammatory tissue reaction. Patient 20 showed the wound area to have a hernia-like appearance.

Two hundred and seventy days post-operation, the insertion wound was covered by scar tissue formation, and no signs of any inflammatory reaction could be distinguished. The ultrasound pictures revealed that the length, width, and depth had dramatically decreased in comparison with the previous examination, and a homogenous, anechoic appearance can be observed.

The macroscopic appearance on day 360 post-operation is that the scar tissue can barely be seen and there is no sign of inflammation in the area; although hair loss in the middle can be seen. The length, width, and depth dramatically decreased in comparison with the previous examination, and a homogenous, anechoic appearance can be observed via ultrasound examination.

On day 450 post-operation, only after clipping the hair of the animal could a very small spot of scar tissue be spotted. The ultrasound inspection revealed a small spotted anechoic homogenous spot. Table 3 summarizes the wound complications with their corresponding patients according to the time periods of this study.

Table 3: Wound complications at different time intervals in patients that suffered complications.

Exam time	Wound complications	Patient number
Day 0	Slight loosening of the gauze bandage fixation	34
Day 28	Inflammation and exudation Inflammation, exudation, and necrosis	All except: 19, 24, 28 3, 12, 17
Day 90	Inflammation and abscess formation	27
Day 180	Hernia-like appearance	20
Day 270	None	
Day 360	None	
Day 450	None	

The following pictures show the wound area with its corresponding ultrasonographic picture.

Figure 18: Day 0 after a successful endoscopic abomasopexy on a cow with left displaced abomasum (patient number 10); the gauze bandage was dirty with debris from the ground of the barn.

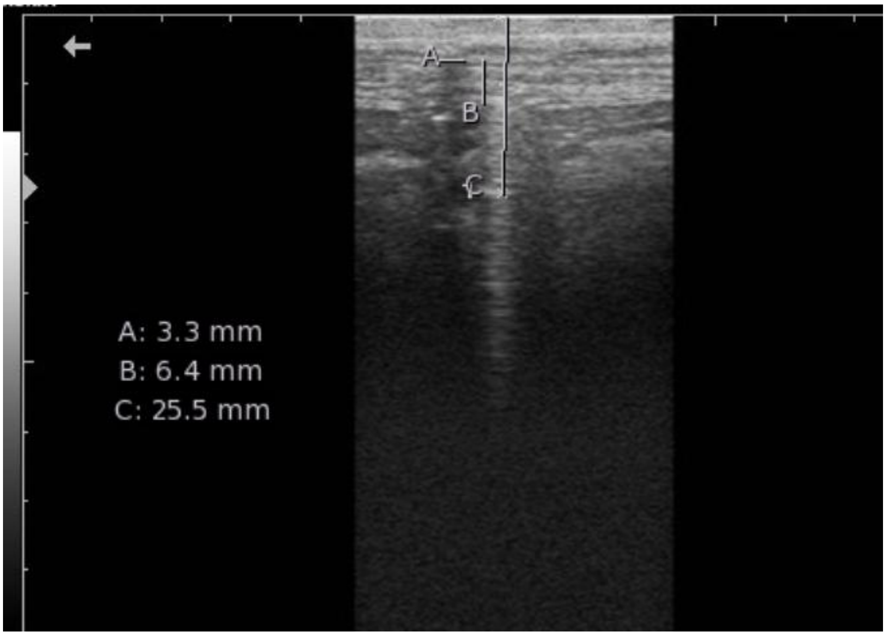
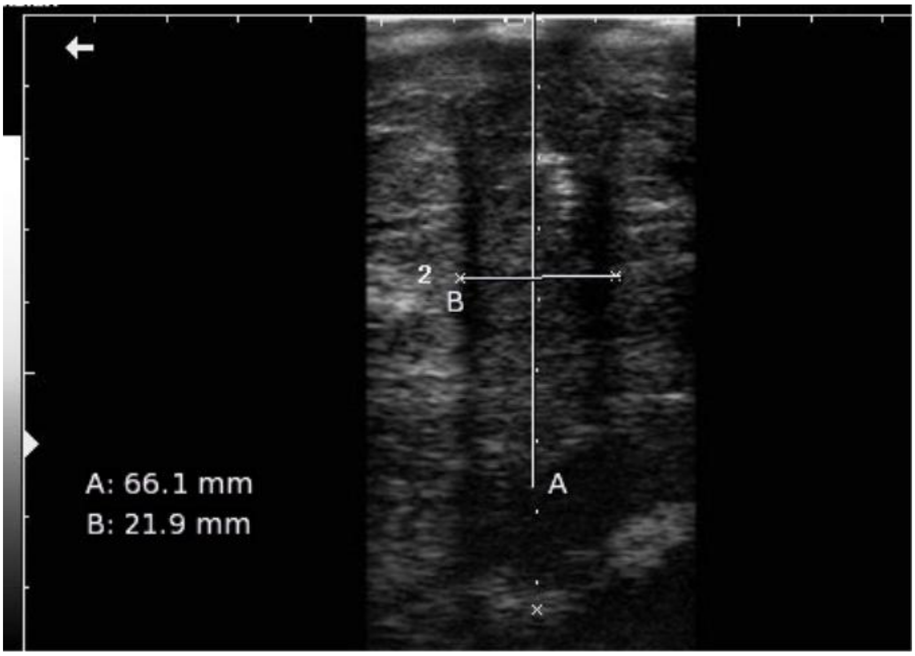


Figure 19: Ultrasound picture (patient number 10) at day 0 after a successful endoscopic abomasopexy on a cow with left displaced abomasum; a mild distortion of the subcutaneous tissue and comet-tail artifact present the location of the toggle-pin. A linear transducer 5 MHz was used. A: length wound area; B: depth of the distorted wound area; and C: sagittal section location of the toggle-pin.

Figure 20: Day 28 post-operation (patient number 16); a purulent exudate and inflammation characteristic can be identified at the site of the wound.



Picture 21: Ultrasound picture of a transverse (medial-lateral) section at day 28 post-operation (patient number 16). The toggle-pin wound insertion area can be seen and in the middle it shows shadowing artifacts indicating the presence of suture materials, and hyper-echoic spots indicating inflammation at the site. A linear transducer 5 MHz was used.

Figure 22: Day 90 post-operation (patient number 5): a purulent exudate and inflammation characteristic can be identified at the site of the wound. The open wound area in the skin decreased radically in size, and the purulent exudates have also reduced.

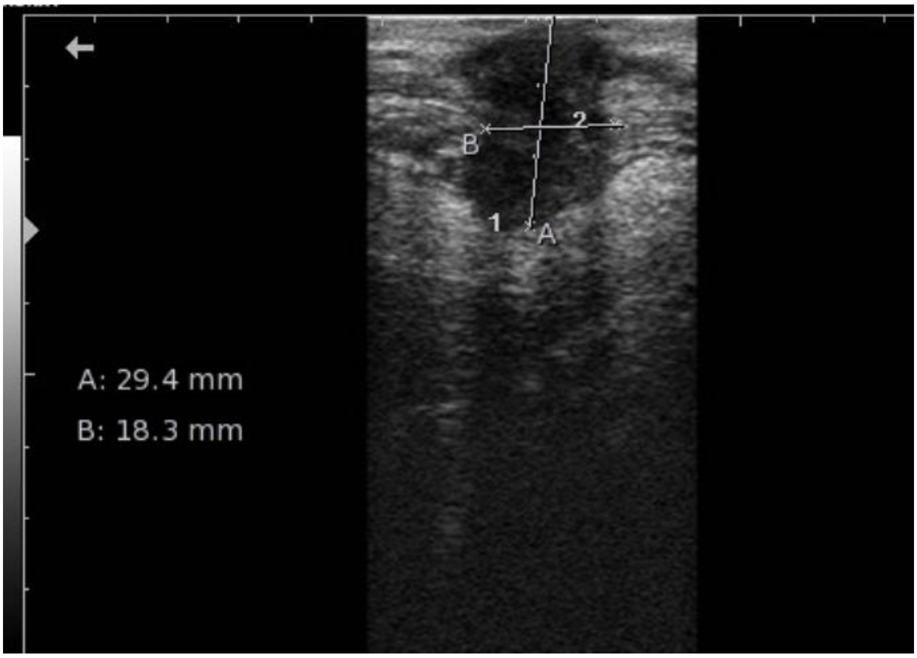


Figure 23: Ultrasound picture of a longitudinal (cranial-caudal) section at day 90 post-operation (patient number 5). The length, width, and depth of this examination picture is decreased in comparison with the earlier period; no sign of the suture materials can be seen, only a hypo-echoic area with small hyper-echoic spots indicating an inflammatory tissue reaction. A linear transducer 5 MHz was used. A: depth; B: length (cranial-caudal).

Figure 24: Day 180 post-operation (patient number 23). There was no sign of purulent exudates and the inflammatory reaction had disappeared. The wound is covered by scar tissue formation.

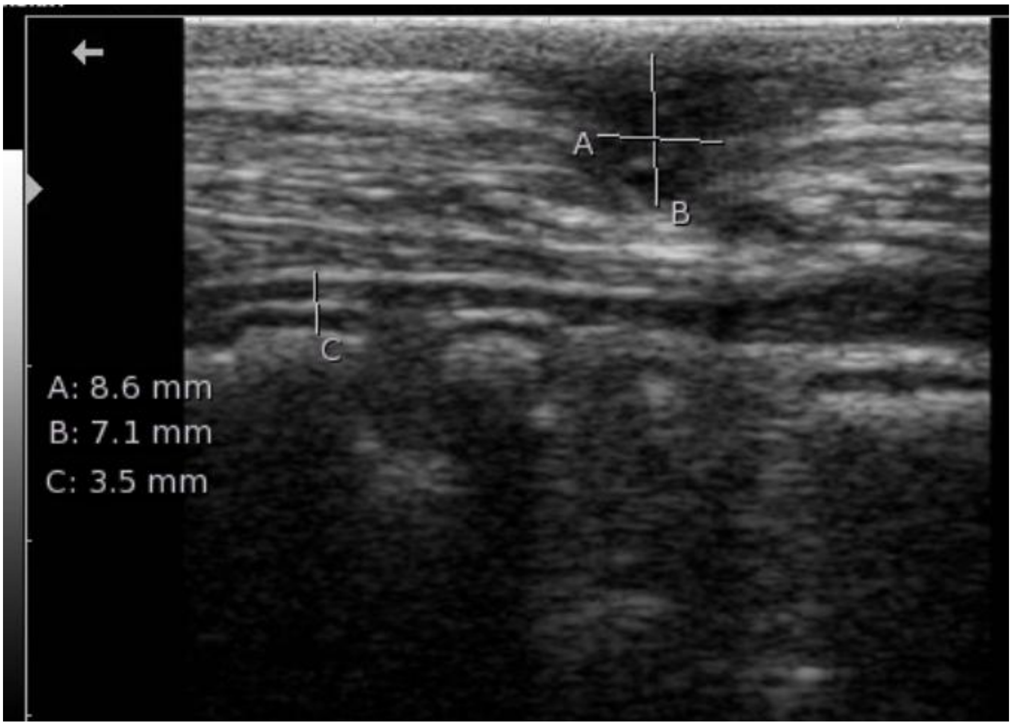


Figure 25: Ultrasound picture of a transverse (lateral-medial) section at day 180 post-operation (patient number 23). The length, width, and depth of this examination picture is decreased in comparison with the earlier period; and no sign of the suture material can be seen, only a hypo-echoic area with small hyper-echoic spots indicating an inflammatory tissue reaction. A linear transducer 5 MHz was used. A: length (lateral-medial); B: depth; C: the abomasum wall.

Figure 26: Day 270 post-operation (patient number 6): the insertion wound is covered by scar tissue formation, and no signs of inflammatory reaction can be distinguished.

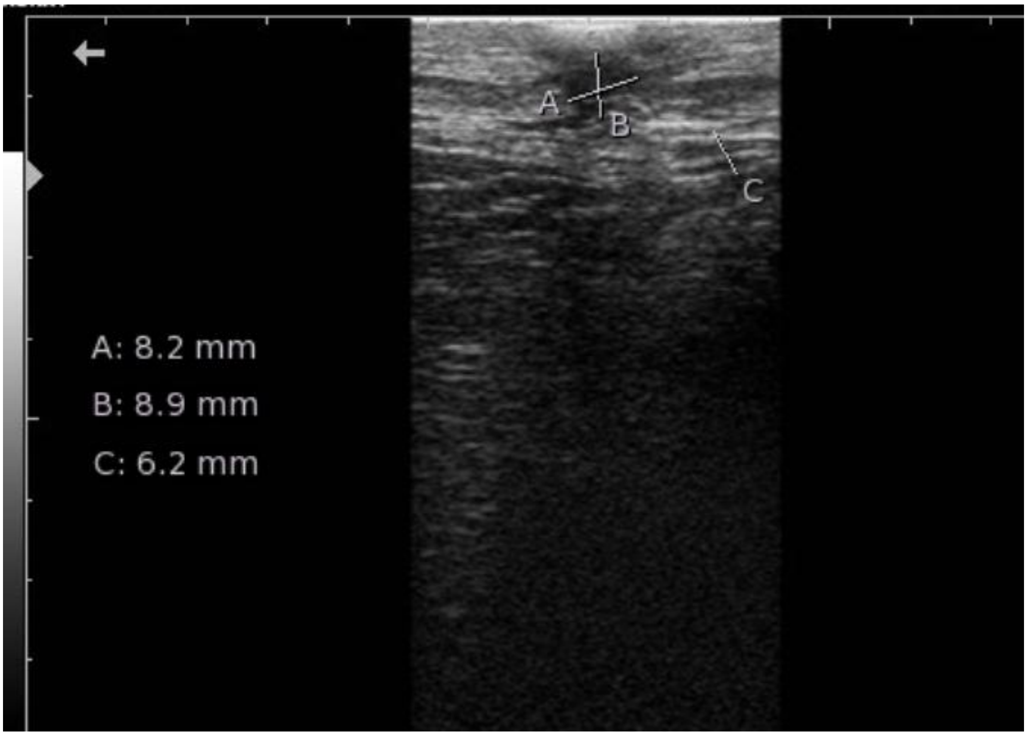


Figure 27: Ultrasound picture of a transverse (lateral-medial) section at day 270 post-operation (patient number 6). The length, width and depth dramatically decreased in comparison with earlier examinations, and an homogenous, anechoic appearance can be seen. A linear transducer 5 MHz was used. A: length (lateral-medial); B: depth; C: the abomasum wall.

Figure 28: Day 360 postoperatively (patient number 26), the scar tissue is barely seen and no sign of inflammation in the area, a hair loss in the middle can be seen.

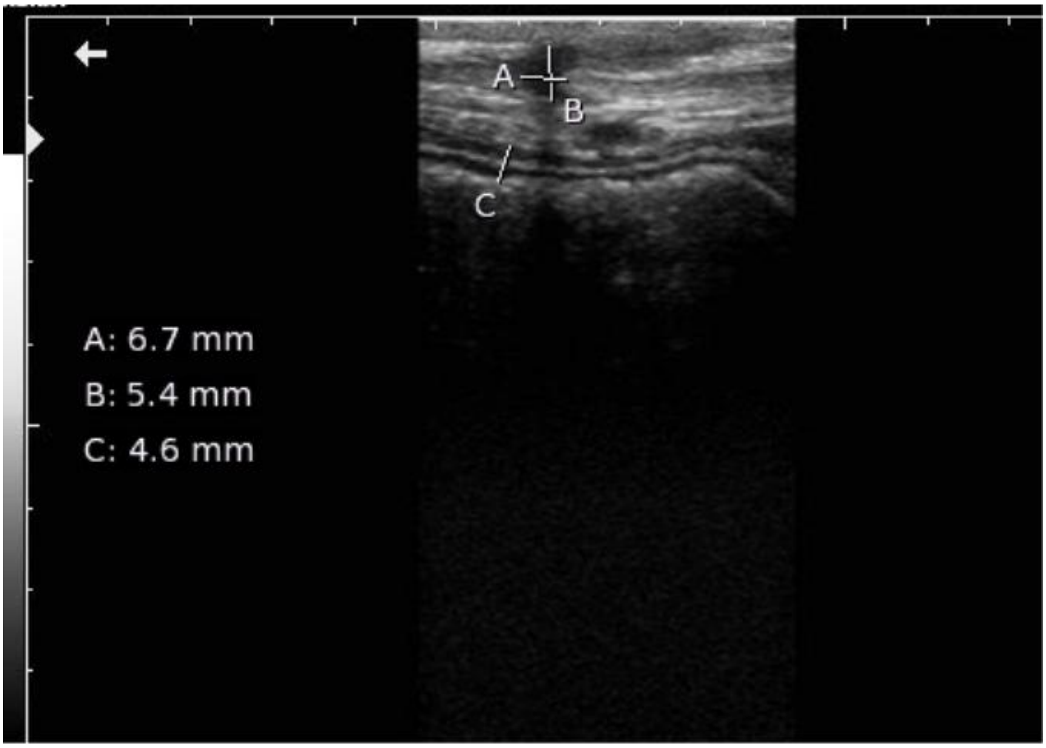


Figure 29: Ultrasound picture of a longitudinal (cranial-caudal) section at day 360 post-operation (patient number 26). The length, width, and depth have dramatically decreased in comparison with the earlier examination; an homogenous, anechoic appearance can be observed. A linear transducer 5 MHz was used. A: length (cranial-caudal); B: depth; C: the abomasum wall.

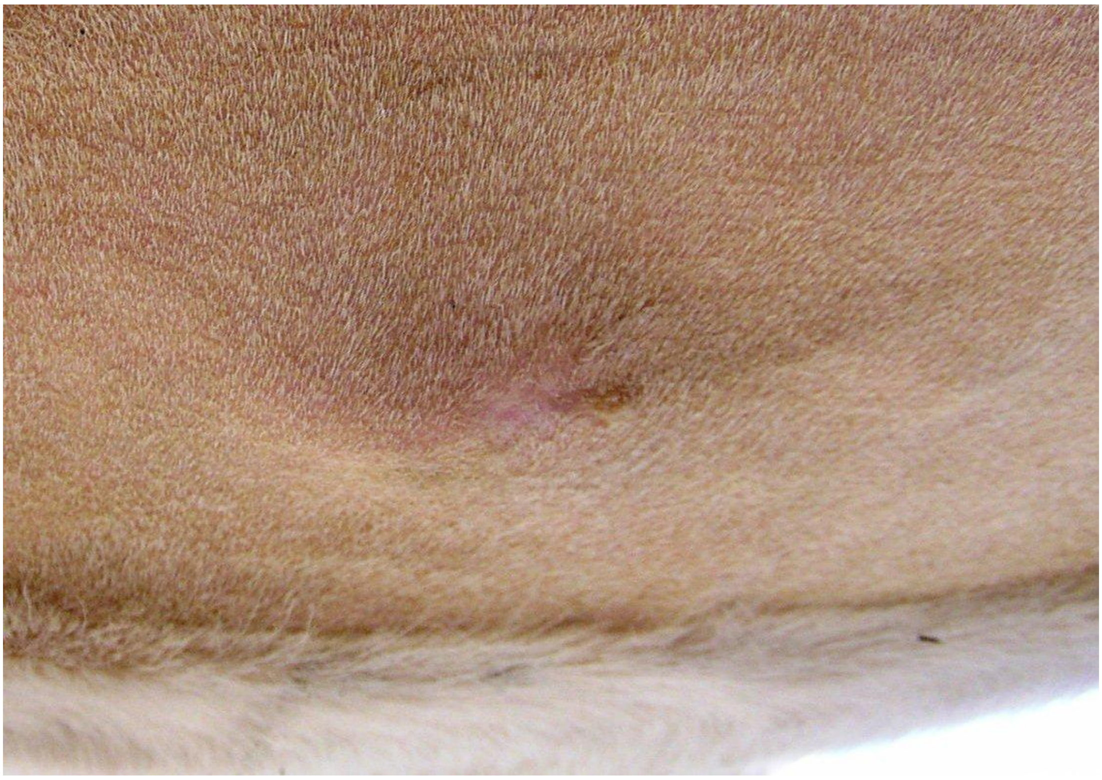


Figure 30: Day 450 post-operation (patient number 20). Only after clipping the hair of the animal can a very small spot of scar tissue be spotted.

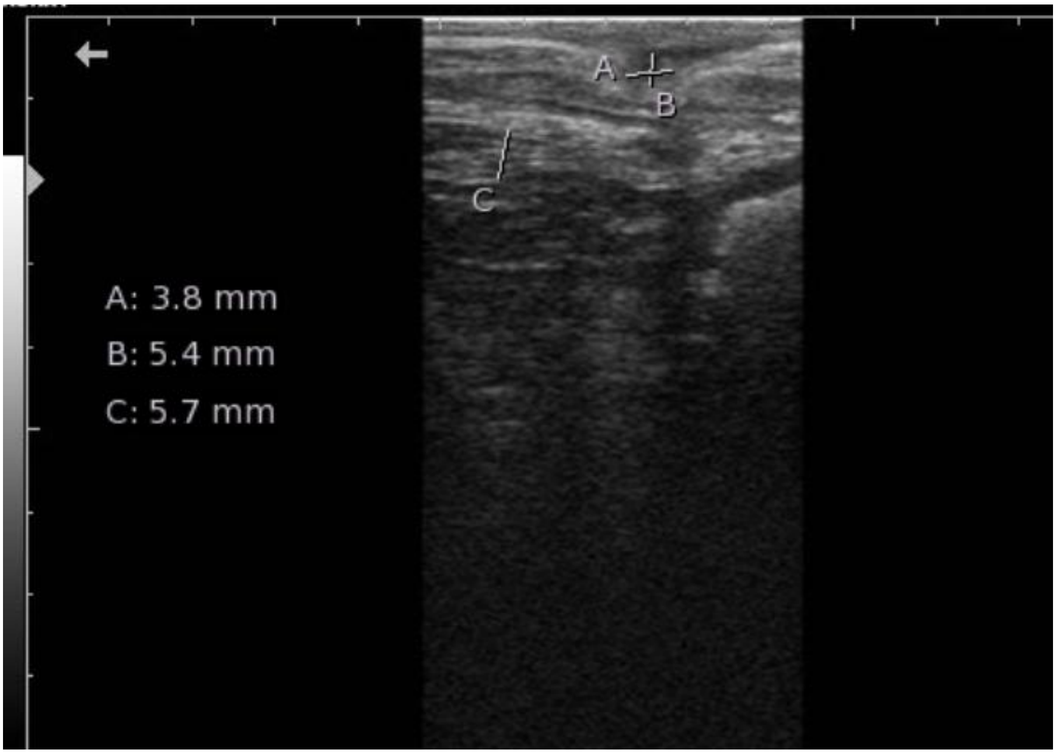


Figure 31: Ultrasound picture of a longitudinal (cranial-caudal) section at day 450 post-operation. A small anechoic homogenous spot can be noticed at the site of adhesion. A linear transducer 5 MHz was used. A: length (cranial-caudal); B: depth; C: the abomasum wall.

The following data shows that the measurements of the length and width in both mentioned aspects with the depth of the inflamed area decrease over time, and that the peak increase began after four weeks post-operation and then decreased over the mentioned examination time periods.

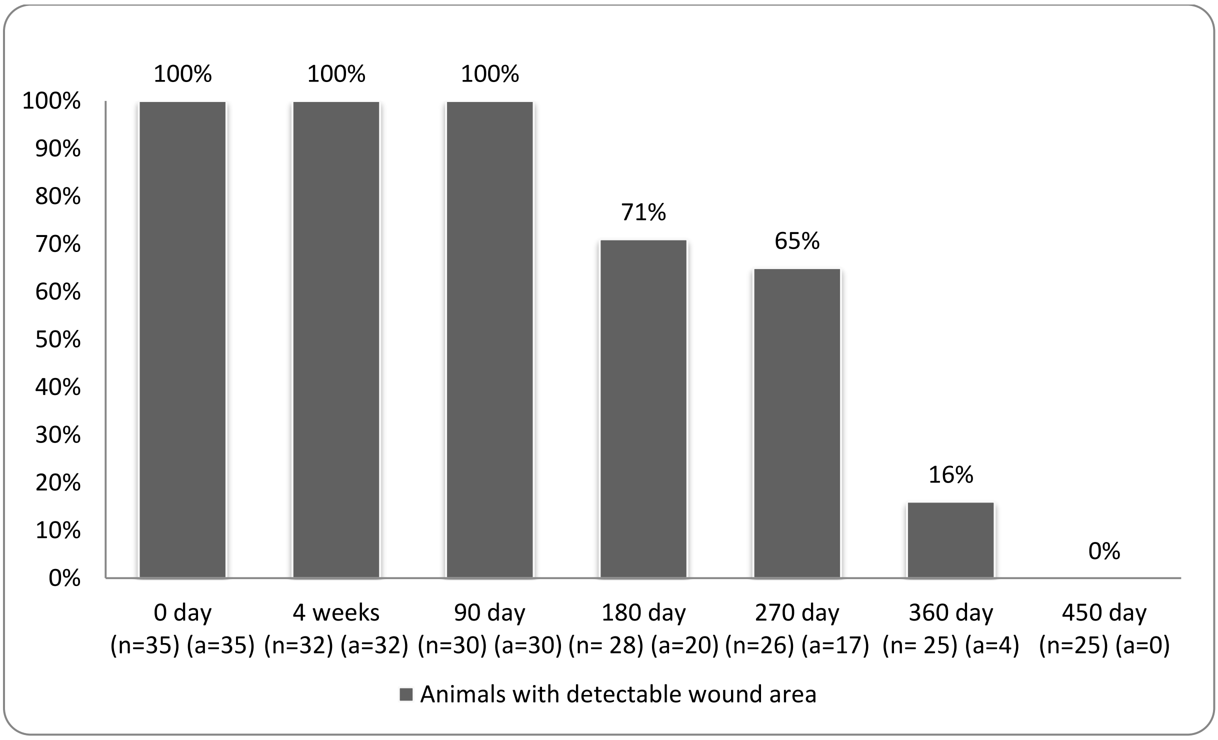


Figure 32: Total number of treated animals and animals with existing wound areas, with current percentage of occurrence in the given time period. (n= X): the total number of the animals; (a= X): animals with detectable adhesions.

The first three examinations of the wound area give 100% occurrence of visible wounds (until 90 days post-operation). The percentage remains constant until the fourth examination (at 180 days post-operation), at which point the percentage decreases to 71% (20 animals out of the total of 28). The fifth investigation with ultrasonography revealed the occurrence of visible wounds in 65% (17 out of 26 animals). The last date (at 360 days post-abomasopexy) showed traces of the wound at the trocar insertion area in 16% (only 4 out of 25 animals). The details of each cow are shown in tables 12 and 14 in the appendices, which also show the history of the patients after the operation.

4.3.1 Examination Day 0

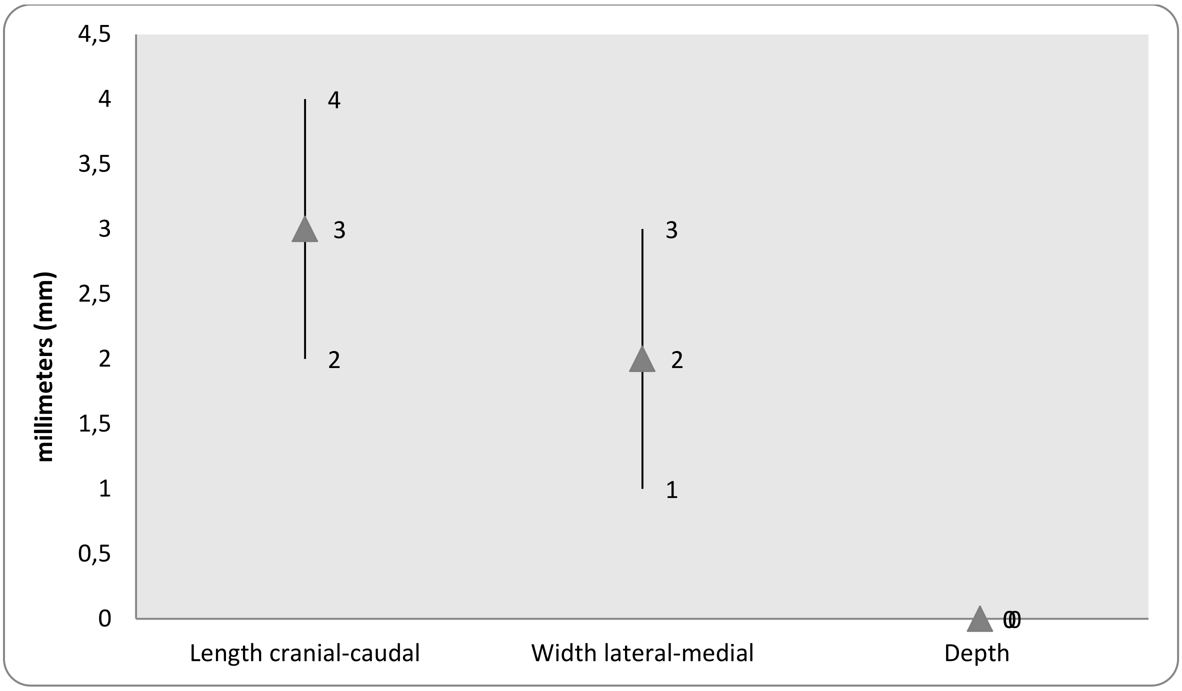


Figure 33: Insertion area in millimeters (mm) at day 0 (▲the mean $\bar{x}$ ± SD) (n=35).

The length (cranial-caudal) values vary from 1.5 to 5.0 mm; the average ($\bar{x}$) was 3.0 ± 1.0 mm SD (standard deviation).

The width (lateral-medial) values vary between 1.50 and 3.50 mm; the average 2.0 ± 1.0 mm $\bar{x}$ ± SD.

The depth at this time period was not possible to measure.

4.3.2 Examination four weeks post-operation

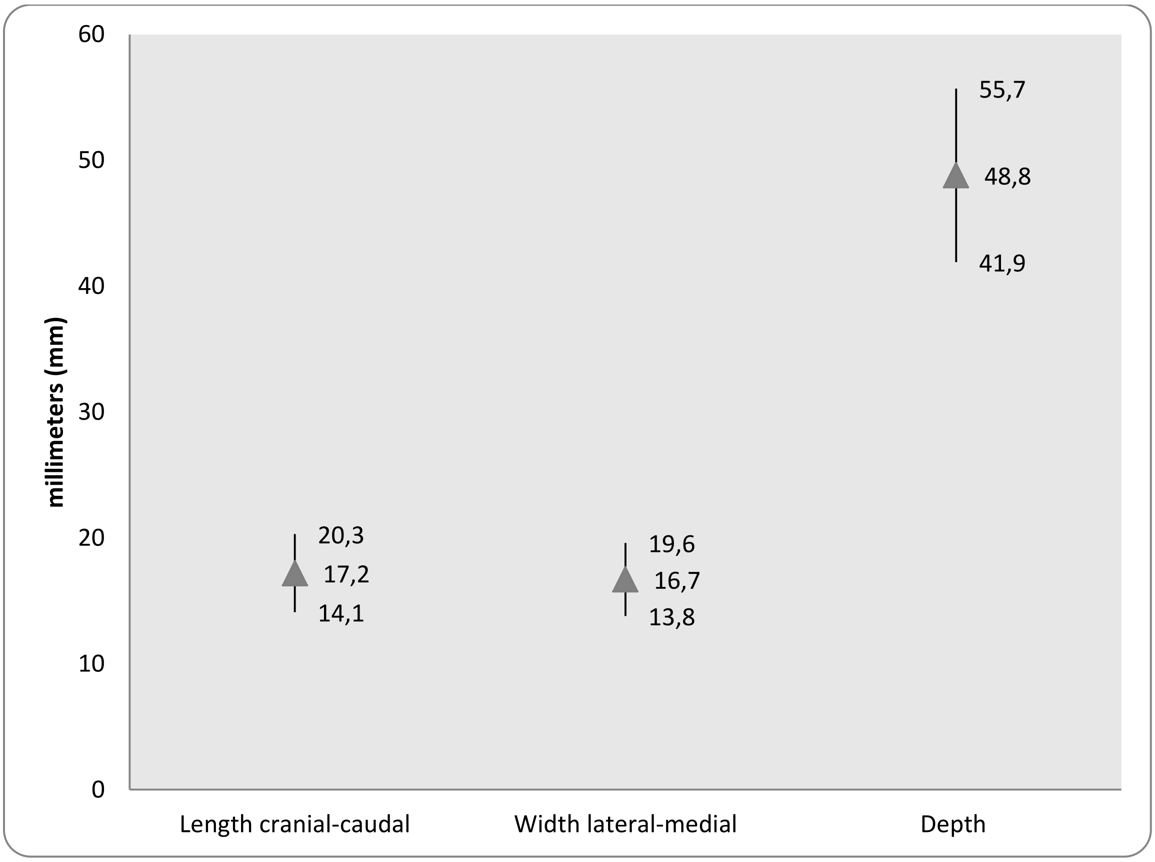


Figure 34: Insertion inflammation area in millimeters (mm) at four weeks (▲the mean $\bar{x}$ ± SD (n=32).

The length (cranial-caudal) values vary from 11.0 to 24.0 mm; the average ($\bar{x}$) was 17.2 ± 3.1 mm SD (standard deviation).

The width (lateral-medial) values vary between 9.5 and 22.5 mm; the average 16.7 ± 2.9 mm $\bar{x}$ ± SD.

The depth values were 35.0–63.8 mm, and the $\bar{x}$ 48.8 ± 6.9 mm SD.

4.3.3 Examination 90 days after the abomasopexy

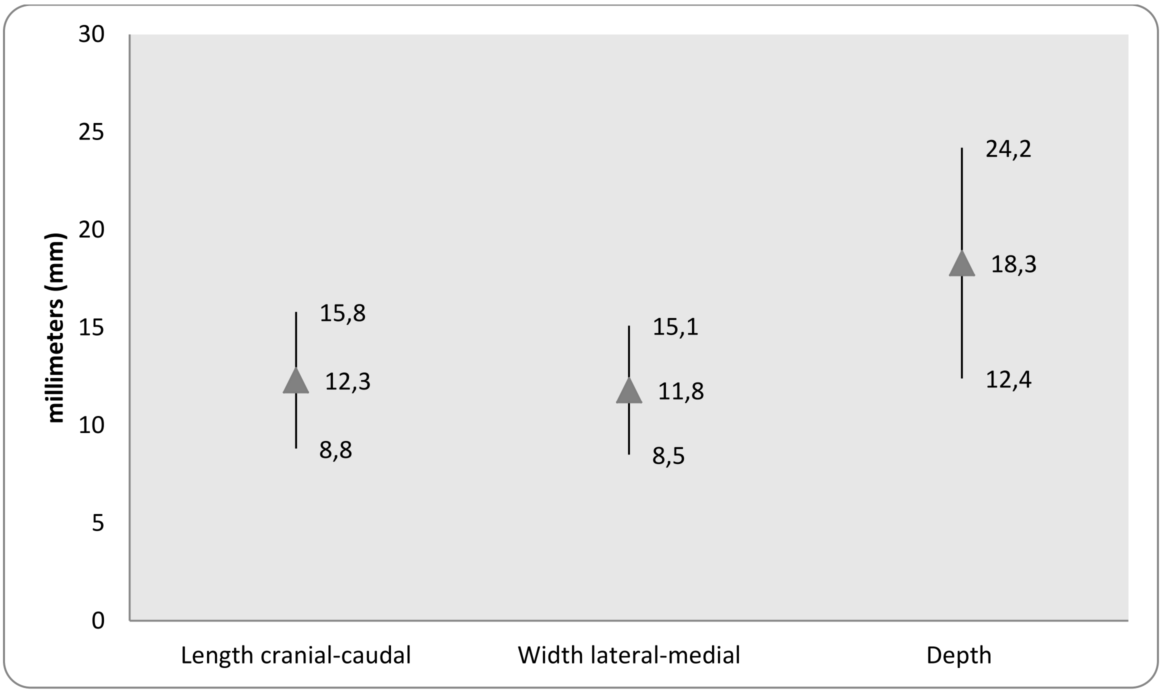


Figure 35: Insertion inflammation area in millimeters (mm) at 90 days (▲ the mean $\bar{x}$ ± SD) (n=30).

The length (cranial-caudal) values vary from 7.5 to 24.1 mm; the average ($\bar{x}$) was 12.3 ± 3.5 mm SD (standard deviation).

The width (lateral-medial) values vary between 5.5 and 22.6 mm; the average 11.8 ± 3.3 mm $\bar{x}$ ± SD.

The depth values were 5.5–25.1 mm, and the $\bar{x}$ 18.3 ± 5.9 mm SD.

4.3.4 Examination 180 days post-operation

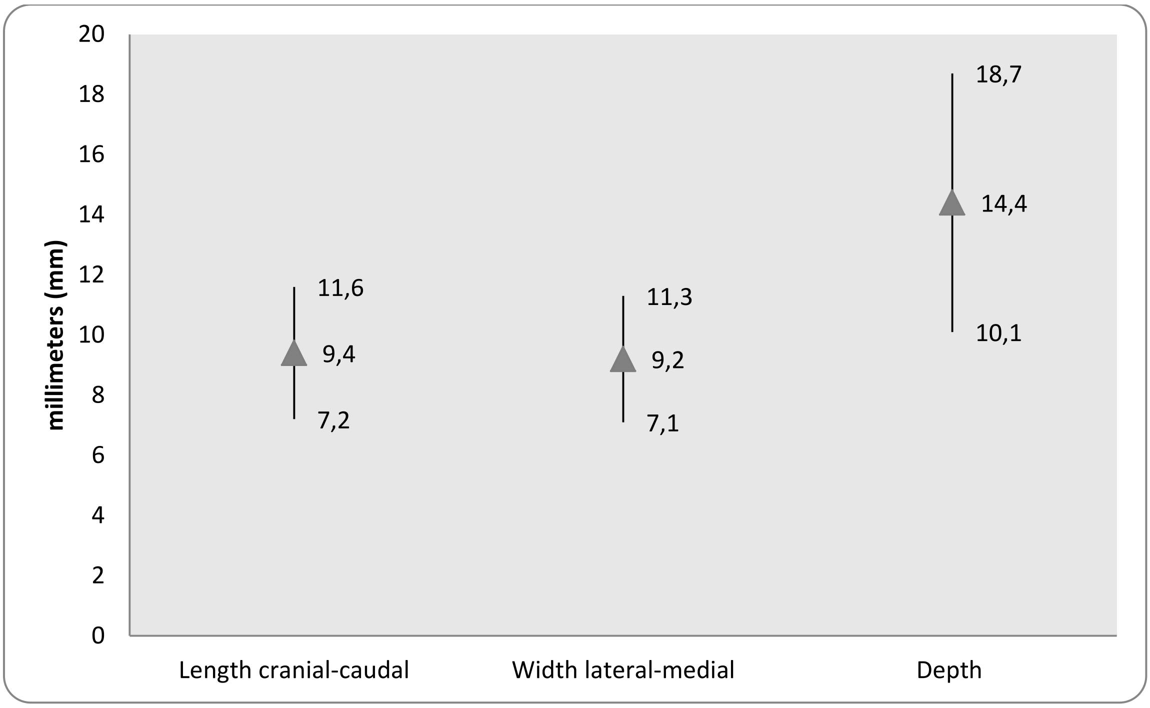


Figure 36: Insertion inflammation area in millimeters (mm) at 180 days (▲the mean $\bar{x}$ ± SD) (n=20).

The length (cranial-caudal) values vary from 6.1 to 14.7 mm; the average ($\bar{x}$) was 9.4 ± 2.2 mm SD (standard deviation).

The width (lateral-medial) values vary between 7.2 and 13.9 mm; the average 9.2 ± 2.1 mm $\bar{x}$ ± SD.

The depth values were 9.11–27 mm, and the $\bar{x}$ 14.4 ± 4.3 mm SD.

4.3.5 Result 270 days post-abomasopexy

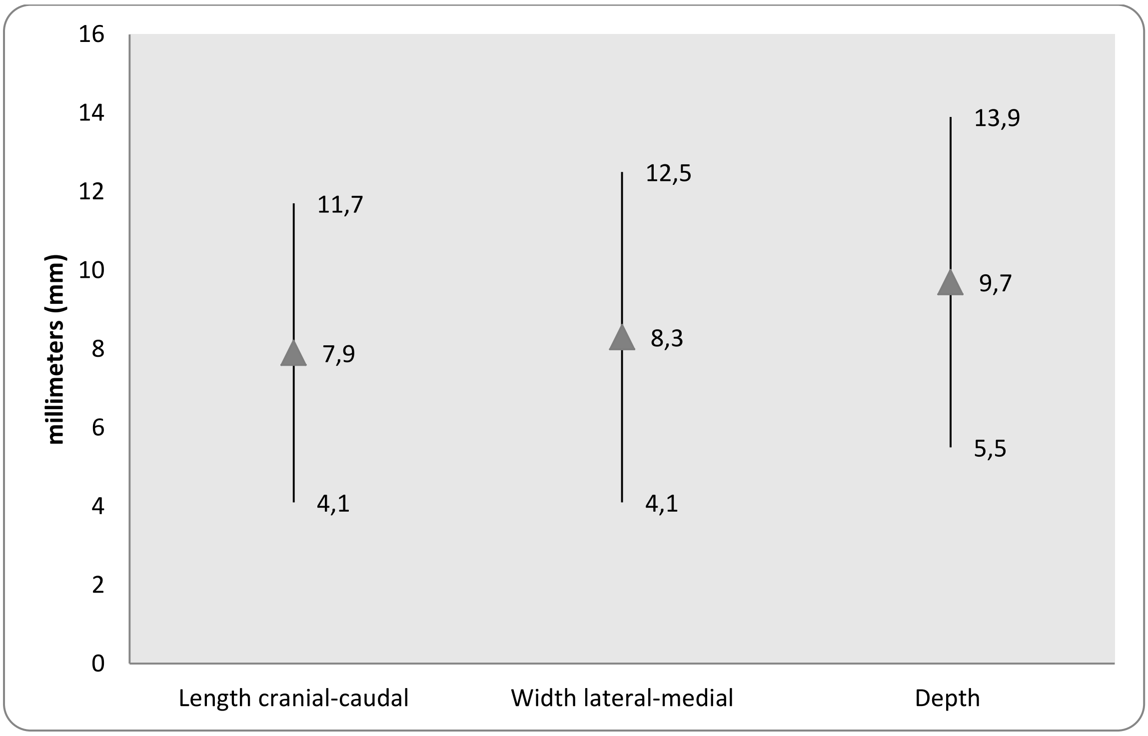


Figure 37: Insertion inflammation area in millimeters (mm) (▲ the mean $\bar{x}$ ± SD) (n=17).

The length (cranial-caudal) values vary from 3.5 to 21.2 mm; the average ($\bar{x}$) was 7.9 ± 3.8 mm SD (standard deviation).

The width (lateral-medial) values vary between 3.4 and 23.2 mm; the average 8.3 ± 4.2 mm $\bar{x}$ ± SD.

The depth values were 4.5–23.1 mm, and the $\bar{x}$ 9.7 ± 4.2 mm SD.

4.3.6 Examination 360 days post-operation

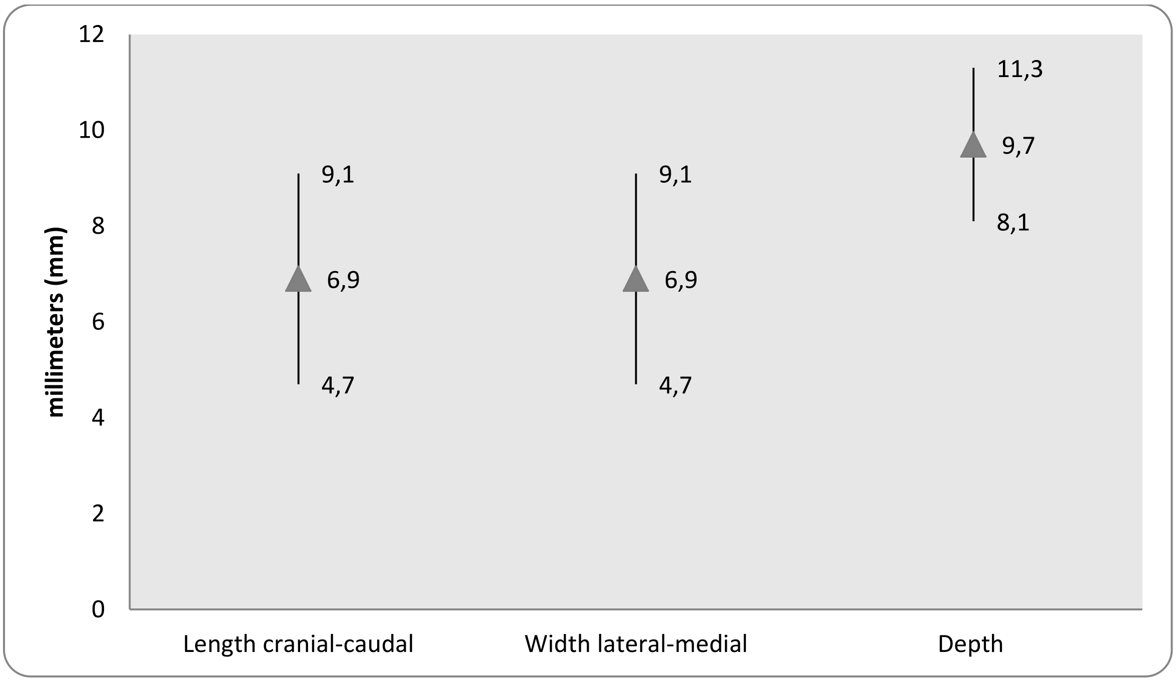


Figure 38: Insertion inflammation area in millimeters (mm) at 360 days (▲the mean $\bar{x}$ ± SD) (n=4).

The length (cranial-caudal) values vary from 3.95 to 9.27 mm; the average ($\bar{x}$) was 6.9 ± 2.2 mm SD (standard deviation).

The width (lateral-medial) values vary between 4.15 and 9.45 mm; the average 6.9 ± 2.2 mm $\bar{x}$ ± SD.

The depth values were 7.5–11.7 mm, and the $\bar{x}$ 9.7 ± 1.6 mm SD.

4.3.7 Examination 450 days post-operation

At this exact time period there was no evidence of a residual wound appearance either under macroscopic examination of the cow or in the ultrasound diagnostic imaging (detailed data of this are categorized in the appendices).

One cow suffered a hernia at the site of the operation but there were no digestive organs in the sac of the hernia.

The following tables show the length, width, and depth of the wound area with its mean and standard deviation at each time period in the study. The values of the length, width, and depth that changed across time periods were found to be statistically significant by using one-way analysis of variance (anova) (see statistical analysis sheets in the appendices).

Table 4: Length of the wound area in animals.

Timetable	n	a	$\bar{x}$ ± SD (mm)	Minimum value (mm)	Maximum value (mm)
0 days	35	35	3.0 ± 1.0	2.0	5.0
4 weeks	32	32	17.2 ± 3.1	14.7	24.0
90 days	30	30	12.3 ± 3.5	10.0	24.0
180 days	28	20	9.4 ± 2.2	8.1	14.7
270 days	26	17	7.9 ± 3.7	21.2	6.2
360 days	25	4	6.8 ± 2.2	3.8	7.3
450 days	25	0	0.0 ± 0.0	0.0	0.0

(n= X): the total number of animals; (a= X): animals with detectable wound insertion area; measurement values given millimeters (mm).

Table 5: Width of the wound area in animals.

Timetable	n	a	$\bar{x}$ ± SD (mm)	Minimum value (mm)	Maximum value (mm)
0 days	35	35	2.0 ± 1.0	2.0	4.0
4 weeks	32	32	16.7 ± 2.9	9.5	22.5
90 days	30	30	11.9 ± 3.3	5.5	22.6
180 days	28	20	9.4 ± 2.1	5.5	13.9
270 days	26	17	8.3 ± 4.2	23.2	3.4
360 days	25	4	6.9 ± 2.2	4.2	9.5
450 days	25	0	0.0 ± 0.0	0.0	0.0

(n= X): the total number of animals; (a= X): animals with detectable wound insertion area; measurement values given in millimeters (mm).

Table 6: Depth of the wound area in animals.

Timetable	n	a	$\bar{x} \pm SD$ (mm)	Minimum value (mm)	Maximum value (mm)
0 days	35	35	0.0 ± 0.0	0.0	0.0
4 weeks	32	32	48.8 ± 6.9	35.0	63.8
90 days	30	30	18.3 ± 5.9	5.5	32.1
180 days	28	20	15.2 ±4.3	9.1	27.0
270 days	26	17	9.7 ± 4.2	4.5	23.1
360 days	25	4	9.5 ± 1.6	7.6	11.7
450 days	25	0	0.0 ± 0.0	0.0	0.0

(n= X): the total number of animals; (a= X): animals with detectable wound insertion area; measurement values given in millimeters (mm).

The values of length, width, and depth within the different examination time periods are statistically significant with ($p < 0.0001$).

4.4 Abomasopexy adhesion area

4.4.1 Content of the adhesion area

The adhesions determined via ultrasound were as follows. Heterogeneous hypo-echoic well-defined mass is located between the abomasum and abdominal wall in the first two examinations (between 3 and 6 months).

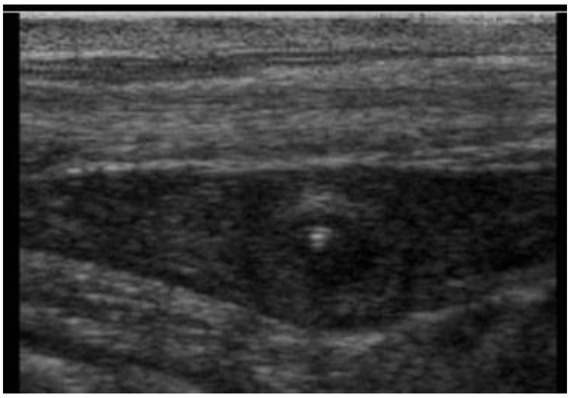
Nine months post-operation, in the center of the adhesion there appears to be a point of calcification (mineralization) tissue, characterized as a hyper-echoic spot in the middle of the adhesion site. The same sonographic appearance is also reported by STRAUSS (2000), RUSHBY et al. (2002), and SUCHETA and BUDE (2002).

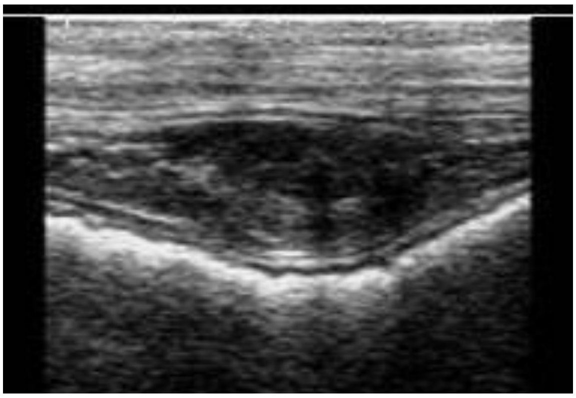
One year (12 months) after the operation, the calcification disappears and there is no longer evidence of a calcification within the adhesion tissue.

4.4.2 Types of adhesions

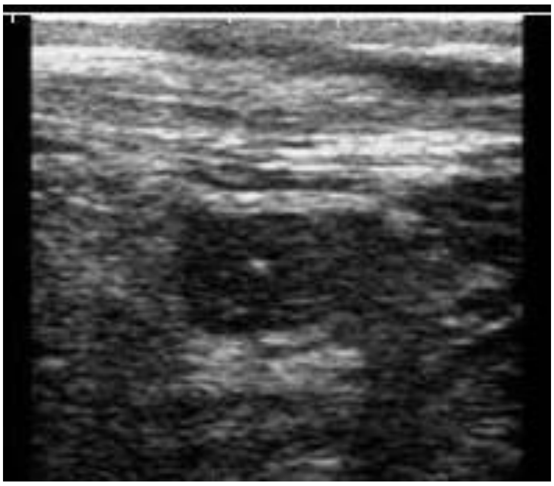
In this study it has been found that the adhesions can be classified according to shape and also according to the presence of a calcification point in the center of the adhesion.

The pictures below show the adhesion site by type.





Figures 39 and 40: A longitudinal ultrasound section shows an oval-shaped adhesion-like appearance. The left picture shows a focal point of calcification (patient number 6), whereas the right picture shows NO presence of calcification (patient number 33). A linear transducer 5 MHz was used.



Figures 41 and 42: A longitudinal ultrasound section shows a round-shaped adhesion-like appearance. The left picture shows a center of calcification (patient number 2) and the right picture shows NO presence of calcification (patient number 20). A linear transducer 5 MHz was used.

4.4.2.1. Types of adhesion according to shape

The shape of an adhesion can be sorted into three categories: oval-, round-, and irregular-shaped adhesions. Twenty-six cows show oval-shaped adhesions; nine cows show round-shaped adhesions; and two cows show irregular-shaped adhesions. Graphs 43 and 44 show the types of adhesion (detailed data are categorized in the appendices).

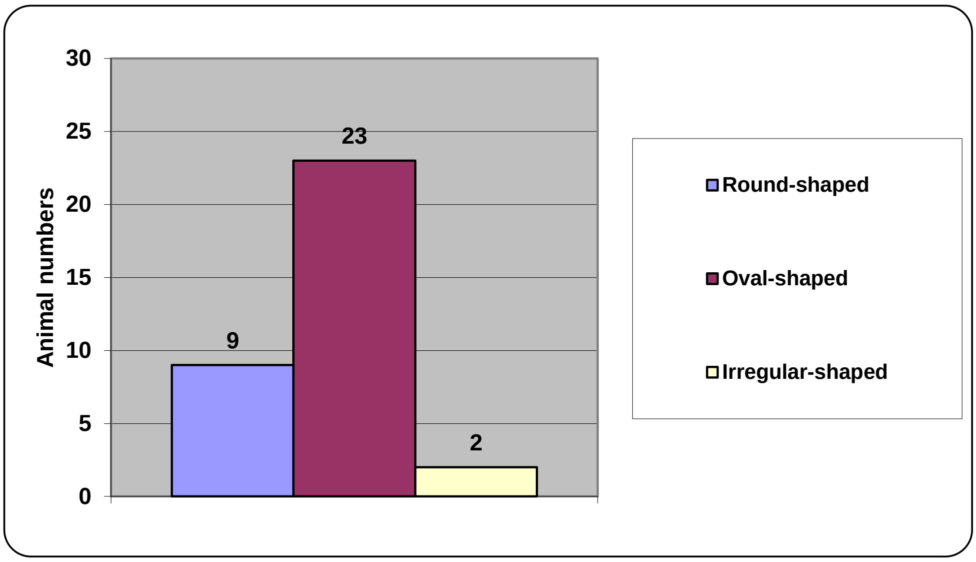


Figure 43: Types of adhesions according to shape; the different kinds of shape can be identified at day 90 post-operation.

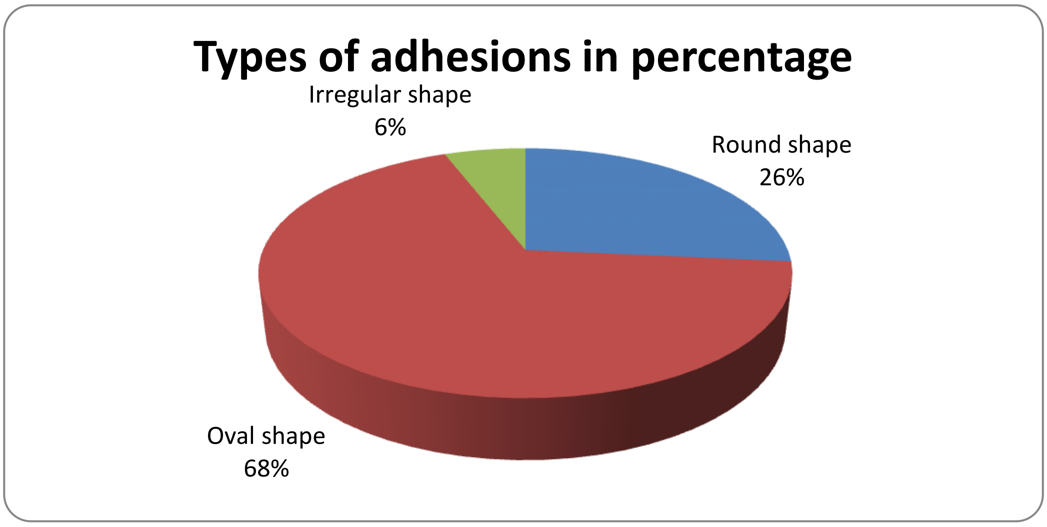


Figure 44: Percentage of occurrences of the different types of adhesions; these kinds of adhesions can be recognized 3 months after the abomasopexy operation.

Sixty-eight per cent of the 34 study animals (one animal died before the adhesion first occurred) had an oval-shape adhesion, 26% had a round-shaped adhesion and the rest of the animals (6%) had an irregular-shaped adhesion (detailed data are categorized in the appendices).

4.4.2.2. Types of adhesion according to calcification appearance

In 19 out of 34 (59%) cows in this study there is a presence of a focal point of calcification in the center of the adhesion; as mentioned previously, these centers of calcification can be characterized as hyper-echoic spots in the middle of the adhesion. Thirteen cows out of the 34 (41%) showed adhesions without a center of calcification.

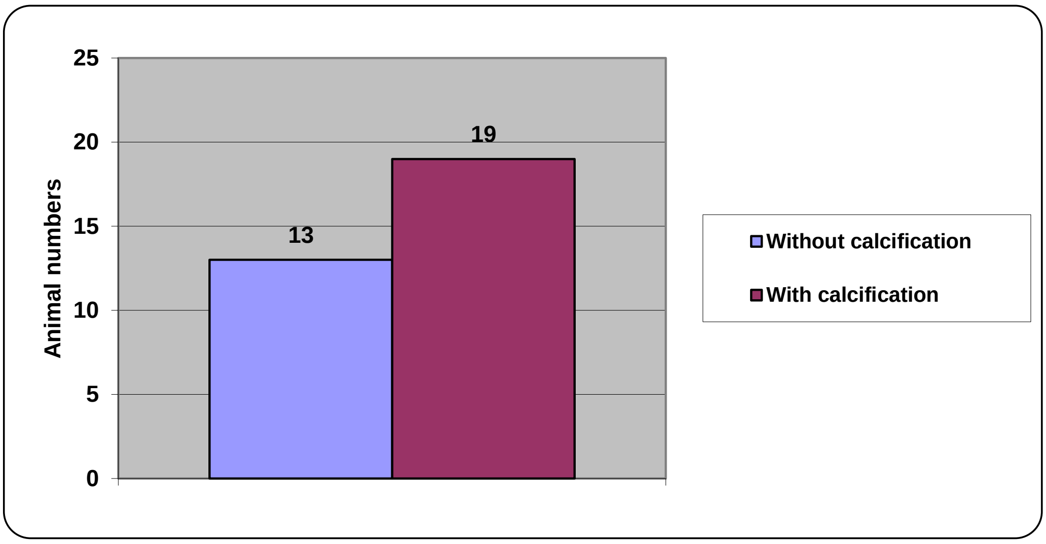


Figure 45: Types of adhesions according to the presence of a calcification spot in the center of the adhesion. The identification of these two types took place 3 months post-operatively.

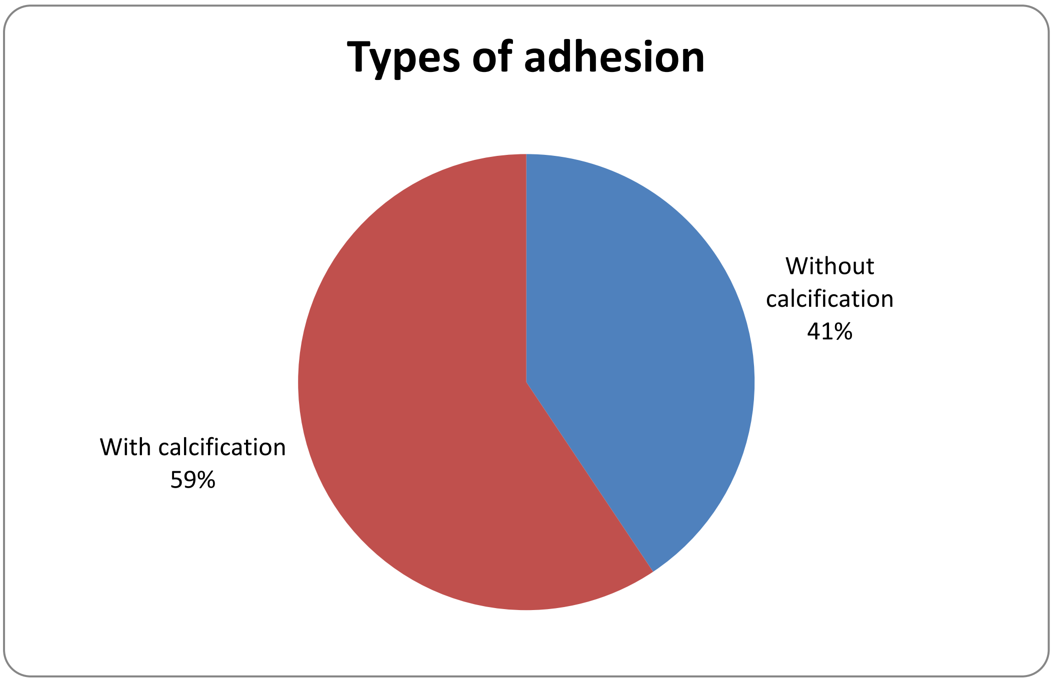


Figure 46: Types of adhesion in percentage according to the presence of a calcification spot in the center of the adhesion. Identification was possible 90 days post-operation.

Detailed data are categorized in table 15 in the appendices.

4.4.3. Measurements of the adhesion area

The length, width, and depth reached peak values 28 days after the operation and then decreased dramatically afterwards; Figure 45 shows the presence of the adhesions in different time periods.

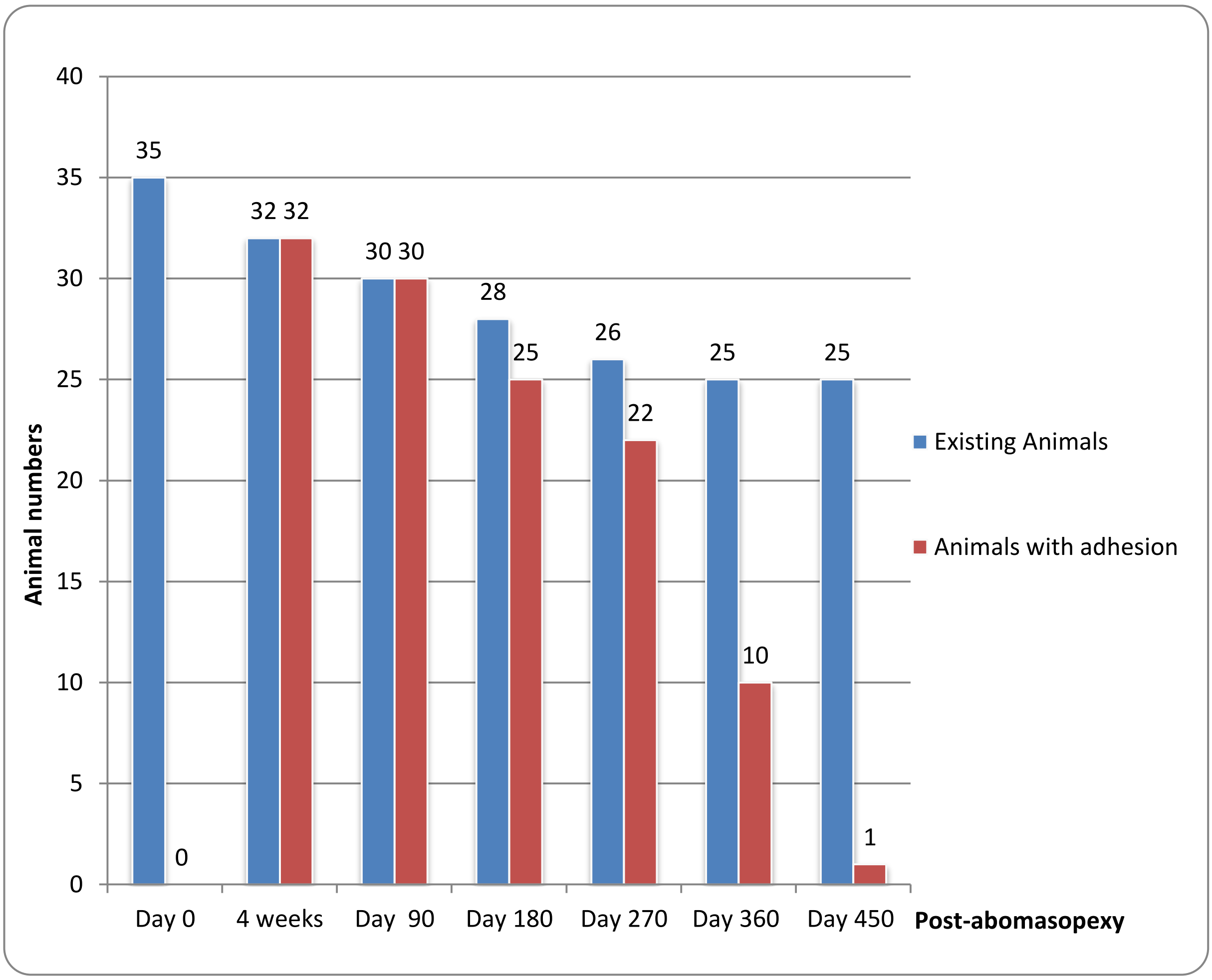


Figure 47: Ultrasonographic verified adhesions between the abomasum and the abdominal wall.

The diagram above shows that at day 0 (the day of operation) n=35 with 100% showing no adhesions at the operation site.

Four weeks post-operation (n=32), 100% of the treated animals show an adhesion at the site of the fixation.

Ninety days after the laparoscopic operation, again, 100% of the operated animals (n=30) show adhesions in the designated area.

One hundred and eighty days post-abomasopexy (n=28) there were 25 animals showing adhesion, that is, 89%.

Two hundred and seventy days post-operation (n=26) there were only 22 animals showing adhesions (85%).

Three hundred and sixty days post-laparoscopic abomasopexy (n=25) there were 10 animals (40%) showing adhesions.

Four hundred and fifty days post-abomasopexy (n=25) there was only one animal (4%) that showed an adhesion. The rest of the treated animals showed no evidence of adhesions at the site of the laparoscopic abomasopexy operation.

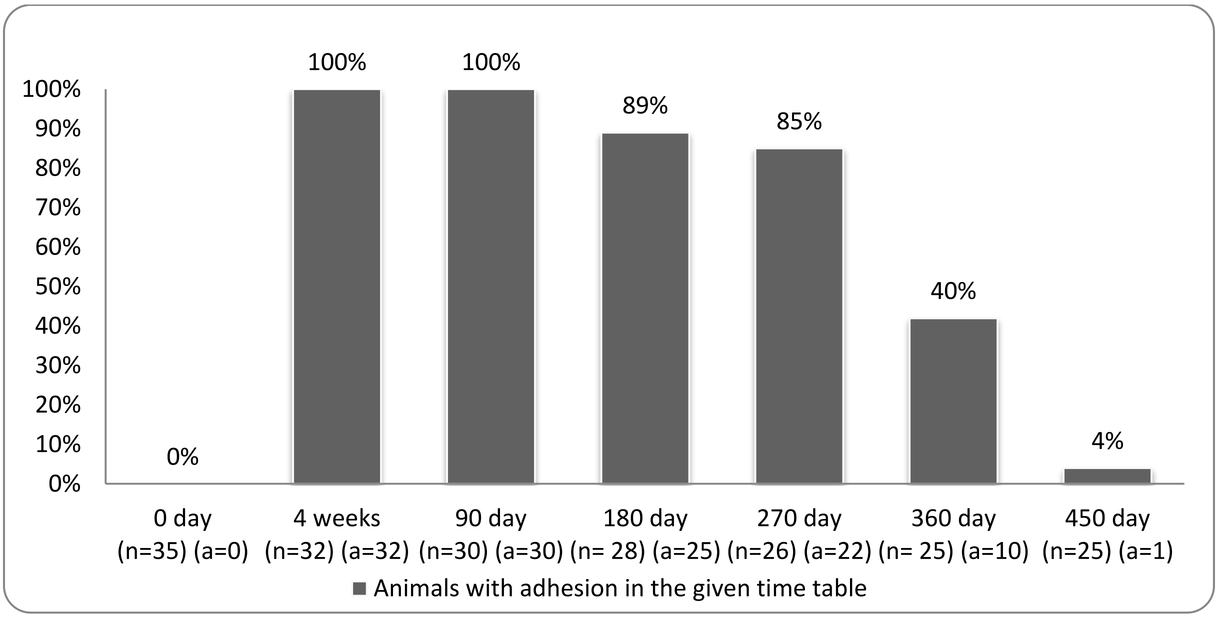


Figure 48: Percentages of the number of animals with adhesions in the abomasopexy fixation area in the examination periods. (n= X): the total number of animals; (a=X): animals with adhesions.

4.4.3.1 Ultrasonographic findings in the fixation area

The ultrasound pictures at day 0 post-operation present the characteristic feature of a comet-tail shadowing artifact of the toggle-pin, which shows the position of the toggle-pin in the abomasum between the abomasum and abdominal wall.

Four weeks post-operation, the ultrasound pictures revealed that the toggle-pin is surrounded by a slightly hypo-echoic tissue (moderately heterogenic tissue), but the appearance of the toggle-pin is as before. Cow number 10 showed that the toggle was surrounded by inflamed materials.

Ultrasound pictures at day 90 post-operation show that the site of adhesion appears as a well-defined slightly heterogeneous hypo-echoic mass visible between the abomasum and the abdominal wall. The upper picture shows a round-shaped adhesion and the lower picture shows an oval-shaped adhesion. At this time cow number 13 relapsed. Cow number 17 showed that the adhesion was situated caudal to the operation wound but for cow number 26 it was located to the left side of the operation area.

On day 180 post-operation the site of adhesion appears via ultrasound as relatively homogenous and hypo-echoic and is decreasing in size compared to the previous examination. The upper picture shows a central focal point of calcification, compared with the lower picture, which is without the calcification point. Cow number 14 showed that the abomasum wall had been divided into two layers; cows 17, 22, and 23 showed that the adhesions were located in different locations from the operation wound, namely, caudal, right, and cranial respectively.

The abomasopexy (adhesion area) at day 270 post-operation shows in the ultrasound that the adhesion is hyper-echoic and severely decreased in size. Two cows show that in the center a hyper-echoic linear structure is visible, which might represent a fibrous tissue or calcification. In comparison with the other pictures taken in the same examination time period, the pictures with calcification in the center show a bigger structure in size than those without it. In cow number 3 the adhesion was located cranial to the wound area; in cow number 25 the picture was the same as for cow number 14 – the abomasal layer was divided into two layers. Cow number 31 only showed fibrin threads but no adhesion was detected and there was no synchronization between the abomasum wall and the abdominal wall.

The adhesion after 360 days showed in the ultrasound pictures as further decreased in size; the subcutaneous tissue shows only mild distortion of the layers. In this examination time period cow number 12 showed the same results as cow number 31 – only fibrin thread, and no adhesion, was detectable.

The area of adhesion at day 450 post-operation shows that the adhesion site is barely visible as a hyper-echoic area and the subcutaneous tissue shows mild distortion of the layers.

The next pictures exhibit the ultrasound finding concerning the adhesion area. They are organized according to the time periods of this study at 0, 28, 90, 180, 270, 360, and 450 days post-operation.

The following tables show the length, width, and depth of the adhesion area with its mean and standard deviation for each time period in the study. There were statistically significant differences between the lengths, widths, and depths values in time periods (see statistical analysis sheets in the appendices), observed by using one-way analysis of variance (anova).

Table 7: Length of the adhesions in animals.

Timetable	n	a	$\bar{x}$ ± SD (mm)	Minimum value (mm)	Maximum value (mm)
0 days	35	0	0	0	0
4 weeks	32	32	50.0 ± 4.1	40.9	57.1
90 days	30	30	25.5 ± 4.6	16.2	37.4
180 days	28	25	14.1 ± 2.8	7.7	19.3
270 days	26	22	9.2 ± 2.8	4.3	15.9
360 days	25	10	5.0 ± 1.0	3.8	7.3
450 days	25	1	3.8 ± 0.0	3.8	3.8

(n= X): the total number of animals; (a= X): animals with adhesions; measurement values given in millimeters (mm)

Table 8: Width of the adhesions in animals.

Timetable	n	a	$\bar{x}$ ± SD (mm)	Minimum value (mm)	Maximum value (mm)
0 days	35	0	0	0	0
4 weeks	32	32	48.4 ± 5.0	32.6	56.2
90 days	30	30	25.9 ± 4.8	15.8	35.2
180 days	28	25	14.4 ± 2.9	8.2	20.5
270 days	26	22	9.4 ± 2.6	4.5	15.3
360 days	25	10	5.1 ± 1.0	3.8	7.9
450 days	25	1	3.6 ± 0.0	3.6	3.6

(n= X): the total number of animals; (a= X): animals with adhesions; measurement values given in millimeters (mm)

Table 9: Depth of the adhesions in animals.

Timetable	n	a	$\bar{x}$ ± SD (mm)	Minimum value (mm)	Maximum value (mm)
0 days	35	0	0	0	0
4 weeks	32	32	33.3 ± 9.4	21.9	50.1
90 days	30	30	18.2 ± 6.1	10.4	34.2
180 days	28	25	10.8 ± 3.2	5.4	16.8
270 days	26	22	5.8 ± 2.4	2.9	11.1
360 days	25	10	3.4 ± 0.9	2.1	4.6
450 days	25	1	3.9 ± 0.0	2.9	2.9

(n= X): the total number of animals; (a= X): animals with adhesions; measurement values given in millimeters (mm)

The values of length, width, and depth within the different examination time periods are statistically significant with ($p < 0.0001$).

4.4.3.1.1 Ultrasound pictures 0 days post-operation

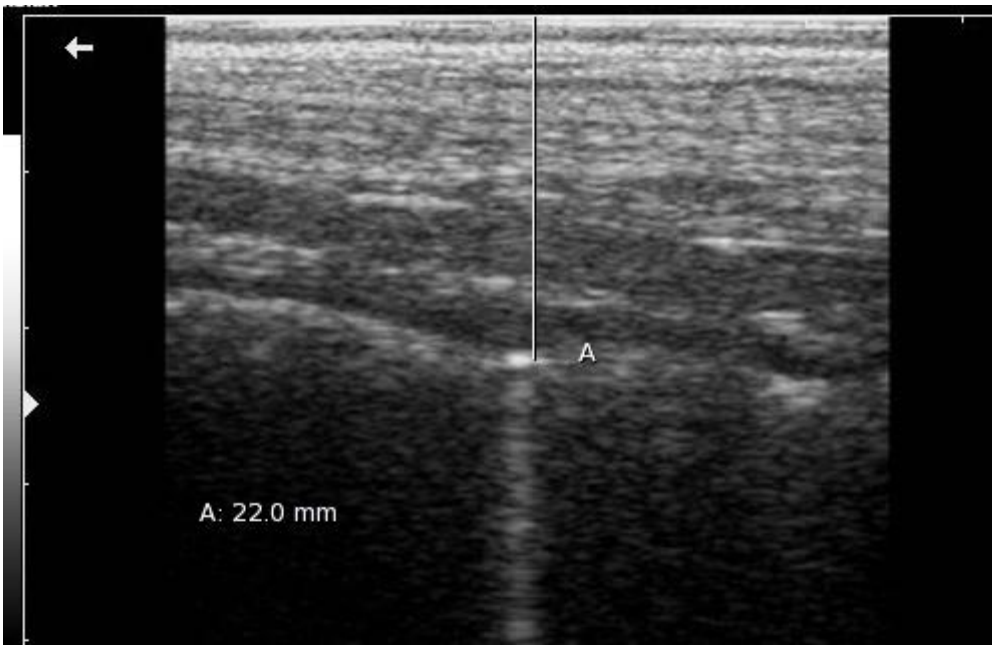


Figure 49: A sagittal ultrasound picture on day 0 post-operation (patient number 4), presenting the characteristic feature of a comet-tail shadowing artifact of the toggle-pin, which shows the position of the toggle-pin (sagittal section) in the abomasum as between the abomasum and the abdominal wall. A linear transducer 5 MHz was used. A: the distance between the abdominal wall and the toggle-pin.

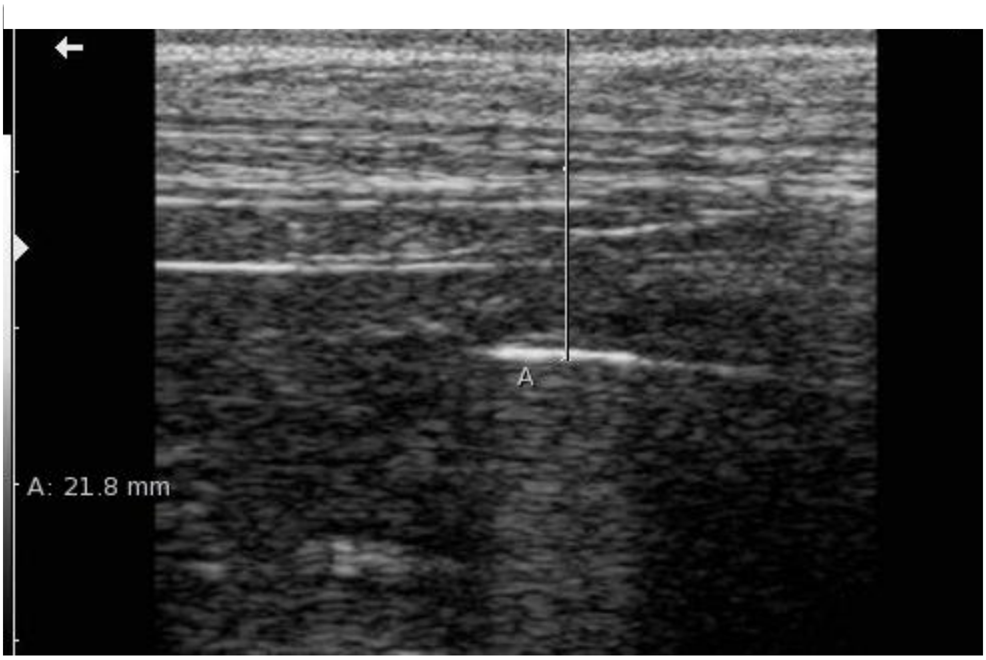


Figure 50: A transverse ultrasound picture on day 0 post-operation (patient number 8), presenting the characteristic feature of a comet-tail shadowing artifact of the toggle-pin, which shows the position of the toggle-pin (transversal section) in the abomasum as between the abomasum and abdominal wall. A linear transducer 5 MHz was used. A: the distance between the abdominal wall and the toggle-pin.

4.4.3.1.2 Ultrasound pictures 4 weeks post-operation

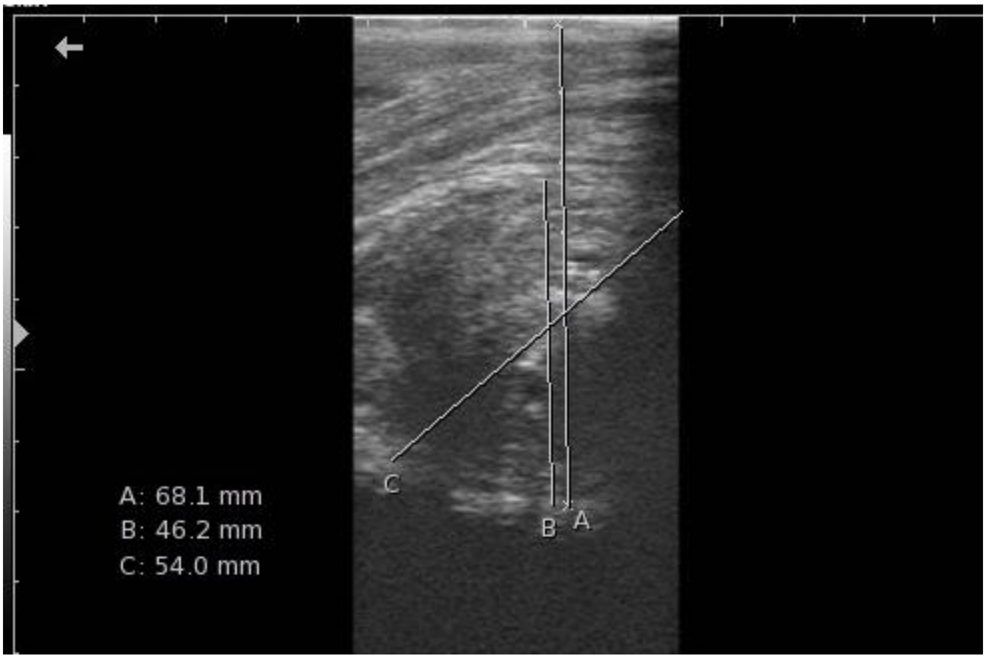


Figure 51: A longitudinal ultrasound picture (cranial-caudal) 4 weeks post-operation (patient number 2), presenting the toggle-pin surrounded by a slightly hypo-echoic tissue (moderately heterogenic tissue); the appearance of the toggle-pin is as before. A linear transducer 5 MHz was used. A: the distance between the abdominal wall and the toggle-pin; B: adhesion depth; C: adhesion length.

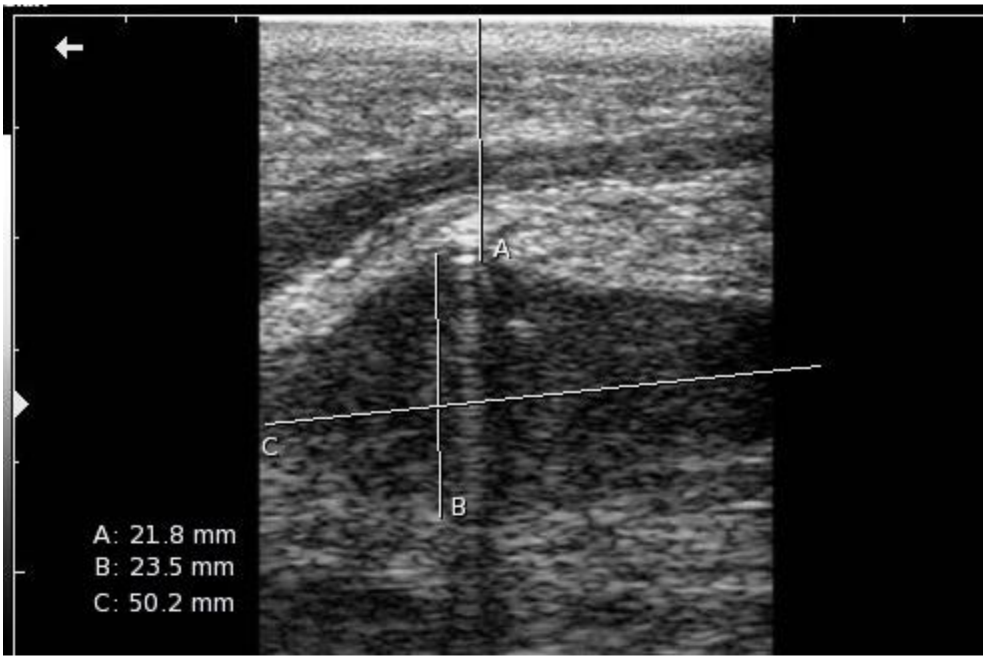


Figure 52: A longitudinal ultrasound picture 4 weeks post-operation (patient number 26), presenting the toggle-pin surrounded by a slightly hypo-echoic tissue (moderately heterogenic tissue); the appearance of the toggle-pin is as before. The abomasal wall also can be seen clearly and slightly elevated in the spot where the toggle-pin was placed. A linear transducer 5 MHz was used. A: the distance between the abdominal wall and the toggle-pin; B: adhesion depth; C: adhesion length (cranial-caudal).

4.4.3.1.3 Ultrasound pictures 90 days post-operation

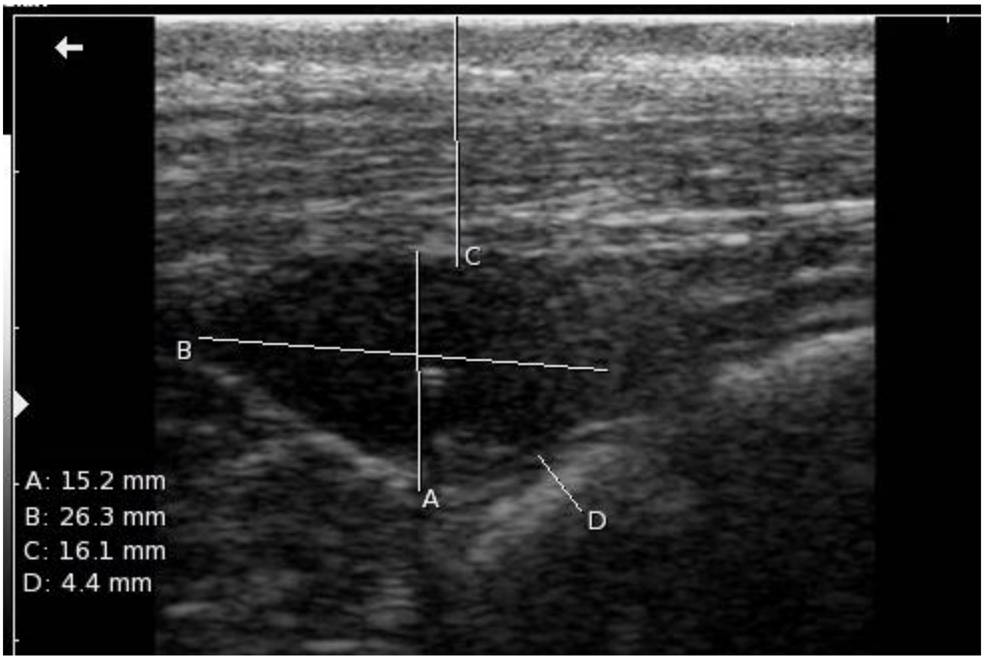


Figure 53: A longitudinal ultrasound picture on day 90 post-operation (patient number 6), presenting the site of adhesion which appears as a well-defined slightly heterogeneous hypo-echoic mass visible between the abomasum and the abdominal wall. The picture shows a round-shaped adhesion. A linear transducer 5 MHz was used. A: adhesion depth; B: adhesion length (cranial-caudal); C: the distance between the abdominal wall and the toggle-pin, D: the abomasum wall.

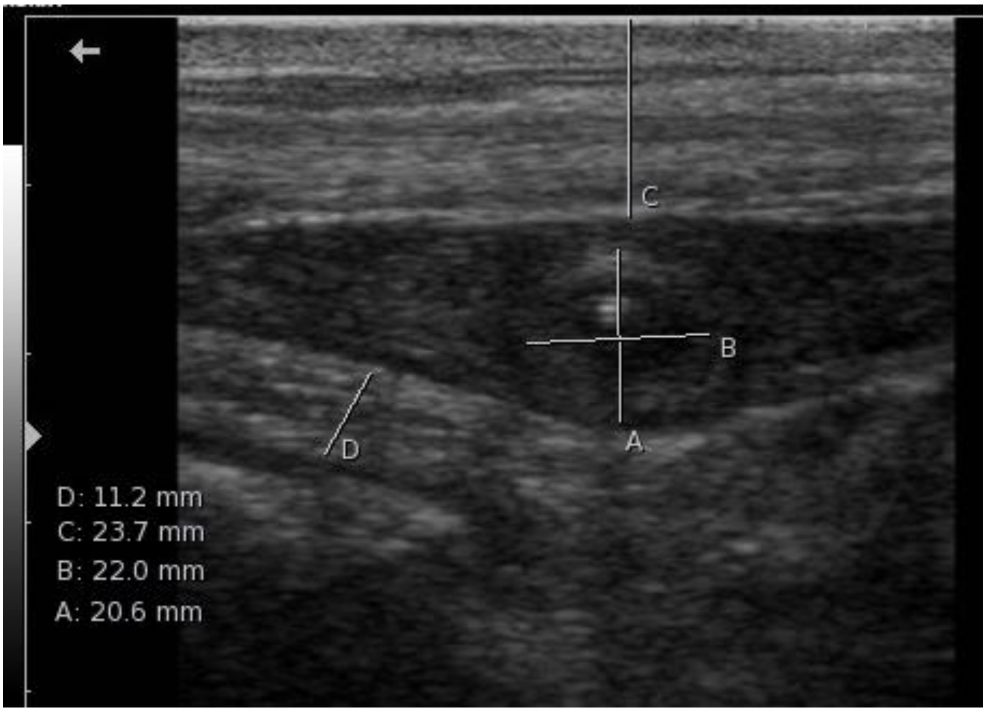


Figure 54: A longitudinal ultrasound picture on day 90 post-operation (patient number 14), presenting the site of adhesion appears which as a well-defined slightly heterogeneous hypo-echoic mass visible between the abomasum and the abdominal wall. The picture shows an oval-shaped adhesion. A linear transducer 5 MHz was used. A: adhesion depth; B: adhesion length (cranial-caudal); C: the distance between the abdominal wall and the toggle-pin; D: the abomasum wall.

4.4.3.1.4 Ultrasound pictures 180 days post-operation

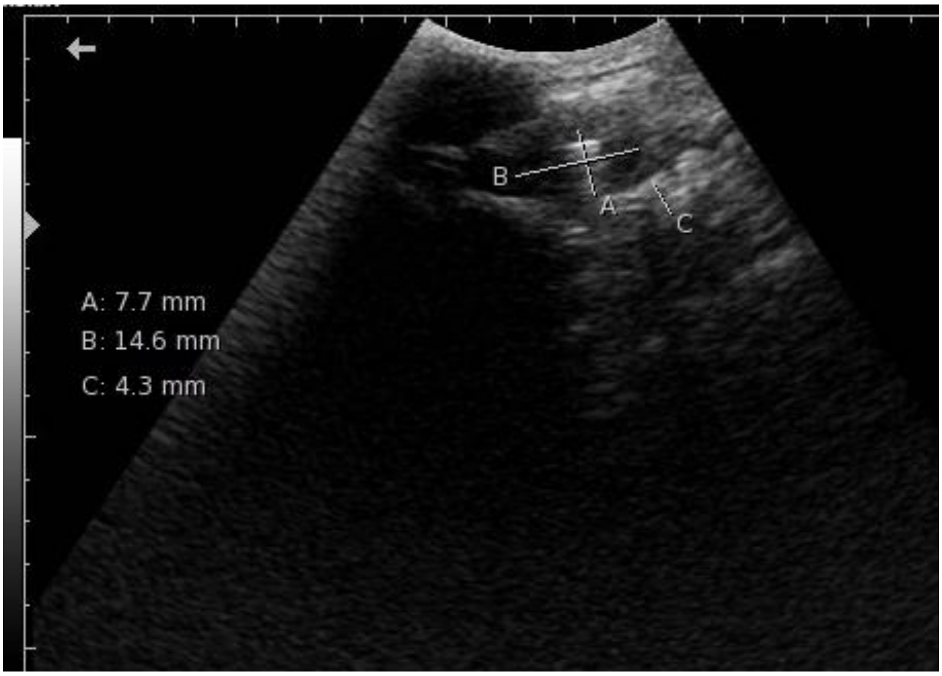


Figure 55: A transverse ultrasound picture on day 180 post-operation (patient number 18), presenting the site of adhesion which is relatively homogenous and hypo-echoic and is decreasing in size compared to the previous examination. The picture shows a focal center of calcification. A convex transducer 3.5 MHz was used. A: adhesion depth; B: adhesion length (lateral-medial); C: the abomasum wall.

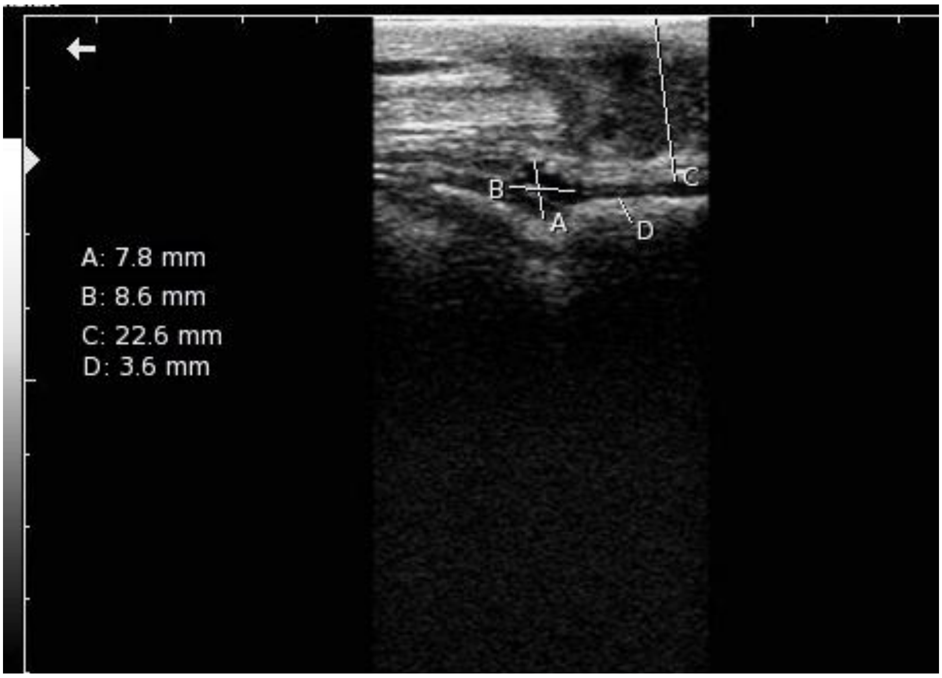


Figure 56: A transverse ultrasound picture on day 180 post-operation (patient number 14), presenting the site of adhesion which is relatively homogenous and hypo-echoic and is decreasing in size compared to the previous examination. The picture shows the adhesion without a focal center of calcification. A linear transducer 7.5 MHz was used. A: adhesion depth; B: adhesion length (lateral-medial); C: the distance between the abdominal and the abomasum walls; D: the abomasum wall.

4.4.3.1.5 Ultrasound pictures 270 days post-operation

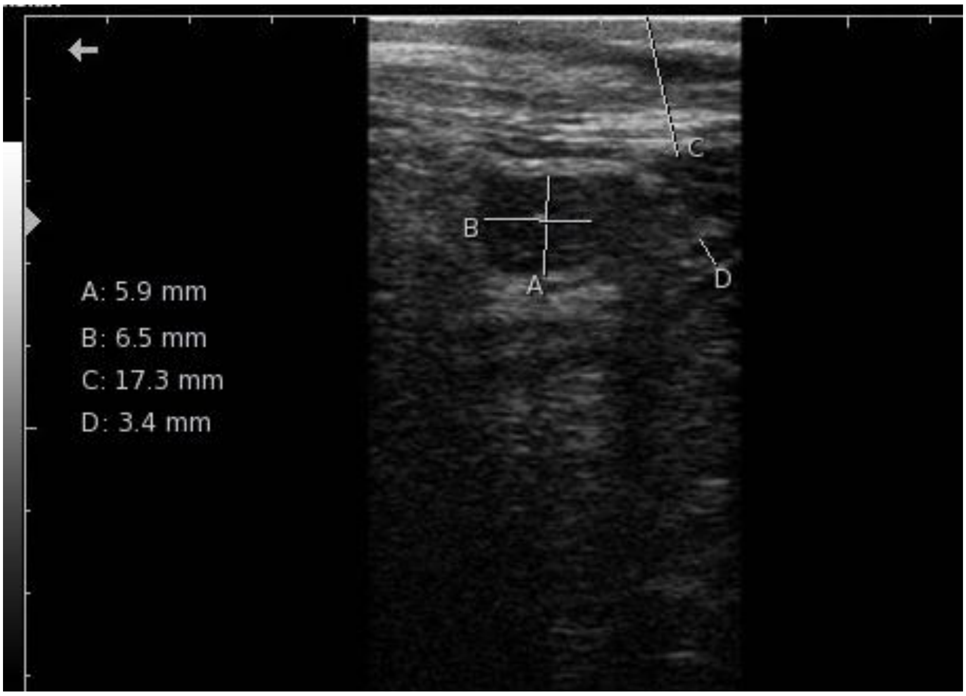


Figure 57: A longitudinal ultrasound picture on day 270 post-operation (patient number 2), presenting the adhesion which is hyper-echoic and severely decreased in size. In the center a hyper-echoic linear structure is visible, which might represent a fibrous tissue or calcification. A linear transducer 5 MHz was used. A: adhesion depth; B: adhesion length (cranial-caudal); C: the abdominal wall; D: the abomasum wall.

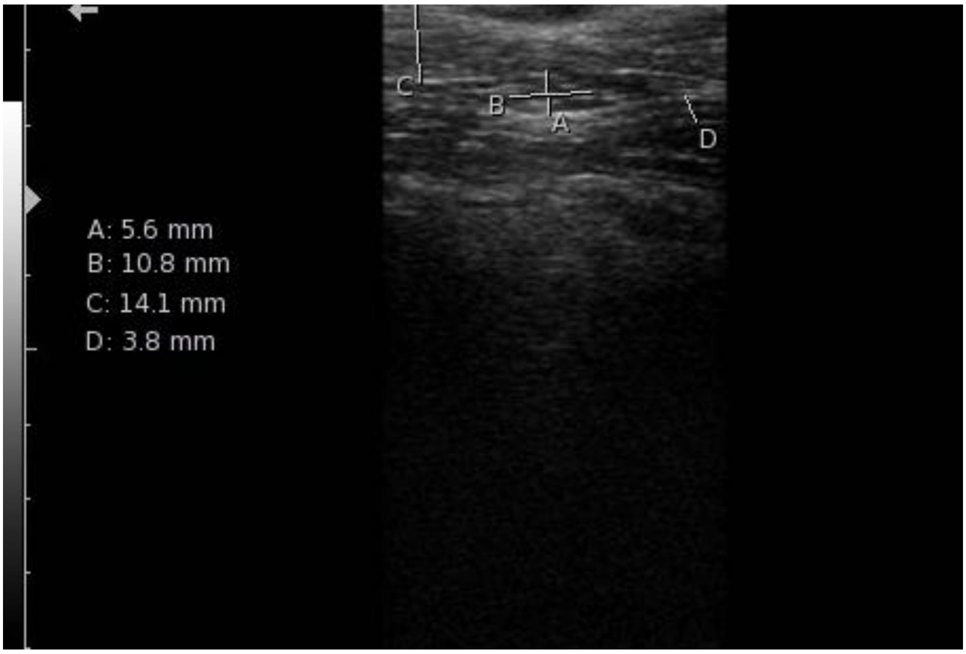


Figure 58: A longitudinal ultrasound picture on day 270 post-operation (patient number 33), presenting the adhesion which is hyper-echoic and severely decreased in size. In comparison with the pictures taken in the same examination time period to these with calcification in the center; these shows bigger in size rather than these without it. A linear transducer 5 MHz was used. A: adhesion depth; B: adhesion length (cranial-caudal); C: the abdominal wall; D: the abomasum wall.

4.4.3.1.6 Ultrasound pictures 360 days post-operation

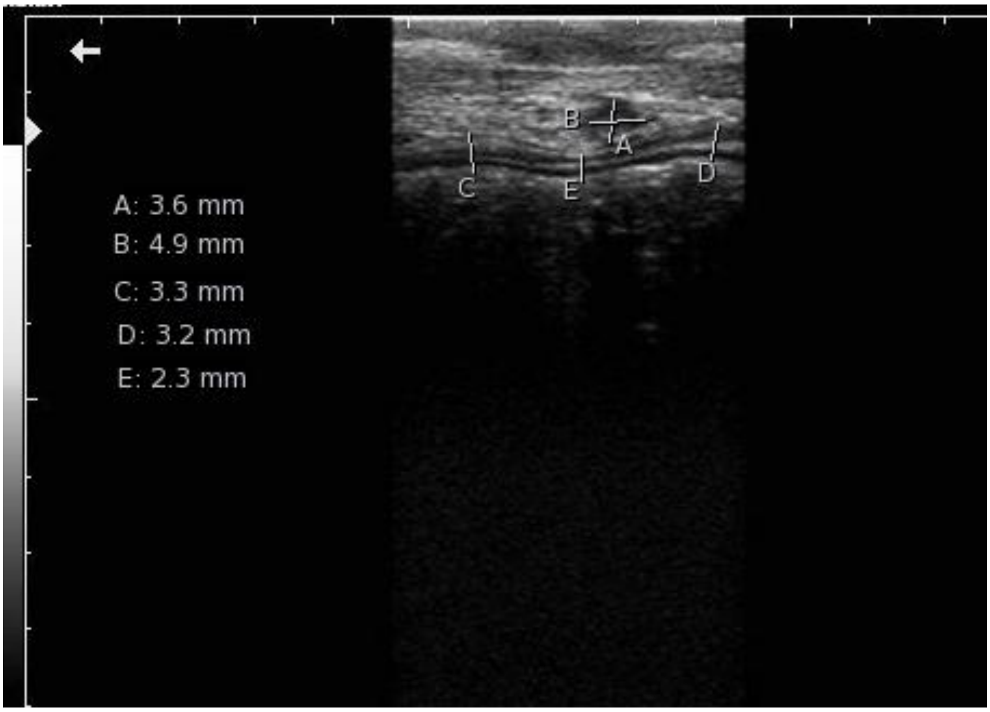


Figure 59: A longitudinal ultrasound picture on day 360 post-operation (patient number 14), presenting the adhesion site which has further decreased in size; the subcutaneous tissue shows mild distortion of the layers. A linear transducer 5 MHz was used. A: adhesion depth, B: adhesion length (cranial-caudal); C: the abomasum wall (cranial to the adhesion); D: the abomasum wall (caudal to the adhesion); E: the abomasum wall adhesion.

Figure 60: A longitudinal ultrasound picture on day 360 post-operation (patient number 20), presenting the adhesion site which has further decreased in size; the subcutaneous tissue shows mild-to-intense distortion of the layers. A linear transducer 5 MHz was used. A: adhesion depth; B: adhesion length (cranial-caudal); C: the abomasum wall.

4.4.3.1.7 Ultrasound pictures 450 days post-operation

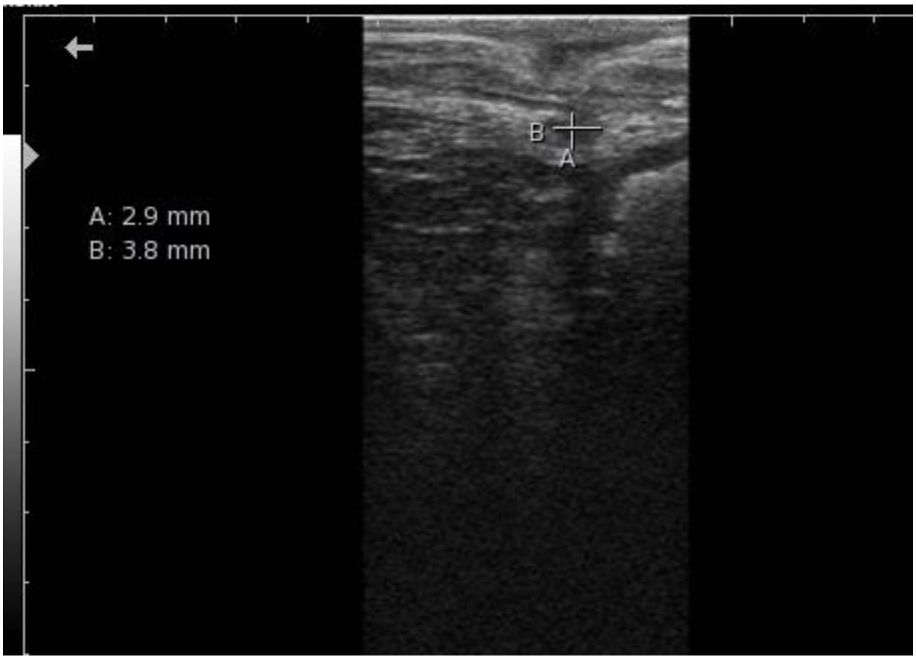


Figure 61: A longitudinal ultrasound picture on day 450 post-operation (patient number 20), presenting the adhesion site which is barely visible as a hyper-echoic area; the subcutaneous tissue shows mild distortion of the layers. A linear transducer 5 MHz was used. A: adhesion depth; B: adhesion length (cranial-caudal).

Detailed data are categorized in the appendices. The data shows that the adhesion measurements and events during the time periods which were included in this study can be summarized as: the peak value is seen four weeks post-operation and then decreases over time, but it has been noticed that the values of the adhesion radically shrink six months after the operation and most of the adhesions fade away nine months post-operation.

4.4.3.2 The results at the fixation adhesion area by examination time period

4.4.3.2.1 The first examination: day 0

At this time the fixation of the abomasum with the abdominal wall began with (n=35).

4.4.3.2.2 The second examination: four weeks after the abomasopexy operation

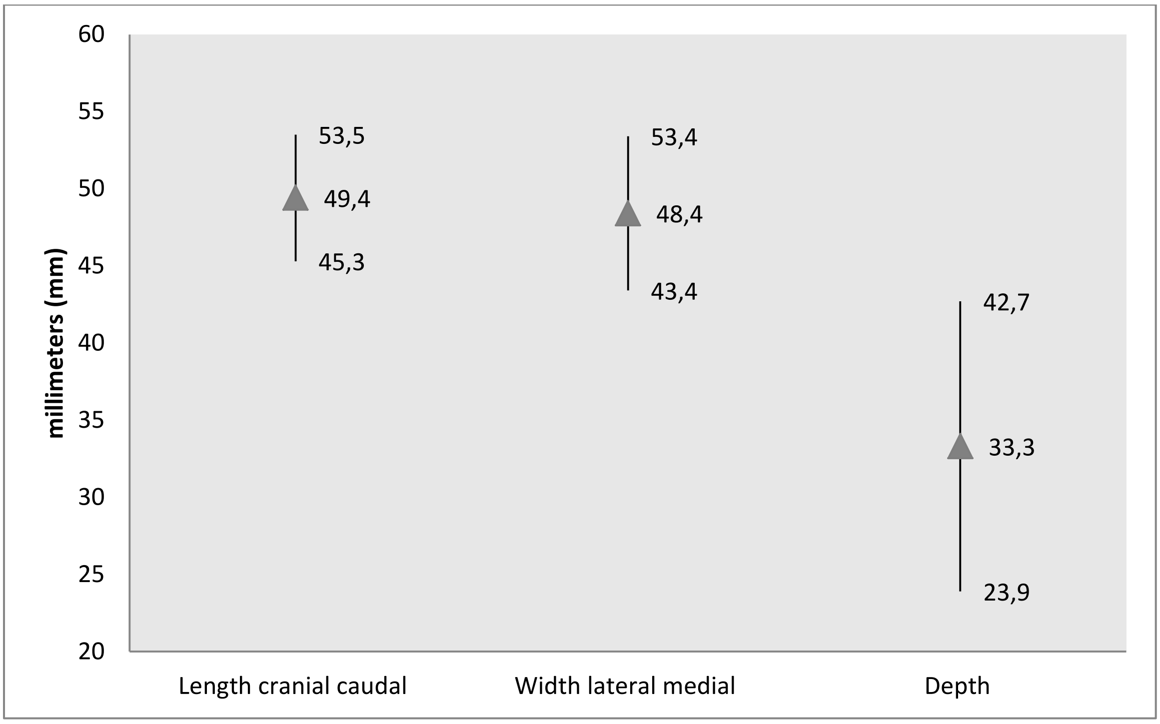


Figure 62: Adhesions after four weeks in the fixation area in millimeters (mm) (▲$\bar{x}$ ± SD).

Four weeks post-operation the total number of animals was 32 (n=32). Here are the results of the three aspects of examination.

First aspect: length (cranial-caudal) of a total of 32 animals with adhesions and the maximum and minimum values are 40.9 mm and 57.1 mm (49.4 ± 4.1 mm) $\bar{x}$±SD.

Second aspect: width (lateral-medial) of a total of 22 animals with adhesions and the maximum and minimum values are 32.6 mm and 56.2 mm (48.4 ± 5.0 mm) $\bar{x}$±SD.

Third aspect: depth of a total of 22 animals with adhesions and the maximum and minimum values are 21.9 mm and 50.1 mm (33.3 ± 9.4 mm) $\bar{x}$±SD.

4.4.3.2.3. The third examination: 90 days post-operation

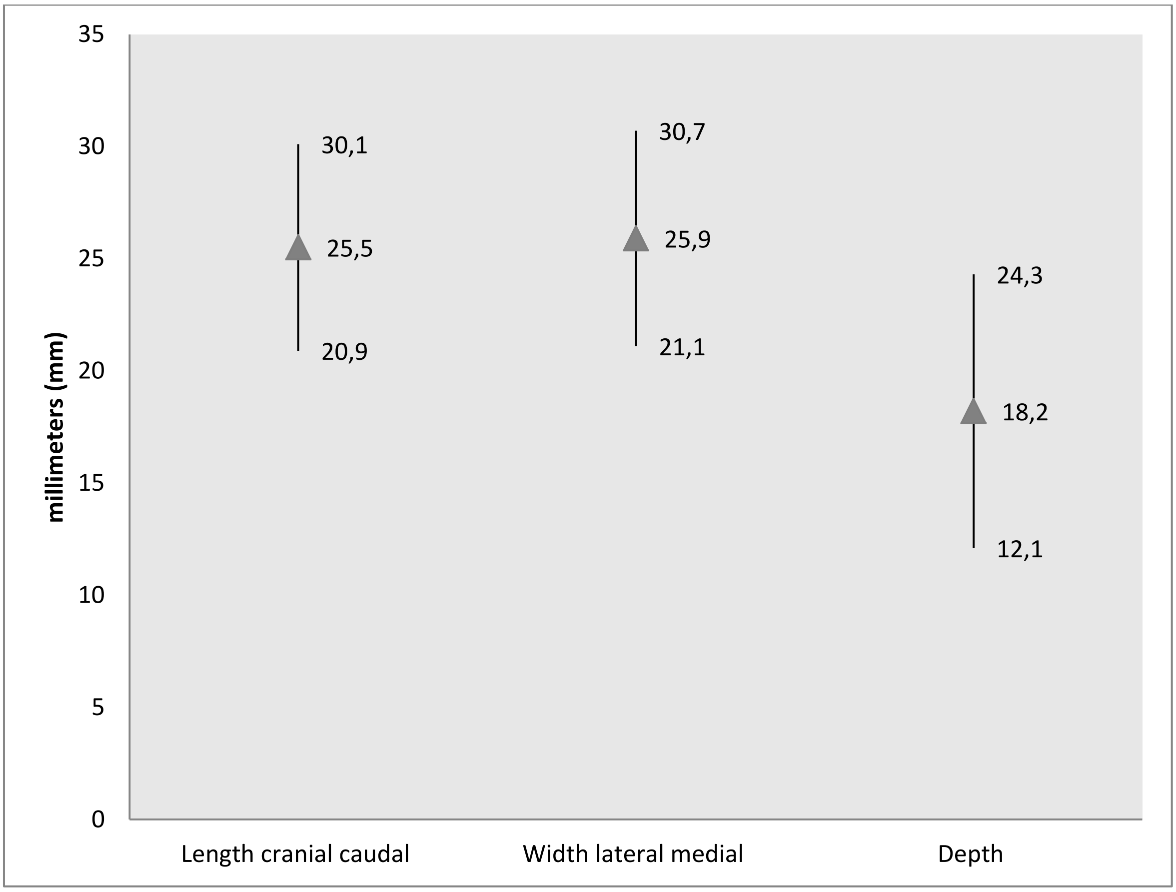


Figure 63: Adhesions after 90 days in the fixation area in millimeters (mm) (▲$\bar{x}$ ± SD).

Ninety days post-operation the total number of animals was 30 (n=30). Here are the results of the three aspects of examination.

First aspect: length (cranial-caudal) of a total of 30 animals with adhesions and the maximum and minimum values are 16.2 mm and 37.4 mm (25.5 ± 4.6 mm) $\bar{x}$±SD.

Second aspect: width (lateral-medial) of a total of 30 animals with adhesions and the maximum and minimum values are 15.8 mm and 35.2 mm (25.9 ± 4.8 mm) $\bar{x}$±SD.

Third aspect: depth of a total of 30 animals with adhesions and the maximum and minimum values are 10.4 mm and 34.2 mm (18.2 ± 6.1 mm) $\bar{x}$±SD.

Detailed data are categorized in the appendices.

4.4.3.2.4 The fourth examination: 180 days after the laparoscopic abomasopexy

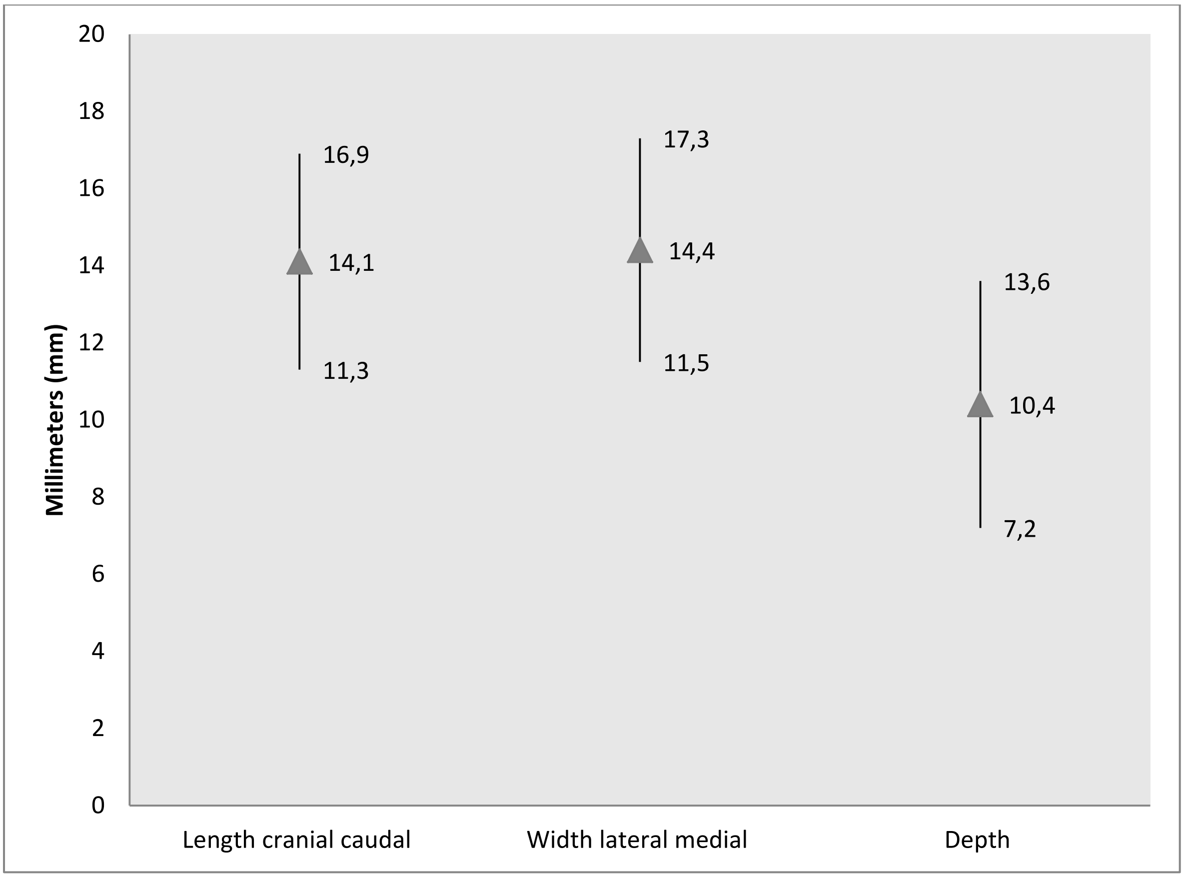


Figure 64: Adhesions after 180 days in the fixation area in millimeters (mm) (▲$\bar{x}$ ± SD).

One hundred and eighty days post-operation the total number of animals was 28. Here are the results of the three aspects of examination.

First aspect: length (cranial-caudal) of a total of 25 (n=25) animals with adhesions out of 28 and the maximum and minimum values are 7.7 mm and 19.3 mm (14.1 ± 2.8 mm) $\bar{x}$±SD.

Second aspect: width (lateral-medial) of a total of 25 animals with adhesions out of 28 and the maximum and minimum values are 8.2 mm and 20.5 mm (14.4 ± 2.9 mm) $\bar{x}$±SD.

Third aspect: depth of a total of 25 animals with adhesions out of 28 and the maximum and minimum values are 5.4 mm and 16.8 mm (10.4 ± 3.2 mm) $\bar{x}$±SD.

4.4.3.2.5 The fifth examination: 270 days post-operation

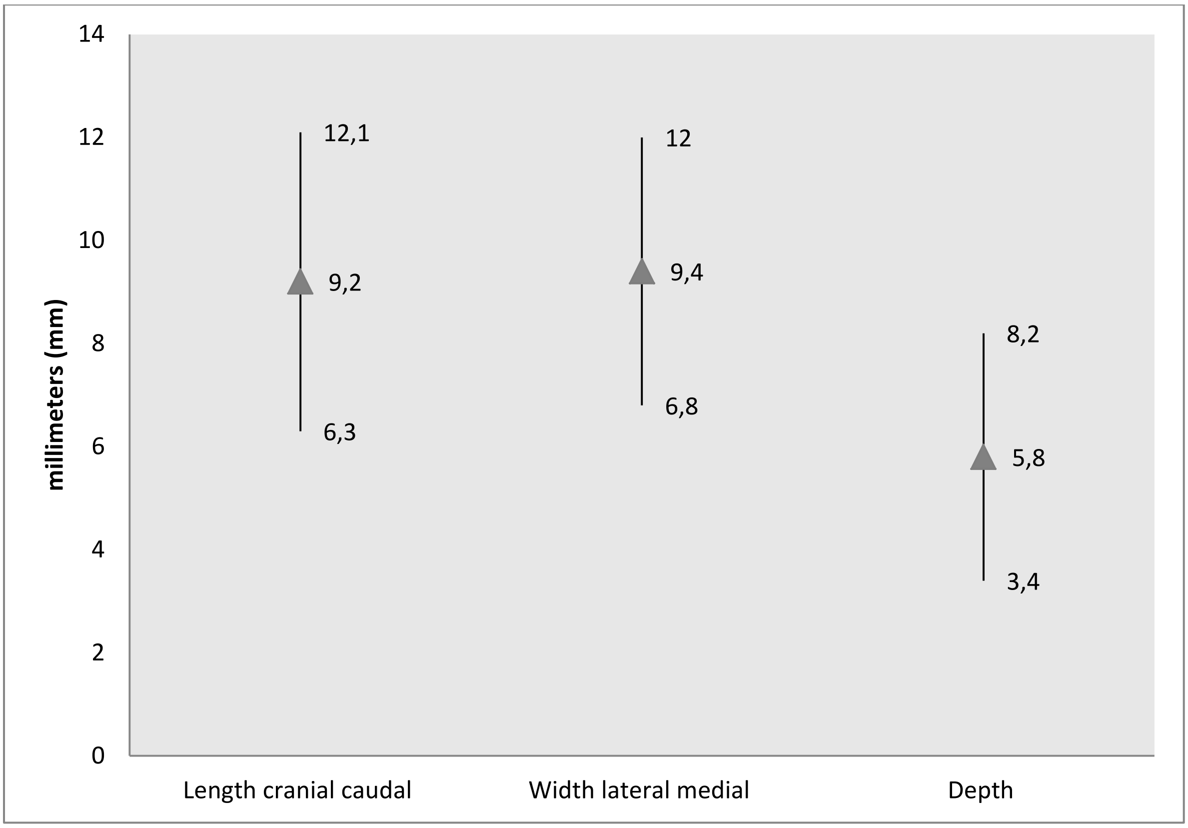


Figure 65: Adhesions after 270 days in the fixation area in millimeters (mm) (▲$\bar{x}$ ± SD).

Two hundred and seventy days post-operation the total number of animals was 26. Here are the results of the three aspects of examination.

First aspect: length (cranial-caudal) of a total of 22 (n=22) animals with adhesions out of 26 and the maximum and minimum values are 4.3 mm and 15.9 mm (9.2 ± 2.9 mm) $\bar{x}$±SD.

Second aspect: width (lateral-medial) of a total of 22 animals with adhesions out of 26 and the maximum and minimum values are 4.5 mm and 15.3 mm (9.4 ± 2.6 mm) $\bar{x}$±SD.

Third aspect: depth of a total of 22 animals with adhesions out of 26 and the maximum and minimum values are 2.9 mm and 11.1 mm (5.8 ± 2.4 mm) $\bar{x}$±SD.

4.4.3.2.6 The sixth examination: 360 days after the toggle-pin abomasopexy operation

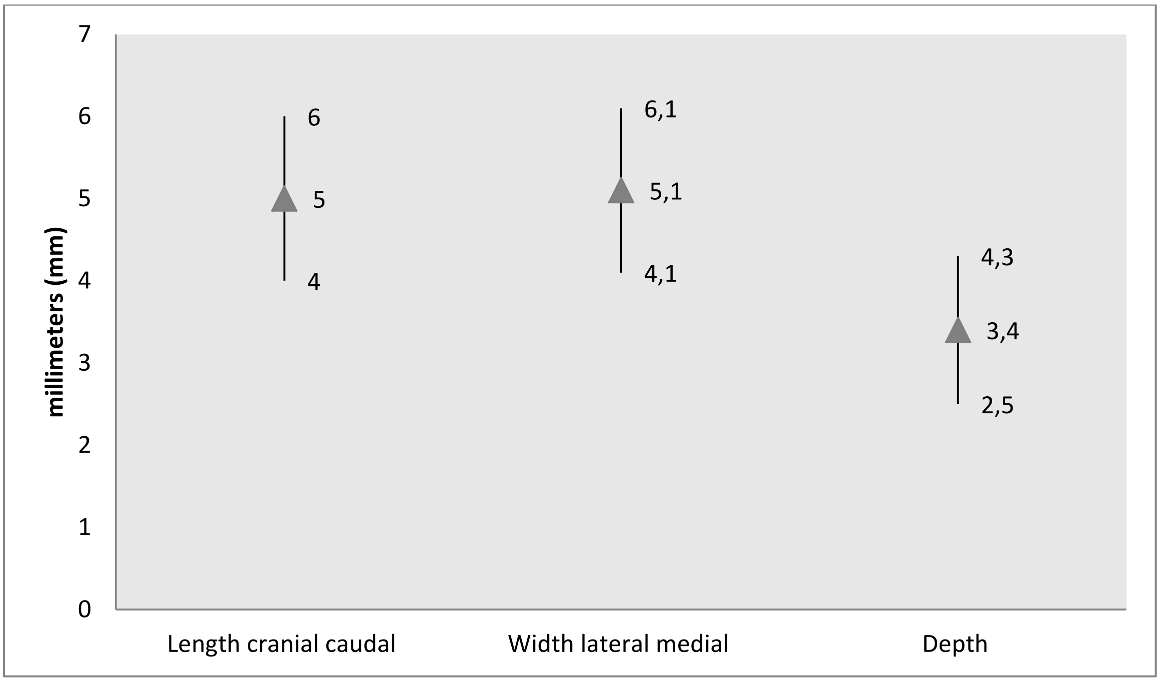


Figure 66: Adhesions after 360 days in the fixation area in millimeters (mm) (▲$\bar{x}$ ± SD).

Three hundred and sixty days post-operation the total number of animals was 25. Here are the results of the three aspects of examination.

First aspect: length (cranial-caudal) of a total of 10 (n=10) animals with adhesions out of 25 and the maximum and minimum values are 3.8 mm and 7.3 mm (5.0 ± 1.0 mm) $\bar{x}$±SD.

Second aspect: width (lateral-medial) of a total of 10 animals with adhesions out of 25 and the maximum and minimum values are 3.8 mm and 7.9 mm (5.1 ± 1.0 mm) $\bar{x}$±SD.

Third aspect: depth of a total of 10 animals with adhesions out of 25 and the maximum and minimum values are 2.1 mm and 4.6 mm (3.4 ± 0.9 mm) $\bar{x}$±SD.

4.4.3.2.7 The seventh examination: 450 days post-laparoscopic abomasopexy

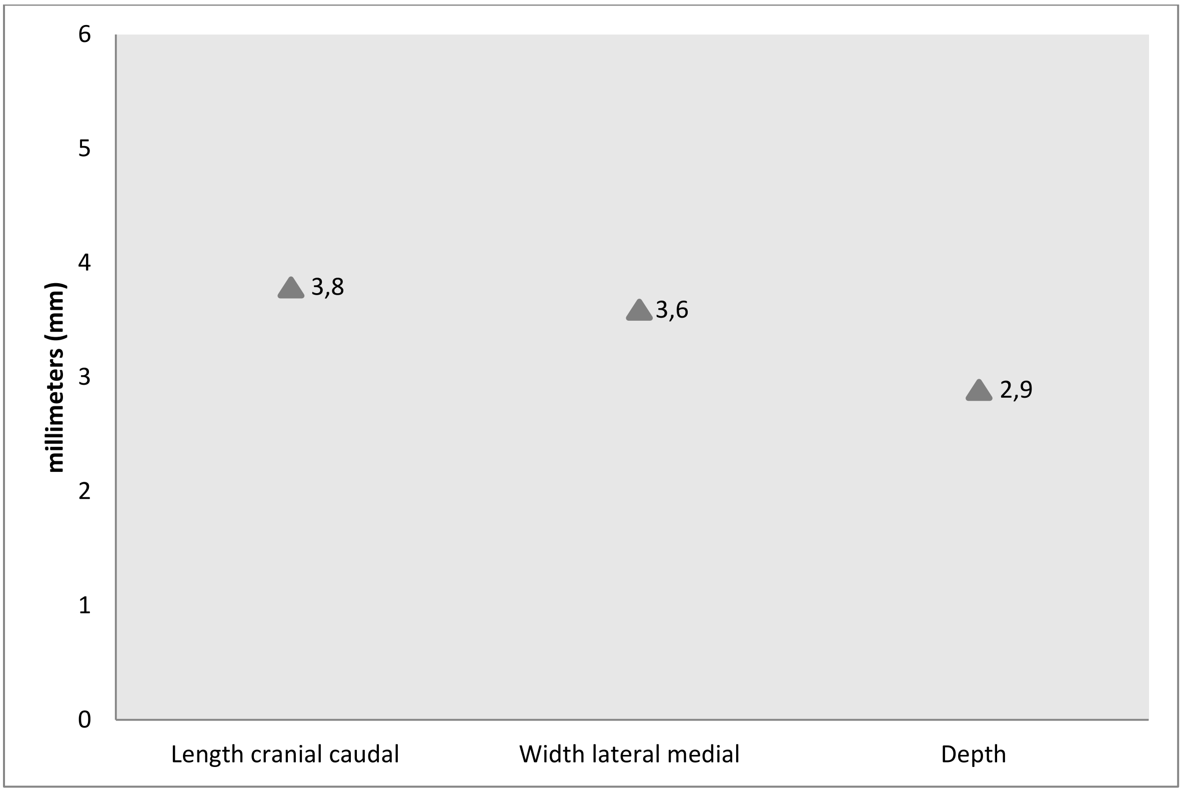


Figure 67: Adhesions after 450 days in the fixation area in millimeters (mm) (▲ $\bar{x}$ ± SD).

Four hundred and fifty days post-operation the total number of animals was 25. Here are the results of the three aspects of examination.

First aspect: length (cranial-caudal) of a total of 1 (n=1) animal with adhesions out of 25 and the maximum and minimum values are 3.8 mm (3.8 ± 0.0 mm) $\bar{x}$±SD.

Second aspect: width (lateral-medial) of a total of 1 animal with adhesions out of 25 and the maximum and minimum values are 3.6 mm (3.6 ± 0.0 mm) $\bar{x}$±SD.

Third aspect: depth of a total of 1 animal with adhesions out of 25 and the maximum and minimum values are 2.9 mm (2.9 ± 0.0 mm) $\bar{x}$±SD.

4.5 Cow survival

The following diagram shows the survival rate of the animals during the study's time periods.

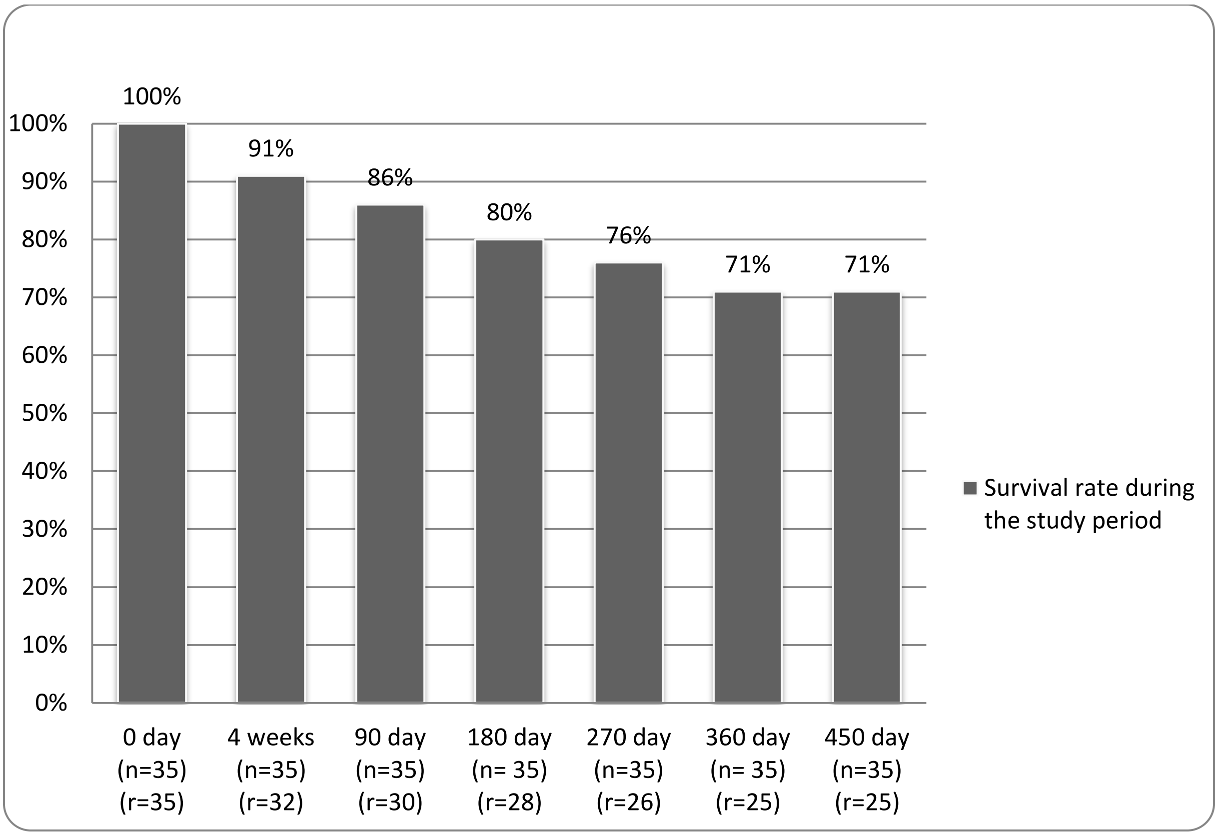


Figure 68: Survival rate among the animals during the study's time periods
(n= X): the total number of animals; (r= X): remaining animals.

The figures show a decrease in the survival rate among the animals treated with laparoscopic abomasopexy from 4 weeks to 360 days post-operation. After four weeks, 3 cows were lost (9%); after 90 days, 5 cows had been lost (14%); after 180 days, 7 cows had been lost (20%); after 270 days, 8 cows had been lost (giving a mortality rate of 24%); and after 360 days and beyond, a total of 10 cows had been lost, putting the final mortality rate at 29%. Causes of death were as follows: 6 cows were slaughtered, 2 died, 1 relapsed and 1 was euthanized. The details are shown in table 10 in the appendices and table 13 shows the history of the animals after the operation.

4.6 Additional ultrasonographic findings

These outcomes were seen via the ultrasonic device at the site of abomasopexy and have been documented.

In one patient, cow number 21, the adhesion is current between the abomasum, the omentum and the abdominal wall as was seen 90 days post-operation; in two cows (numbers 6 and 15) it was shown that the omentum and a blood vessel appearance are located between the abomasum wall and the abdominal wall directly under the abomasopexy site, which was also detected 90 days after the operation.

Another patient, cow number 32, at four weeks post-operation showed, at the site between the toggle-pin and the abdominal wall, a slight deviation in the wound insertion area of about 0.14 mm; hyper-echoic tissue spots indicating an inflammation reaction with shadowing artifacts showed the remaining suture materials between the gauze bandage and the toggle-pin, this patient during the same time period demonstrated that at the adhesion site appeared hyper-echoic spots with reverberation artifacts, and anechoic fluid packets are visible in subcutaneous tissue which appears slightly heterogeneous. The spots most likely represent gas due to infection of the site or can mean that gas has displaced toggle-pin.

A further patient (cow number 17), also in the four week post-operation assessment, illustrates that the abomasum lumen is visible curving away from the transducer, and the toggle-pin is visible surrounded by hypo-echoic tissue and appeared located outside of the abomasum; but nevertheless there is a synchronized movement between the abdominal wall and the abomasum indicating adhesion between the two.

In the center of the adhesion site a hyper-echoic linear can be identified as a calcification center; the abomasum wall under the calcified area is also hyper-echoic and enlarged in size. These findings were seen in cow number 5, 270 days post-operation.

A comet-tail shadowing artifact was seen, giving a “toggle-pin presence indication”, in which the abomasum wall can be identified, which indicates the location of the pin outside the abomasum. This was seen in the 270th day post-operation assessment of cow number 21.

In cow number 18 an ultrasound picture is characterized by an inflamed area which appears like a heterogeneous hypo-echoic area, seen between the layers of the abomasum in the abomasopexy area (the abomasum wall divided into two layers); small spots of a hyper-echoic nature can also be noticed.

The following ultrasound pictures show the special sonographic finding.

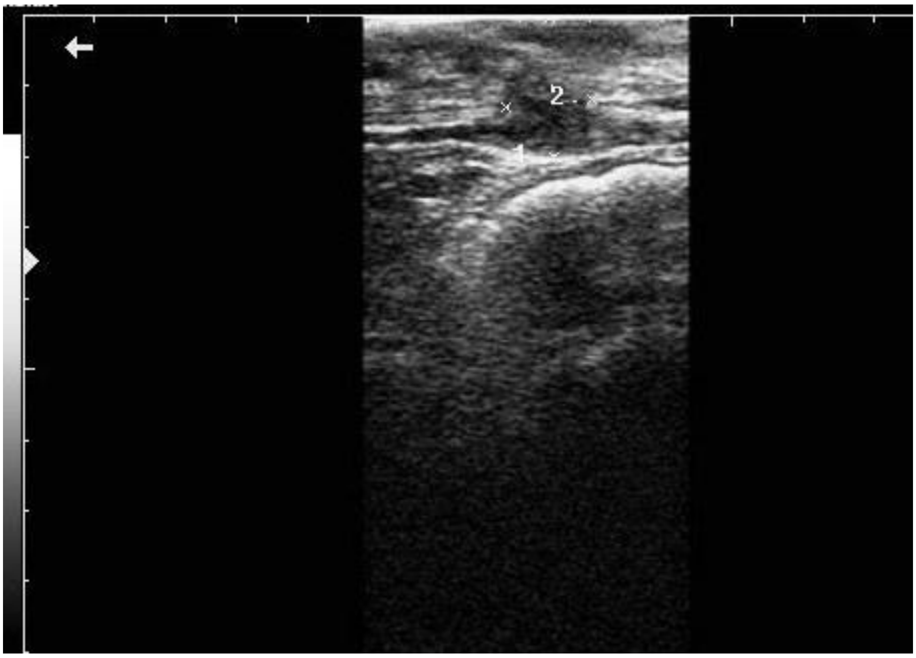


Figure 69: A transverse ultrasound picture on day 90 post-operation. The adhesion is present between the abomasum, the omentum and the abdominal wall (cow number 21). A linear transducer 5 MHz was used.

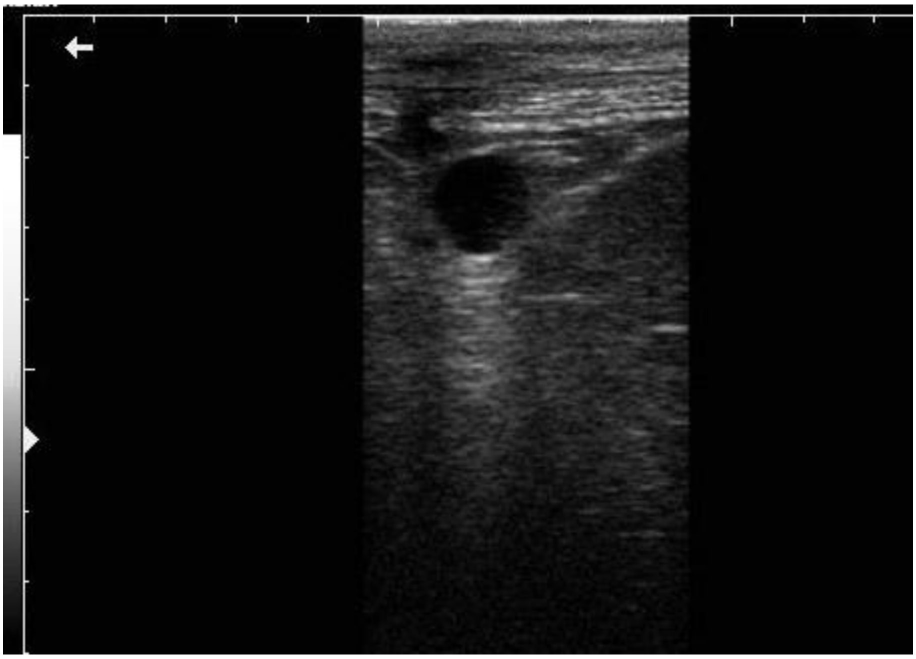


Figure 70: A transverse ultrasound picture on day 90 post-operation. The omentum and a blood vessel appear to be located between the abomasum wall and the abdominal wall directly under the abomasopexy site (cow number 6). A linear transducer 5 MHz was used.

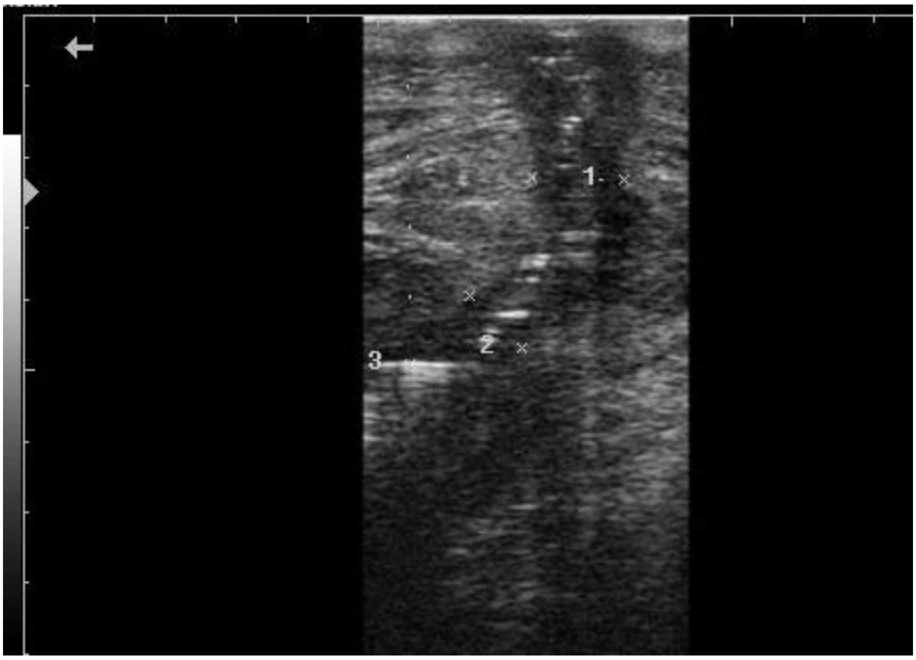


Figure 71: A transverse ultrasound picture on day 28 post-operation (cow number 32). The adhesion site appears as hyper-echoic spots with reverberation artifacts, and anechoic fluid packets are visible in subcutaneous tissue which appears slightly heterogeneous. A slight deviation in the wound insertion area of about 0.14mm and hyper-echoic tissue spots indicate an inflammation reaction with shadowing artifacts showing the remaining suture materials between the gauze bandage and the toggle-pin. A linear transducer 5 MHz was used.

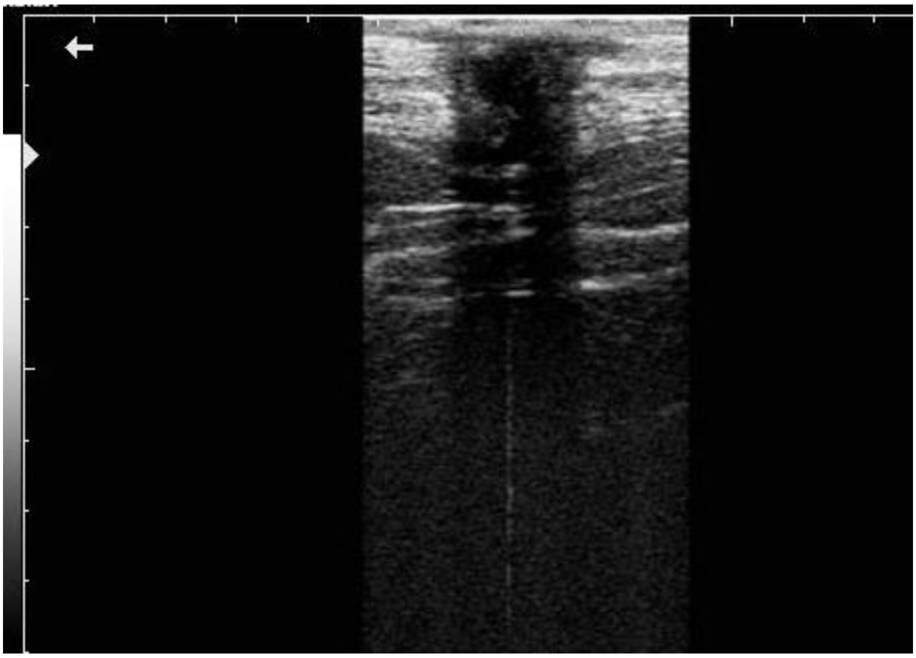


Figure 72: A longitudinal ultrasound picture on day 28 post-operation (cow number 17). The abomasum lumen is visible curving away from transducer, and the toggle-pin is visible surrounded by hypo-echoic tissue and appears to be located outside of the abomasum; but nevertheless there is a synchronize movement between the abdominal wall and the abomasum indicating adhesion between the two. A linear transducer 5 MHz was used.

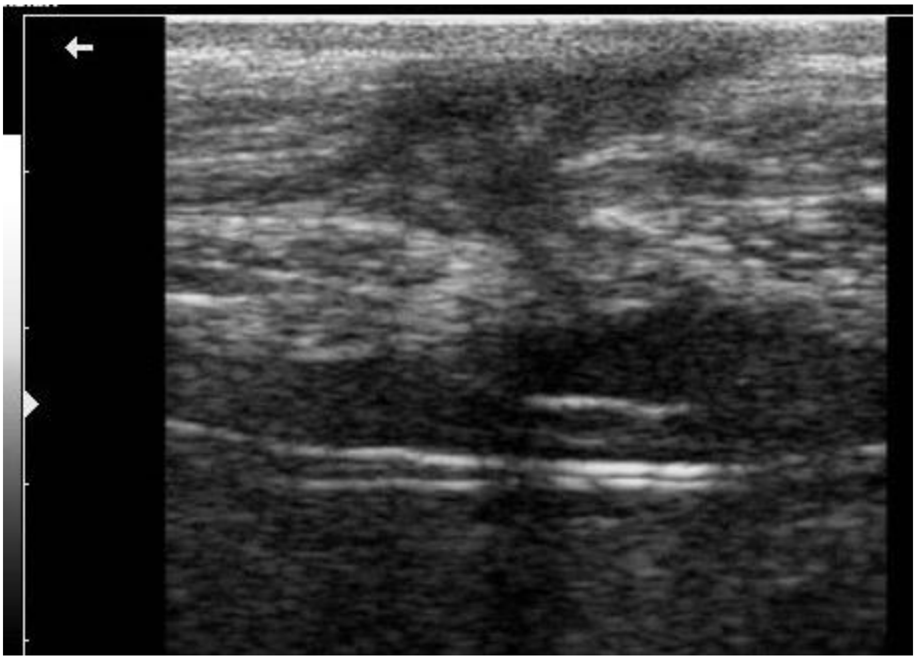


Figure 73: A longitudinal ultrasound picture on day 270 post-operation (cow number 5). In the center of the adhesion site a hyper-echoic linear can be identified as a calcification or a fibrous tissue; the abomasum wall under the calcification area is also hyper-echoic and enlarged in size. A linear transducer 7.5 MHz was used.

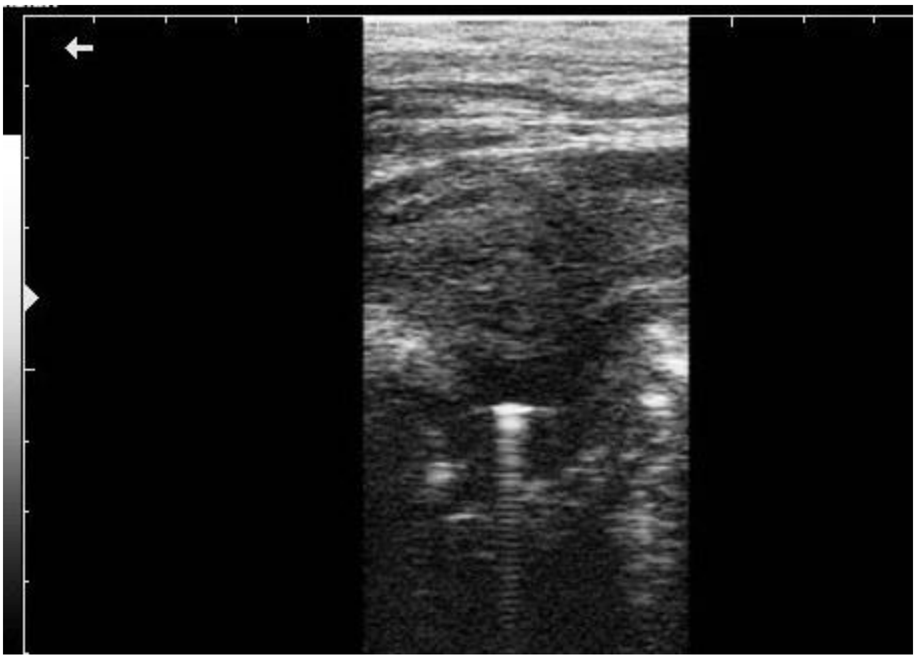


Figure 74: A transverse ultrasound picture on day 270 post-operation (cow number 21). A comet-tail shadowing artifact can be seen that indicates the presence of the toggle-pin. The abomasum wall can be identified, which indicates the location of the pin outside the abomasum. A linear transducer 5 MHz was used.

Figure 75: A transverse ultrasound picture (cow number 18). The inflamed area is characterized as a heterogeneous hypo-echoic area which can be seen between the layers of the abomasum in the abomasopexy area (the abomasum wall divided into two layers); small spots of a hyper-echoic nature can also be noticed. A linear transducer 5 MHz was used.

5 Discussion

5.1 Generals

The ultrasonography examination provided exact information concerning the various developments of fibrous tissue formation. Moreover, ultrasonography made it possible to determine the location and extent of the lesions accurately.

The rapid advances in ultrasound imaging have resulted not only from continual improvements in equipment and new technologies, but also from expanded diagnostic capabilities.

The modern portable ultrasound machines provide the veterinary practitioner with an inexpensive, non-invasive tool with which to examine certain internal structures. These include the viscera that are in contact with the body wall. Transabdominal ultrasonographic examination can investigate: the peritoneal cavity; liver; spleen; rumen; reticulum; abomasum; intestines; and right kidney. Transabdominal ultrasonographic examination is most useful in the diagnosis of focal peritonitis (SCOT 2009).

Ultrasonography as a diagnostic technique provides reliable information concerning the abomasum, its contents and the condition of its wall and should make it possible to identify and assess any changes in adjacent structures (OK et al., 2002).

5.2 Materials and methods and selection of the animal patients

In this work there were 35 cows suffering from left displaced abomasum which were randomly selected for admission to the clinic to be diagnosed and operated on with laparoscopic abomasopexy. To minimize the individual variation between the total number of animals in this study, if a cow suffered from a complication during the abomasopexy they were not then selected as a study animal. Such problems could include: false trocar insertion in the rumen; delayed insertion of the toggle-pin to the abomasum; and finding the toggle-pin suture in the pre-located area without migration around and over the omentum.

5.3 Choice of the laparoscopic abomasopexy operation

In the clinic of ruminants and swine the laparoscopic abomasopexy operation of choice is the Janowitz method (SEEGER and DOLL 2007); in addition, this is also the operation of choice because of its outcomes and results via the help of ultrasonographic imaging devices in this work.

5.4. Procedures of ultrasonography

5.4.1 Procedures of ultrasonographic examination

Preparation of the site of the ultrasonographic investigation involved the hair over the region of the examination (fixation area) being clipped and remaining hair removed; these procedures were also described by BRAUN et al. (1997), OK et al. (2002), and ABDELAAL et al. (2009).

The sonographic examination was carried out in the standing position; this method was also designated by BRAUN et al. (1997), BRAUN (2003) and OK et al. (2002). In another method, for example in operations on dogs, the ultrasonographic evaluation of adhesion after a gastropexy is maintained in the lateral recumbency position (WACKER et al., 1998).

5.4.2 Type and frequency of the ultrasonographic transducer

In this work the best picture quality and best detailed ultrasonographic examination was maintained by 5 MHz, as suggested by WILD (1995); by trying to use the transducer frequency at 10 MHz, the sonographic picture loses its detail and the examinations were not possible because the area between the abdominal wall and the wall of the abomasum (the fixation area) and the adjacent structures and organ were difficult to examine. Also, by this frequency the focal point will go deeper into the tissue and not focus on the fixation area, as confirmed by OK et al. (1997), and BRAUN et al. (1997).

But BRAUN (2004) and BRAUN et al. (2009) suggested that the most effective diagnostic work in bovine ultrasonographic examination could be made by a 3.5 MHz transducer frequency; however, this is for investigating internal diseases and not lesions between the abdominal wall and the abomasum. Since in this work the main object is to determine the abdominal adhesions at the fixation area between the abdominal wall and the abomasum wall, 3.5MHz was not chosen.

The best frequencies of choice for soft tissue are between 5 and 10 MHz as suggested by HOFER (2005).

The use of a linear transducer in this study was also suggested by BRAUN (1997) and BRAUN (2004).

5.5 Wound insertion area

5.5.1 Outcome of the wound insertion area

The wound insertion area was made because of insertion of the trocar into the abdominal wall. This area was used to fix the bandage gauze with its suture material to maintain the required pressure between the toggle-pin and the abdominal wall, to enable the performance of the abomasopexy (JANOWITZ 1998).

As a result of the insertion of the toggle-pin inside the abomasum and the manipulation to find and fix the suture material to the abdominal wall via the gauze bandage, a small amount of leakage of abomasal content into the wound area materialized. This matter could cause the initiation of local peritonitis, thus leading to a wound area infection and adhesions; this is also suggested by GOKCE et al. (2007). That the perforation of the wall of the reticulum allows leakage of ingesta and bacteria, which contaminate the peritoneal cavity, resulting in local or diffuse peritonitis, is also confirmed by ABDELAAL et al. (2009).

The macroscopic appearance of the wound insertion area at the site of fixation in all cows shows severe signs of inflammation; also, the tissue in the location of the gauze bandage was showing signs of degeneration and necrosis. These are caused by two main factors: the first is the pressure that is maintained between the toggle-pin and the abdominal wall, and the second is the movement of the abomasum itself. JANOWITZ (1998) suggested that the free space to be used as a playground for the abomasum’s movement in the toggle-pin suture material is 5 cm; with regard to the macroscopic findings of the skin at the fixation area it has been suggested to increase the free space to more than 5 cm gradually by controlling the outcome of the adhesions (of course without compromising the adhesions between the abomasum and abdominal wall), in order to provide maximum protection against reoccurrence of the abomasum displacement.

One cow shows, 270 days post-operation, an increase in the wound area but after three months the swelling disappeared.

A rapid decrease in skin wound area was also noticed in bulls after making a skin incision to evaluate the influence of certain drugs injected subcutaneously (SARDARI et al., 2007).

5.5.2 The ultrasonographic findings of the wound area

The area showed a formation of inflammatory materials, and in the center of the area, debris of the suture materials can be also identified; inflammation is a normal bodily reaction to the presence of a foreign body. In the aspect values during the examination time periods of 4 weeks, 90, 180, and 360 days, the depth shows up bigger than the length and width within the same examination time periods. This may be due to the presence of the suture materials which, embedded within the wound insertion area, enhance the tissue reaction to a foreign body, as BRAUN et al. (1998) suggested for the presence of a foreign body in cases of traumatic reticuloperitonitis.

At four weeks post-operation the area shows an abscess-like formation and exudation in the wound area in 32 patients; three of them also suffered from necrosis in the wound area. The inflammation and the abscess picture appeared sonographically as described by BRAUN (1998), BRAUN (2005), and ABDELLAAL (2009).

In this study one cow suffered from a hernia formation at the site of the insertion wound, thus indicating the first occurrence of a hernia after a laparoscopic abomasopexy: no literature has been found that mentioned a hernia formation after this kind of operation.

5.6 Outcome of the adhesion area

The adhesion-fibrous tissue area can be characterized and manifested as echogenic deposits and also as echogenic constricting bands or high-level echoes, as is suggested by BRAUN et al. (1998), OMIDI (2008), and SCHMIDT (2007).

The peak of the adhesions in length, width, and depth occurred four weeks post-operation. This could be due to the presence of foreign body materials at the site of the operation (like the toggle-pin and the suture material that holds it and fixes it to the abdominal wall). This result is a normal bodily defense mechanism as a reaction to the presence of foreign body materials, just like in cases of traumatic reticuloperitonitis as suggested by BISCHOFF (1998) and BANI ISMAIL et al. (2007).

Mc GINTY et al. (2005) suggested that a foreign body that attached to the abdominal wall such as a mesh could also cause adhesions in the abdomen of the porcine, or in cases of a foreign body in traumatic reticulo peritonitis as explained by CIHAN et al. (2004).

The decrease in tissue reaction after removing the gauze bandage (after four weeks) was a normal outcome as described GOLDENBERG et al. (2005): in the abdomen, where there is no foreign body, the inflammatory response was minimal to moderate.

The abomasopexy and manipulation in the abdominal cavity (which might also be defined as abdominal operations, foreign materials, or any form of damage of the peritoneal surface) might be causes of adhesions as suggested by GÜMBEL et al. (2005) and ZOGRAFOS et al. (2005).

Experimental injury to the serosal surface of the jejunoileal junction in pony foals caused formation of adhesions at the site of operation (BOURÉ et al. 2002).

Observations of the outcome of the dimensions of the adhesions in correspondence to length, width, and depth decreasing over time show that these could be interpreted as normal outcomes of the adhesions as suggested by ZOGRAFOS et al. (2005).

As the suture materials' pressure continues on the tissue between the abdominal wall and the abomasum and the presence of the wound in the insertion area, this might be a cause of the activation of fibroblast. In this case the persistent activation of fibroblast results in induced proliferation and production of connective tissue constituents such as cytokines, connective tissue growth factor, some interleukins, and others. These factors cause the fibroblasts to alter the matrix environment, which differs from normal connective tissue not only in terms of biochemical compositions, but also in terms of mechanical properties (ECKES 2005).

Fibroblasts' involvement was also noticed in the work of GOLDENBERG et al. (2005) which made a histological analysis to adhesions in rabbits, which also revealed the presence of fibroblast cells in the tissue.

By moving throughout the examination time periods it has been noticed that after the removal of the suture materials (at four weeks) the fixation area decreased in size dramatically but also gradually; this could be interpreted as being due to the fact that the pressure that was maintained between the abdominal wall and the abomasal wall disappeared, and also due to the removal of the foreign body from the operation site as in BRAUN et al. (1998). After removal of a reticular abscess (foreign body) from five cows, all the cows were clinically healthy.

The decrease in the adhesions between the abdominal wall and the intestine were also reported and, via sonography, investigated; the results of the study show that after four months' control of the initial diagnosis of the adhesions, they decreased in size but did not completely disappear (TUCKER et al., 1996).

Four hundred and fifty days post-operation only one cow presented the existence of an adhesion in the abomasopexy fixation area; in returning to the data of the type of adhesion and the presence of adhesion for this cow, it must be observed that there was no center of calcification but that the cow was one of four cows that showed after 360 days evidence of tissue reaction at the wound insertion area; this could be the cause of the persistence of the adhesion at 450 days post-operation.

5.7 Toggle appearance and visibility

The toggle-pin was easy to examine because of its metal structural nature and with distinguishing characteristic such as hyper-echoic area and comet-tail artifact. This feature enabled the ultrasonographic examination to differentiate it from its surrounding tissues and to differentiate from the adhesions which give the same ultrasonographic pictures as metal implants in the musculoskeletal system as suggested by DICKIE (2006).

Sonographic images of the toggle-pin show many changes in location. This might be due to the abomasal movement and also to the tension between the toggle-pin and the gauze bandage.

One cow showed a toggle-pin presence after 270 days (cow number 21). This cow was considered to be one of 10 cows with persistent adhesion at 360 days after the abomasopexy operation, and this could be the reason why the toggle-pin persisted in appearing in that location. However, on the next examination, at 450 days post-operation, the sonographic examination revealed no trace of the toggle-pin at the site.

5.8 Cow survival

The dropping-out causes differ from cow to cow as shown in detail in the appendices; the mortality rate at 360 days post-operation is the same as a rate observed at day 306 of another study which treated 188 cows with toggle-pin abomasopexy sutures, made by RAIZMAN and SANTOS (2002).

Two cows in the present study died suddenly, and unfortunately the owners of the animals neither made any notification nor any pathological examination, merely reporting that they were healthy and then in the morning they were found dead. ROTH and KING (1991) reported on the sudden death of 5 cows out of 50 suffering from traumatic reticulo-peritonitis (TRP); one of them suffered from a ruptured abscess in the abdomen and the other four from chronic active epicarditis and pericarditis, fibrous adhesions of the reticulum to the diaphragm. HAJIGHAHARAMANI and GHANE (2010) also reported one case of sudden death caused by TRP.

5.9 Relapse risks

One cow showed a relapse after three months (3% of a total of 35 cows treated with laparoscopic abomasopexy); the same result was also reported by JANOWITZ (1998) and SEEGER (2005).

In a study made by KÖTTER (2005) to evaluate the laparoscopic abomasopexy method at field level (200 cows), only three cows shows a relapse of a left displaced abomasums after the subsequent calving (KÖTTER 2005).

Another study by MULON et al. (2006), which made a ventral laparoscopic abomasopexy on 18 cows, showed no relapse rate among the treated animals (the study period was 15.5 months).

5.10 Conclusions and suggestions

5.10.1 Conclusions

The ultrasonographic examination can diagnose and measure the adhesions between the abomasum and the abdominal wall.

The ultrasonographic diagnosis of the adhesions between the abomasum wall and the abdominal wall revealed that the laparoscopic abomasopexy can offer 48% protection for cows suffering from left displaced abomasum after 360 days; after that the percentage decreased rapidly, so after 450 days only 4% protection from the left abomasum displacement could be provided. During the study period only one cow showed a relapse after a laparoscopic abomasopexy.

Sixteen cows, after a successful operative laparoscopic abomasopexy, gave birth to sixteen healthy calves without any problems concerning the digestive system.

5.10.2 Suggestions

Some of the cows suffered at the gauze bandage application site mild to extreme signs of inflammation and also signs of pressure and necrosis. By the suggestion of a control study to try different lengths of the suture material between the toggle-pin and the gauze bandage, the normal value that is now used is 5 cm. Along with controlling the outcome of the adhesions between the abomasum and the abomasum wall, this might affect the inflammation signs at the point of pressure and to the whole area of fixation and could manipulate the end effect of the adhesion formation between the abomasum and the abdominal wall.

6 Summary

Objective: The laparoscopic abomasopexy by Janowitz (1998) requires a fibrinous connective tissue adhesion between the abdominal and the abomasal wall to prevent the recurrence of abomasal displacement. After resolution of the inflammatory process, however, these adhesions are organized and receded, much as in the case of traumatic reticuloperitonitis given by BISCHOFF (1998), BROWN et al. (1998), and TUCKER et al. (1996).

This order and regression makes the patients suffer from abomasal displacement which, treated with laparoscopic abomasopexy, is responsible for relapses (see JANOWITZ (1998), SEEGER (2005), and KÖTTER (2005)).

Specific studies on the development of such inflammatory adhesions after laparoscopic abomasopexy have not yet been performed. It was therefore the objective of this study to determine the local findings in the field by using ultrasound to examine the abomasum fixation area in patients suffering from left-side abomasal displacement, which operated according to the Janowitz procedure to the 450th day post-operation.

Materials and methods: Thirty-five German Holstein cows, of a mean age of 4.8 years (2 to 7.4 years) were admitted to the clinic suffering from left-sided abomasal displacement. The operative treatment was carried out according to the endoscopic process described by JANOWITZ (1998).

The sonographic examinations were carried out starting immediately after surgery (day 0), mainly using 5 MHz linear transducer **(**DP-3300 Vet – MINDRAY®[4]**)**. Further investigations followed after four weeks, three months and six months, then at three-month intervals until the disappearance of the adhesions. In each case the abomasum fixation area in transverse and longitudinal directions was investigated.

Results: At day 0 the adhesions were not yet detectable. However, these adhesions were at four weeks and three months after surgery found in all studied patients. Thereafter, the number of animals with detectable adhesions decreased, so that the adhesions between the abdominal and the abomasum wall at six months post-operation were at 89% (25 out of 28 animals); after nine months were at 85% (22 out of 26 animals); and after twelve months were only 40% (10 out of 25 animals) of the patients. Fifteen months after the laparoscopic abomasal fixation only 1 of the 25 remaining study animals (4%) showed adhesions. The toggle-pin visibility was detectable directly and four weeks post-operation. In two animals the

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toggle-pin presented itself after the 90^{th} day; the toggle-pin was detectable ultrasonographically even on the 180^{th} and 270^{th} days post-operation.

In the majority of cases (68%) the shape of the adhesion area was oval; round-shaped adhesion formed in about 26% of cases and the remaining 6% of patients' adhesion shapes was irregular. A calcification in the center of the adhesion was ultrasonographically detected in 59% of the study subjects. During the study period only one cow showed a relapse of abomasum displacement three months post-operation.

Conclusions: Ultrasound examination can recognize and identify the adhesions of an abomasum fixation area. These adhesions reach their maximum four weeks after surgery, then the adhesions continue to fall, although at considerable individual variations. Since the time of next calving is usually about 12 months after surgery, only 40% of the study subjects with ultrasonographically detected fixation (adhesions) were traced to the subsequent lactation term. This was observed in 19 subjects in this study, which suggests that the occurrence of abomasal displacement still must be added to other predisposing factors, which are not given in any lactation.

7 Zusammenfassung

Gegenstand dieser Untersuchungen: Bei der laparoskopischen Abomasopexie nach Janowitz (1998) beruht die Rezidivprophylaxe auf den sich zwischen Bauch- und Labmagenwand ausbildenden fibrinösen Adhäsionen und bindegewebigen Verwachsungen. Nach Abklingen des Entzündungsprozess können diese Verwachsungen jedoch organisiert und zurückgebildet werden, ähnlich wie dies bei der Reticuloperitonitis traumatica der Fall ist (BISCHOFF 1998; BRAUN et al., 1998; TUCKER et al., 1996).
Diese Um- und Rückbildungen werden im Falle der per Abomasopexie behandelten Labmagenverlagerung für das Auftreten von Rezidiven verantwortlich gemacht (JANOWITZ 1998; SEEGER 2005; KÖTTER 2005).
Konkrete Untersuchungen über die Ausbildung, Ausdehnung und Entwicklung solcher entzündlicher Adhäsionen nach laparoskopischer Abomasopexie wurden bislang nicht durchgeführt. Es war daher das Ziel diese Studie, bei Klinikpatienten mit einer nach dem Janowitz-Verfahren operierten linksseitigen Labmagenverlagerung die Entwicklung der lokalen Befunde im Bereich der Abomasopexiestelle anhand regelmäßiger sonographischer Untersuchungen bis zum 450. Tag post operationen zu verfolgen.

Material und Methoden: Bei den 35 Probanden handelte es sich um deutsche Holstein-Kühe, Durchschnittsalter 4,8 Jahre (2 bis 7,4 Jahre), die zur Operation einer linksseitigen Labmagenverlagerung in die Klinik eingeliefert wurden. Die Operation wurde nach dem von Janowitz (1998) beschriebenen endoskopischen Verfahren durchgeführt.
Die sonographischen Untersuchungen wurden, beginnend unmittelbar nach Beendigung der Operation (Tag 0), überwiegend mittels 5 MHz-Linearschallkopf durchgeführt **(**DP-3300 Vet - MINDRAY®[5]**)**. Weitere Untersuchungen folgten 4 Wochen, 3 Monate und 6 Monate nach der Operation, danach in 3-monatigen Abständen bis zum Verschwinden der Adhäsionen. Dabei wurde jeweils der Abomasopexiebereich in Quer- und Längsrichtung untersucht.

Ergebnisse: Zum Zeitpunkt „Tag 0“ waren noch keine Adhäsionen nachweisbar. Jedoch fanden sich diese 4 Wochen und 3 Monate nach der Operation bei allen untersuchten Patienten. Danach nahm die Zahl der Tiere mit nachweisbaren Adhäsionen langsam ab. Zum Zeitpunkt 6 Monate p. op. finden sich solche noch bei 89 % (25 von 28 Tieren), nach 9 Monaten bei 85 % (22 von 26 Tieren) und nach 12 Monaten nur noch bei 40 % (10 von 25 Tieren) der Patienten. 15 Monate nach der Abomasopexie konnte nur noch bei 1 von 25

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verbliebenen Studientieren (4 %) eine Adhäsion zwischen Bauchwand und Labmagen nachgewiesen werden. Das als Gegenlager in den Labmagen eingebrachte Metallstäbchen (toggle pin) war bis 4 Wochen nach der Operation sonographisch nachweisbar. Danach wurde der Toggle-Faden durchtrennt, und so war am 90. Tag p. op. nur noch in zwei Fällen, bei einer dieser beiden Tiere auch noch am 180. und am 270. Tag p. op. der Toggle im Bereich der Abomasopexiestelle zu erkennen. In der Mehrzahl der Fälle (68 %) war die Adhäsionsfläche oval, bei 26 % eher rund und bei den restlichen 6 % der Patienten unregelmäßig geformt. Bei 59 % der Probanden war eine Kalzifizierung im Zentrum der Adhäsionen nachweisbar. Im Untersuchungszeitraum konnte nur in einem Fall ein Rezidiv nachgewiesen werden, und zwar 3 Monate nach der Operation.

Schlussfolgerungen: Mittels Ultraschalluntersuchung lassen sich die Adhäsionen im Bereich der Abomasopexiestelle gut erkennen und beurteilen. Diese Adhäsionen erreichen ihr Maximum 4 Wochen nach der Operation, anschließend bilden sich kontinuierlich zurück, bei allerdings erheblicher individueller Variation**.** Da zum Zeitpunkt der nächsten Kalbung - in der Regel etwa 12 Monate nach der Operation - nur noch bei 40 % der Probanden eine ausreichende Fixation des Labmagens über nachweisbare Adhäsionen sichergestellt sein dürfte, kann sich der Labmagen in der nachfolgenden Laktation wiederum verfolgen. Dies wurde in der vorliegend Studie allerdings bei keinen der zu diesen Zeitpunkt noch vorhandenen 19 Probanden beobachtet, was dafür spricht, dass für das Auftreten einer solchen Labmagenverlagerung noch weitere prädisponierende Faktoren hinzukommen müssen, welche nicht in jeder Laktation gegeben sind.

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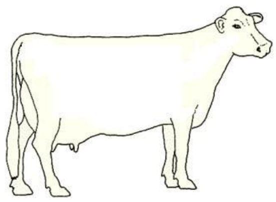
9 Appendices

Examination platform

This is a sample of one of the forms were used in Ultrasound examination in the farms:

Examination platform

Owner information:



Ear mark:

Date Examination						
Cranial- Caudal*						
Lateral- Medial*						
Depth (Thickness)*						
Remarks						

*Corresponds to the fixation

Animal patient's data the observations

Breeds of the animals regarding the place of origin

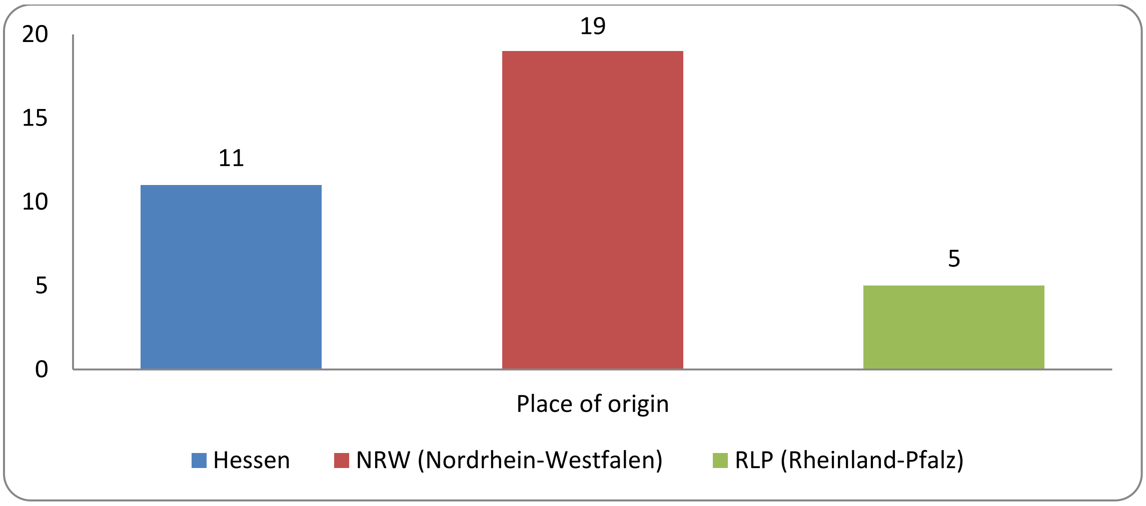


Figure 76: The distribution of the animals regarding to the place of origin

Table 10: Animal patient's data the observations

Animal number	Earmark	Clinic number	Date of birth	Date of operation	Remarks
1	-39535	261	02.03.2002	08.05.2007	Ok
2	-29786	274	16.12.2002	16.05.2007	Ok
3	-43239	307	08.03.2004	31.05.2007	Ok
4	-06669	283	27.02.2002	21.05.2007	Euthanized in 24.09.2007
5	-89662	288	17.11.2004	22.05.2007	Ok
6	-73423	295	20.07.2001	25.05.2007	Ok
7	-23026	303	10.07.2004	30.05.2007	Ok
8	-42787	308	13.07.2002	31.05.2007	Deceased in 30.10.2007
9	-48393	304	17.11.1999	30.05.2007	Slaughter 10.10.2007
10	-83558	301	27.01.2005	29.05.2007	Ok
11	-34539	314	09.12.2004	06.06.2007	Ok
12	-60115	332	06.07.2002	18.06.2007	Ok
13	-55720	341	14.07.2001	22.06.2007	Relapse 18.09.2007
14	-04452	342	15.08.2001	22.06.2007	Ok
15	-08372	359	22.09.2004	28.06.2007	Ok
16	-49133	362	30.06.2002	29.06.2007	Ok
17	-55790	337	07.07.2001	21.06.2007	Ok
18	-08092	343	21.11.2002	22.06.2007	Ok
19	-03679	366	08.12.2001	02.07.2007	Slaughtered 15.07.2007
20	-44060	384	12.01.2001	09.07.2007	Ok
21	-23939	386	19.03.2000	10.07.2007	Ok
22	-24999	394	08.03.2004	15.07.2007	Ok
23	-15474	396	20.01.2001	15.07.2007	Ok
24	-29395	401	15.02.2002	19.07.2007	Slaughtered 25.07.2007
25	-67504	410	13.06.2001	24.07.2007	Ok
26	-89456	417	26.01.2001	30.07.2007	Ok
27	-08123	434	05.10.2003	08.08.2007	Slaughtered 15.04.2008
28	-00336	439	17.09.2002	10.08.2007	Slaughtered 25.08.2007
29	-86000	460	15.01.2004	18.08.2007	Ok
30	-79176	463	22.11.2004	26.08.2007	Ok
31	-35142	465	17.09.2003	26.08.2007	Slaughtered 03.07.2008
32	-42764	488	05.05.2002	29.08.2007	Deceased in 20.02.2008
33	-61885	534	16.07.2003	21.09.2007	Ok
34	-57323	361	20.10.2005	10.10.2007	Ok
35	-51756	574	23.07.2004	25.10.2007	Ok

Table 11: Animal patient’s data –breed, age and weight

Animal number	Earmark	Breed	Clinic number	Age (Year)	Weight (Kg) estimated
1	-39535	SBH	261	5	450
2	-29786	SBH	274	5	550
3	-43239	SBH	307	3	550
4	-06669	SBH	283	5	600
5	-89662	SBH	288	3	550
6	-73423	SBH	295	6	600
7	-23026	RBH	303	3	500
8	-42787	SBH	308	5	650
9	-48393	RBH	304	8	550
10	-83558	RBH	301	2	454
11	-34539	SBH	314	3	650
12	-60115	RBH	332	5	650
13	-55720	SBH	341	6	580
14	-04452	RBH	342	6	650
15	-08372	SBH	359	3	550
16	-49133	SBH	362	5	600
17	-55790	RBH	337	6	600
18	-08092	RBH	343	5	550
19	-03679	RBH	366	5	650
20	-44060	SBH	384	6	600
21	-23939	SBH	386	6	580
22	-24999	SBH	394	7	600
23	-15474	SBH	396	6	600
24	-29395	SBH	401	5	650
25	-67504	SBH	410	5	600
26	-89456	SBH	417	6	600
27	-08123	SBH	434	4	650
28	-00336	SBH	439	5	600
29	-86000	SBH	460	3	600
30	-79176	RBH	463	3	530
31	-35142	SBH	465	3	600
32	-42764	SBH	488	4	500
33	-61885	RBH	534	5	600
34	-57323	SBH	361	2	500
35	-51756	SBH	574	3	550
SBH: 25 cow, RBH:10			Mean	4,628571	579,8285714
SBH (Schwarzbunte Holstein):Black Holsteins , RBH (Rotbunte Holstein) Red Holsteins					

Table 12: Animal patient's data –case history

Animal number	Earmark	Clinic number	Date of operation	Case history (remarks)
1	-39535	261	08.05.2007	Calved 4 days before
2	-29786	274	16.05.2007	Calved 5 days before
3	-43239	307	31.05.2007	Calved 5 days before
4	-06669	283	21.05.2007	Calved 14 days before
5	-89662	288	22.05.2007	Calved 30 days before
6	-73423	295	25.05.2007	Calved 16 days before (twins)
7	-23026	303	30.05.2007	Pregnant: 4.5 months
8	-42787	308	31.05.2007	Calved 7 days before
9	-48393	304	30.05.2007	Calved 8 days before
10	-83558	301	29.05.2007	Calved 23 days before
11	-34539	314	06.06.2007	Calved 10 days before
12	-60115	332	18.06.2007	Calved 16 days before
13	-55720	341	22.06.2007	Calved 30 days before
14	-04452	342	22.06.2007	Calved 25 days before
15	-08372	359	28.06.2007	Calved 28 days before
16	-49133	362	29.06.2007	Calved 14 days before
17	-55790	337	21.06.2007	Calved 14 days before (retained placenta)
18	-08092	343	22.06.2007	Calved 35 days before (heavy calf)
19	-03679	366	02.07.2007	Calved 10 days before
20	-44060	384	09.07.2007	Calved 18 days before
21	-23939	386	10.07.2007	Calved 14 days before
22	-24999	394	15.07.2007	Calved 7 days before
23	-15474	396	15.07.2007	Calved 4 days before
24	-29395	401	19.07.2007	Calved 14 days before
25	-67504	410	24.07.2007	Calved 12 days before (twins)
26	-89456	417	30.07.2007	Calved 14 days before
27	-08123	434	08.08.2007	Calved 12 days before
28	-00336	439	10.08.2007	Calved 4 days before
29	-86000	460	18.08.2007	Pregnant: 5 months
30	-79176	463	26.08.2007	Calved 10 days before
31	-35142	465	26.08.2007	Calved 7 days before
32	-42764	488	29.08.2007	Calved 11 days before
33	-61885	534	21.09.2007	Calved 10 days before
34	-57323	361	10.10.2007	Calved 14 days before
35	-51756	574	25.10.2007	Calved 14 days before

Calving: 33 cows, values varies between 4 - 35 days.

Pregnant: 2 cows, values varies between 4.5 - 5 Months.

Table 13: Animals data – after the operation (during the study period)

Animal number	Earmark	Clinic number	Date of operation	Case history (remarks)
1	-39535	261	08.05.2007	No remarks
2	-29786	274	16.05.2007	No remarks
3	-43239	307	31.05.2007	No remarks
4	-06669	283	21.05.2007	Euthanized
5	-89662	288	22.05.2007	No remarks
6	-73423	295	25.05.2007	Calved 14 months after
7	-23026	303	30.05.2007	No remarks
8	-42787	308	31.05.2007	Found deceased in the barn
9	-48393	304	30.05.2007	Slaughtered (udder injury)
10	-83558	301	29.05.2007	Calved 13 months after
11	-34539	314	06.06.2007	Calved 12 months after
12	-60115	332	18.06.2007	Stillborn 12 months after
13	-55720	341	22.06.2007	Relapse
14	-04452	342	22.06.2007	Calved 10 months after
15	-08372	359	28.06.2007	Calved 13 months after
16	-49133	362	29.06.2007	No remarks
17	-55790	337	21.06.2007	Stillborn 6 months after
18	-08092	343	22.06.2007	No remarks
19	-03679	366	02.07.2007	Slaughtered (not wanted any more)
20	-44060	384	09.07.2007	Calved 14 months after
21	-23939	386	10.07.2007	Calved 12 months after
22	-24999	394	15.07.2007	Calved 13 months after
23	-15474	396	15.07.2007	Calved 12 months after
24	-29395	401	19.07.2007	Slaughtered (mastitis)
25	-67504	410	24.07.2007	Calved 13 months after
26	-89456	417	30.07.2007	Calved 14 months after
27	-08123	434	08.08.2007	Calved 6 months after and then slaughtered
28	-00336	439	10.08.2007	Slaughtered (claw problems)
29	-86000	460	18.08.2007	Calved 5 months after
30	-79176	463	26.08.2007	No remarks
31	-35142	465	26.08.2007	Stillborn with no milk yield (then slaughtered)
32	-42764	488	29.08.2007	Found deceased in the barn
33	-61885	534	21.09.2007	Calved 10 months after
34	-57323	361	10.10.2007	Calved 12 months after
35	-51756	574	25.10.2007	Calved 11 months after

Table 14: Toggle-pin position in animals in various examination periods

Animal	Day 0	Day 28	Day 90	Day 180	Day 270	Day 360	Day 450
1	Ce	Cr	nV	nV	nV	nV	nV
2	Ce	Ca	nV	nV	nV	nV	nV
3	Ce	Cr	nV	nV	nV	nV	nV
4	Ce	Me	D	D	D	D	D
5	Ce	Ca	nV	nV	nV	nV	nV
6	Ce	Me	nV	nV	nV	nV	nV
7	Ce	Cr	Cr-La	nV	nV	nV	nV
8	Ce	Cr-La	nV	D	D	D	D
9	Ce	Cr-Me	nV	nV	D	D	D
10	Ce	Cr	nV	nV	nV	nV	nV
11	Ce	Cr-Me	nV	nV	nV	nV	nV
12	Ce	Cr	nV	nV	nV	nV	nV
13	Ce	Ce	D	D	D	D	D
14	Ce	Cr	nV	nV	nV	nV	nV
15	Ce	Cr	nV	nV	nV	nV	nV
16	Ce	Cr	nV	nV	nV	nV	nV
17	Ce	Ca-Me	nV	nV	nV	nV	nV
18	Ce	Ca-Me	nV	nV	nV	nV	nV
19	Ce	D	D	D	D	D	D
20	Ce	Cr	nV	nV	nV	nV	nV
21	Ce	Cr-Me	Cr-Me	Cr-Me	Cr-Me	nV	nV
22	Ce	Ca-La	nV	nV	nV	nV	nV
23	Ce	Ca-La	nV	nV	nV	nV	nV
24	Ce	D	D	D	D	D	D
25	Ce	Ca	nV	nV	nV	nV	nV
26	Ce	Ca	nV	nV	nV	nV	nV
27	Ce	Ca	nV	nV	D	D	D
28	Ce	D	D	D	D	D	D
29	Ce	Ca-Me	nV	nV	nV	nV	nV
30	Ce	Ca-Me	nV	nV	nV	nV	nV
31	Ce	Cr-Me	nV	nV	nV	D	D
32	Ce	Ca	nV	D	D	D	D
33	Ce	Cr-La	nV	nV	nV	nV	nV
34	Ce	Cr-Me	nV	nV	nV	nV	nV
35	Ce	Cr-Me	nV	nV	nV	nV	nV

The Position is corresponds to the fixation area
Ce =Central
Cr =Cranial
Ca =Caudal
Me =Medial
La =Lateral
nV =not Visible
D= Dropped out animal

Table 15a and 15b: Data concerns the insertion inflammation area.

Table – 15 a: Insertion wound area -toggled

Animal	Day 0			Day 28			Day 90			Day 180		
	L c-c	**W l-m**	**Depth**	**L c-c**	**W l-m**	**Depth**	**L c-c**	**W l-m**	**Depth**	**L c-c**	**W l-m**	**Depth**
1	2,00	3,00	0,00	18,10	20,10	43,20	10,50	12,30	19,50	8,09	8,27	10,80
2	2,00	2,50	0,00	16,70	17,20	52,20	15,50	14,30	22,00	6,88	6,50	17,20
3	1,50	3,00	0,00	17,50	14,20	45,00	11,50	12,40	19,50	0,00	0,00	0,00
4	3,00	2,00	0,00	20,10	18,50	38,10	D			D		
5	5,00	2,00	0,00	15,50	15,80	49,60	10,70	11,30	27,50	8,50	8,70	27,00
6	2,50	1,50	0,00	18,80	18,40	49,70	10,10	10,40	19,90	7,90	8,50	9,11
7	1,50	1,80	0,00	15,30	15,70	63,80	12,30	11,50	16,00	0,00	0,00	0,00
8	2,00	3,00	0,00	20,10	20,50	55,90	10,40	11,20	19,50	D		
9	3,50	2,00	0,00	14,70	15,50	51,20	8,50	7,40	21,50	6,10	5,50	16,10
10	3,00	2,00	0,00	16,40	15,30	47,70	13,20	11,90	14,50	0,00	0,00	0,00
11	2,50	2,00	0,00	19,50	18,50	55,10	9,90	10,40	15,20	7,60	8,20	15,20
12	4,00	2,50	0,00	16,40	12,00	55,50	10,50	12,20	16,50	0,00	0,00	0,00
13	1,50	3,00	0,00	10,50	9,50	40,50	D			D		
14	3,00	2,50	0,00	18,10	17,50	55,10	9,50	9,10	19,80	8,47	7,20	19,60
15	1,50	2,00	0,00	14,70	15,50	40,00	12,50	10,30	12,70	9,50	8,40	12,50
16	3,00	3,00	0,00	17,80	19,30	63,80	24,10	19,00	20,00	7,90	10,50	12,00
17	2,50	2,00	0,00	19,20	16,70	55,50	13,30	11,50	15,50	0,00	0,00	0,00
18	3,00	2,50	0,00	16,70	17,40	45,60	9,90	10,20	20,00	0,00	0,00	0,00
19	2,50	3,00	0,00	D			D			D		
20	2,00	3,50	0,00	18,70	15,30	40,50	7,50	5,50	13,00	10,50	10,75	9,73
21	1,50	1,90	0,00	11,00	11,50	35,00	11,50	9,70	18,50	0,00	0,00	0,00
22	3,50	2,50	0,00	21,70	19,20	50,20	12,40	12,20	17,50	10,50	7,45	14,10
23	3,00	2,50	0,00	15,20	17,50	55,00	11,20	12,00	10,10	8,40	9,73	9,75
24	3,00	1,50	0,00	D			D			D		
25	2,50	3,00	0,00	18,90	19,20	48,30	12,30	12,40	31,30	9,50	7,89	17,50
26	3,00	2,50	0,00	14,80	14,90	50,10	10,10	9,50	5,50	12,40	9,50	12,10
27	1,50	2,00	0,00	24,00	22,50	51,50	19,20	18,90	32,10	14,40	13,80	20,00
28	2,00	1,50	0,00	D			D			D		
29	2,00	3,50	0,00	20,10	18,20	37,30	10,50	11,20	16,50	9,90	10,50	15,50
30	3,50	2,00	0,00	21,20	19,50	50,10	11,50	11,70	18,90	0,00	0,00	0,00
31	2,50	2,00	0,00	13,30	15,50	44,30	21,20	22,60	25,10	14,70	13,90	16,70
32	1,50	2,00	0,00	16,00	13,00	47,10	13,00	9,70	19,20	D		
33	2,50	2,5	0,00	16,10	16,50	49,40	12,20	13,50	19,80	8,20	8,80	11,20
34	3,00	3,5	0,00	11,30	13,40	43,80	12,70	12,10	15,60	9,75	10,50	15,50
35	1,50	2,0	0,00	21,60	20,70	51,70	10,00	9,90	5,52	8,50	9,00	10,70

(L c-c = Length cranial-caudal), (W l-m = Width lateral-medial), All numbers are in millimeter (mm)
D= Dropped out animal

Table 15b continuation of the data

Table 15b: Insertion wound area -toggled									
Animal	Day 270			Day 360			Day 450		
	L c-c	**W l-m**	**Depth**	**L c-c**	**W l-m**	**Depth**	**L c-c**	**w l-m**	**Depth**
1	3,50	3,40	7,60	0,00	0,00	0,00	0,00	0,00	0,00
2	4,50	4,80	10,50	0,00	0,00	0,00	0,00	0,00	0,00
3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
4	D			D			D		
5	7,50	7,60	15,00	0,00	0,00	0,00	0,00	0,00	0,00
6	7,80	8,10	9,00	0,00	0,00	0,00	0,00	0,00	0,00
7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
8	D			D			D		
9	D			D			D		
10	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
11	6,20	6,80	4,80	0,00	0,00	0,00	0,00	0,00	0,00
12	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
13	D			D			D		
14	6,10	5,80	6,40	0,00	0,00	0,00	0,00	0,00	0,00
15	5,00	5,10	4,50	0,00	0,00	0,00	0,00	0,00	0,00
16	7,50	7,60	11,50	0,00	0,00	0,00	0,00	0,00	0,00
17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
18	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
19	D			D			D		
20	9,50	9,70	9,50	8,70	8,60	7,50	0,00	0,00	0,00
21	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
22	8,50	7,90	9,28	0,00	0,00	0,00	0,00	0,00	0,00
23	8,10	9,10	9,00	0,00	0,00	0,00	0,00	0,00	0,00
24	D			D			D		
25	4,50	5,10	5,50	0,00	0,00	0,00	0,00	0,00	0,00
26	7,45	8,35	9,14	5,36	6,20	8,50	0,00	0,00	0,00
27	D			D			D		
28	D			D			D		
29	10,20	10,40	7,93	0,00	0,00	0,00	0,00	0,00	0,00
30	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
31	0,00	0,00	0,00	D			D		
32	D			D			D		
33	7,63	8,10	10,70	3,95	4,15	10,20	0,00	0,00	0,00
34	9,50	9,50	11,10	0,00	0,00	0,00	0,00	0,00	0,00
35	21,20	23,20	23,10	9,27	9,45	11,70	0,00	0,00	0,00

(L c-c = Length cranial-caudal), (W l-m = Width lateral-medial), All numbers are in millimeter (mm)
D= Dropped out animal

Table 16: Types of adhesion according to shape: Oval, round and irregular-shaped

Animal	**Shape**	**Animal**	**Shape**
1.	Oval-shaped	**19.**	Deceased
2.	Round-shaped	**20.**	Round-shaped
3.	Oval-shaped	**21.**	Oval-shaped
4.	Oval-shaped	**22.**	Oval-shaped
5.	Irregular-shaped	**23.**	Irregular-shaped
6.	Oval-shaped	**24.**	Deceased
7.	Oval-shaped	**25.**	Oval-shaped
8.	Round-shaped	**26.**	Oval-shaped
9.	Round-shaped	**27.**	Round-shaped
10.	Oval-shaped	**28.**	Deceased
11.	Oval-shaped	**29.**	Round-shaped
12.	Oval-shaped	**30.**	Oval-shaped
13.	Oval-shaped	**31.**	Round-shaped
14.	Round-shaped	**32.**	Oval-shaped
15.	Oval-shaped	**33.**	Oval-shaped
16.	Oval-shaped	**34.**	Oval-shaped
17.	Oval-shaped	**35.**	Round-shaped
18.	Oval-shaped		

Table 17: Types of adhesion according to the presence of calcification

Animal	Details	Animal	Details
1.	Without-calcification	**19.**	Deceased
2.	Calcification	**20.**	Without-calcification
3.	Without-calcification	**21.**	Without-calcification
4.	Without-calcification	**22.**	Calcification
5.	Without-calcification	**23.**	Calcification
6.	Calcification	**24.**	Deceased
7.	Without-calcification	**25.**	Calcification
8.	Calcification	**26.**	Calcification
9.	Calcification	**27.**	Without-calcification
10.	Calcification	**28.**	Deceased
11.	Without-calcification	**29.**	Calcification
12.	Calcification	**30.**	Calcification
13.	Calcification	**31.**	Calcification
14.	Without-calcification	**32.**	Calcification
15.	Calcification	**33.**	Without-calcification
16.	Calcification	**34.**	Without-calcification
17.	Without-calcification	**35.**	Calcification
18.	Calcification		

Table 18a: Adhesion fixation area

Animal	Day 0			Day 28			Day 90			Day 180		
	L c-c	W l-m	Depth	L c-c	W l-m	Depth	L c-c	W l-m	Depth	L c-c	W l-m	Depth
1	nD			51.2	52.3	26.8	22.6	21.7	12.5	7.7	8.2	5.4
2	nD			54.0	53.6	46.2	26.3	28.2	25.8	16.4	14.5	13.9
3	nD			53.1	51.2	34.9	32.4	34.2	20.2	19.3	20.4	15.3
4	nD			40.9	42.4	28.3	D			D		
5	nD			43.1	32.6	41.7	24.5	22.7	23.2	12.4	15.6	12.1
6	nD			51.6	47.3	23.8	26.3	25.7	15.2	15.2	16.2	8.4
7	nD			53.4	52.0	35.6	30.2	35.2	17.3	14.7	15.6	9.5
8	nD			52.3	50.3	50.1	23.9	24.2	26.1	D		
9	nD			54.5	56.2	48.6	31.4	32.1	34.2	11.7	12.1	11.6
10	nD			47.1	48.3	27.4	24.5	24.9	20.3	15.8	16.2	10.7
11	nD			47.5	46.7	32.6	21.7	22.6	16.2	13.0	11.2	9.8
12	nD			54.7	50.2	25.9	31.1	32.5	20.3	12.8	13.4	8.1
13	nD			53.6	52.0	27.3	D			D		
14	nD			45.7	39.8	40.3	22.0	21.5	20.6	7.8	8.4	8.6
15	nD			48.9	44.3	23.4	23.1	22.6	15.2	18.3	17.2	7.1
16	nD			52.6	51.6	29.1	20.3	20.4	11.5	nD		
17	nD			51.1	47.7	30.1	21.8	22.3	10.8	10.8	11.7	6.3
18	nD			56.5	53.2	24.9	27.3	30.2	10.4	12.9	12.3	7.3
19	nD			D						D		
20	nD			57.1	52.3	47.9	25.4	26.8	24.9	12.9	13.2	12.7
21	nD			45.2	44.4	25.2	25.1	24.4	15.5	13.9	14.3	10.2
22	nD			52.4	43.6	31.9	21.2	22.6	14.3	13.2	14.2	7.4
23	nD			47.3	39.5	27.8	37.4	29.4	17.7	17.2	20.5	15.2
24	nD			D			D			D		
25	nD			46.4	53.2	26.3	31.2	30.6	12.4	17.3	15.6	7.2
26	nD			50.2	50.9	23.5	20.6	21.1	10.6	12.2	12.3	8.0
27	nD			44.3	45.8	46.2	22.1	23.5	22.6	15.9	15.5	15.1
28	nD			D			D			D		
29	nD			48.7	52.6	49.4	31.5	32.6	31.7	15.7	16.3	14.4
30	nD			45.2	51.1	30.6	20.3	20.2	12.5	nD		
31	nD			47.4	48.2	46.8	16.2	15.8	15.7	nD		
32	nD			55.1	48.8	21.9	25.4	27.8	17.6	D		
33	nD			52.3	51.3	23.1	26.4	25.8	15.3	15.3	16.4	8.6
34	nD			49.8	51.7	22.6	30.2	32.7	12.0	14.5	12.9	9.1
35	nD			45.7	42.4	44.5	22.8	23.1	22.9	16.7	15.6	16.8

(L c-c = Length cranial-caudal), (W l-m = Length lateral-medial). All numbers are in millimeter (mm), (D=Dropped out animal), (nD=no Data).

Table 18b continuation of the data - adhesion fixation area

Animal	Day 270			Day 360			Day 450		
	L c-c	W l-m	Depth	L c-c	W l-m	Depth	L c-c	W l-m	Depth
1	4.3	4.5	2.9	nD			nD		
2	6.5	6.2	5.9	nD			nD		
3	14.3	14.4	7.7	4.2	4.8	2.8	nD		
4	D			D			D		
5	5.3	6.4	4.5	nD			nD		
6	8.1	8.9	3.6	nD			nD		
7	10.8	11.3	6.5	nD			nD		
8	D			D			D		
9	D			D			D		
10	9.1	8.8	4.3	4.6	5.1	2.1	nD		
11	7.1	8.2	4.6	nD			nD		
12	6.2	6.9	3.7	nD			nD		
13	D			D			D		
14	10.9	11.5	11.1	4.9	4.2	3.6	nD		
15	15.9	15.3	6.5	7.3	7.9	4.6	nD		
16	nD			nD			nD		
17	nD			nD			nD		
18	7.2	6.9	4.2	nD			nD		
19	D			D			D		
20	10.6	10.3	9.8	3.8	3.8	4.0	3.8	3.6	2.9
21	10.9	11.7	4.8	6.0	5.2	2.3	nD		
22	7.1	6.8	3.6	nD			nD		
23	7.6	8.3	5.3	nD			nD		
24	D			D			D		
25	7.7	8.2	3.2	nD			nD		
26	10.1	9.7	4,8	5.8	5.1	2.4	nD		
27	D			D			D		
28	D			D			D		
29	10.8	10.4	9.3	4.3	4.8	4.5	nD		
30	nD						nD		
31	nD			D			D		
32	D			D			D		
33	10.7	11.2	6.5	4.5	5.1	3.1	nD		
34	9.7	10.3	4.9	nD			nD		
35	10.4	9.7	10.1	5.0	4.8	4.1	nD		

(L c-c = Length cranial-caudal), (W l-m = Length lateral-medial). All numbers are in millimeter (mm), (D=Dropped out animal), (nD=no Data).

Table 19: Detailed data of the insertion wound area of all the animal patients

0day		30day			90day			180day			270day			360day		
0,15	0,15	10,50	9,50	35,00	7,50	5,50	5,50	6,10	5,50	9,11	3,50	3,40	4,50	3,95	4,15	7,50
0,15	0,15	11,00	11,50	37,30	8,50	7,40	5,52	6,88	6,50	9,73	4,50	4,80	4,80	5,36	5,20	8,50
0,15	0,15	11,30	12,00	38,10	9,50	9,10	10,10	7,60	7,20	9,75	4,50	5,10	5,50	8,70	8,60	10,20
0,15	0,18	13,30	13,00	40,00	9,90	9,50	12,70	7,90	7,45	10,70	5,00	5,10	6,40	9,27	9,45	11,70
0,15	0,19	14,70	13,40	40,50	9,90	9,70	13,00	7,90	7,89	10,80	6,10	5,80	7,60			
0,15	0,20	14,70	14,20	40,50	10,00	9,70	14,50	8,09	8,20	11,20	6,20	6,80	7,93	6,82	6,85	9,48
0,15	0,20	14,80	14,90	43,20	10,10	9,9	15,20	8,20	8,27	12,00	7,45	7,60	9,00	2,230773	2,226825	1,606821
0,15	0,20	15,20	15,30	43,80	10,10	10,20	15,50	8,40	8,40	12,10	7,50	7,60	9,00			
0,20	0,20	15,30	15,30	44,30	10,40	10,30	15,60	8,47	8,50	12,50	7,50	7,90	9,14			
0,20	0,20	15,50	15,50	45,00	10,50	10,40	16,00	8,50	8,70	14,10	7,63	8,10	9,28			
0,20	0,20	16,00	15,50	45,60	10,50	10,40	16,50	8,50	8,80	15,20	7,80	8,10	9,50			
0,20	0,20	16,10	15,50	47,10	10,50	11,20	16,50	9,50	9,00	15,50	8,10	8,35	10,50			
0,20	0,20	16,40	15,70	47,70	10,70	11,20	17,50	9,50	9,50	15,50	8,50	9,10	10,70			
0,20	0,20	16,40	15,80	48,30	11,20	11,30	18,50	9,75	9,73	16,10	9,50	9,50	11,10			
0,25	0,20	16,70	16,50	49,40	11,50	11,50	18,90	9,90	10,50	16,70	9,50	9,70	11,50			
0,25	0,20	16,70	16,70	49,60	11,50	11,50	19,20	10,50	10,50	17,20	10,20	10,40	15,00			
0,25	0,20	17,50	17,20	49,70	11,50	11,70	19,50	10,50	10,50	17,50	21,20	23,20	23,10			
0,25	0,25	17,80	17,40	50,10	12,20	11,90	19,50	12,40	10,75	19,60						
0,25	0,25	18,10	17,50	50,10	12,30	12,00	19,50	14,40	13,80	20,00	7,92	8,27	9,68			
0,25	0,25	18,10	17,50	50,20	12,30	12,1	19,80	14,70	13,90	27,00	3,798737	4,179113	4,222598			
0,25	0,25	18,70	18,20	51,20	12,40	12,20	19,80									
0,30	0,25	18,80	18,40	51,50	12,50	12,20	19,90	9,38	9,40	15,20						
0,30	0,25	18,90	18,50	51,70	12,70	12,30	20,00	2,196806	2,052906	4,29						
0,30	0,25	19,20	18,50	52,20	13,00	12,40	20,00									
0,30	0,25	19,50	19,20	55,00	13,20	12,40	21,50									
0,30	0,30	20,10	19,20	55,10	13,30	13,5	22,00									
0,30	0,30	20,10	19,30	55,10	15,50	14,30	25,10									
0,30	0,30	20,10	19,50	55,50	19,20	18,90	27,50									
0,30	0,30	21,20	20,10	55,50	21,20	19,00	31,30									
0,30	0,30	21,60	20,50	55,90	24,10	22,60	32,10									
0,35	0,30	21,70	20,70	63,80												
0,35	0,30	24,00	22,50	63,80	12,26	11,88	18,27									
0,35	0,35				3,509956	3,282447	5,882313									
0,40	0,35	17,19	16,70	48,81												
0,50	0,35	3,125875	2,858702	6,86												
0,25	0,24															
0,081491	0,056537															

The data shows the maximum, minimum values and the average ($\bar{x}$) and the SD (Standard deviation)

Table 20: Detailed data of the fixation adhesion area of all the animal patients

30day				90day				180day				270day				360day				450day		
40,90	32,60	21,90		16,20	15,80	10,40		7,70	8,20	5,40		4,30	4,50	2,90		3,80	3,80	2,10		3,80	3,60	2,90
43,10	39,50	22,60		20,30	20,20	10,60		7,80	8,40	6,30		5,30	6,20	3,20		4,20	4,20	2,30				
44,30	39,80	23,10		20,30	20,40	10,80		10,80	11,20	7,10		6,20	6,40	3,60		4,30	4,80	2,40		3,80	3,60	2,90
45,20	42,40	23,40		20,60	21,10	11,50		11,70	11,70	7,20		6,50	6,80	3,60		4,50	4,80	2,80		0	0	0
45,20	42,40	23,50		21,20	21,50	12,00		12,20	12,10	7,30		7,10	6,90	3,70		4,60	4,80	3,10				
45,70	43,60	23,80		21,70	21,70	12,40		12,40	12,30	7,40		7,10	6,90	4,20		4,90	5,10	3,60				
45,70	44,30	24,90		21,80	22,30	12,50		12,80	12,30	8,00		7,20	8,20	4,30		5,00	5,10	4,00				
46,40	44,40	25,20		22,00	22,60	12,50		12,90	12,90	8,10		7,60	8,20	4,50		5,80	5,10	4,10				
47,10	45,80	25,90		22,10	22,60	14,30		12,90	13,20	8,40		7,70	8,30	4,60		6,00	5,20	4,50				
47,30	46,70	26,30		22,60	22,60	15,20		13,00	13,40	8,60		8,10	8,80	4,80		7,30	7,90	4,60				
47,40	47,30	26,80		22,80	22,70	15,20		13,20	14,20	8,60		9,10	8,90	4,80								
47,50	47,70	27,30		23,10	23,10	15,30		13,90	14,30	9,10		9,70	9,70	4,90		5,04	5,08	3,35				
48,70	48,20	27,40		23,90	23,50	15,50		14,50	14,50	9,50		10,10	9,70	5,30		0,995188	1,03034	0,886848				
48,90	48,30	27,80		24,50	24,20	15,70		14,70	15,50	9,80		10,40	10,30	5,90								
49,80	48,80	28,30		24,50	24,40	16,20		15,20	15,60	10,20		10,60	10,30	6,50								
50,20	50,20	29,10		25,10	24,90	17,30		15,30	15,60	10,70		10,70	10,40	6,50								
51,10	50,30	30,10		25,40	25,70	17,60		15,70	15,60	11,60		10,80	11,20	6,50								
51,20	50,90	30,60		25,40	25,80	17,70		15,80	15,60	12,10		10,80	11,30	7,70								
51,60	51,10	31,90		26,30	26,80	20,20		15,90	16,20	12,70		10,90	11,50	9,30								
52,30	51,20	32,60		26,30	27,80	20,30		16,40	16,20	13,90		10,90	11,70	9,80								
52,30	51,30	34,90		26,40	28,20	20,30		16,70	16,30	14,40		14,30	14,40	10,10								
52,40	51,60	35,60		27,30	29,40	20,60		17,20	16,40	15,10		15,90	15,30	11,10								
52,60	51,70	40,30		30,20	30,20	22,60		17,30	17,20	15,20												
53,10	52,00	41,70		30,20	30,60	22,90		18,30	20,40	15,30		9,15	9,36	5,81								
53,40	52,00	44,50		31,10	32,10	23,20		19,30	20,50	16,80		2,784438	2,639744	2,392231								
53,60	52,30	46,20		31,20	32,50	24,90																
54,00	52,30	46,20		31,40	32,60	25,80		14,14	14,39	10,35												
54,50	52,60	46,80		31,50	32,70	26,10		2,820082	2,918482	3,187365												
54,70	53,20	47,90		32,40	34,20	31,70																
55,10	53,20	48,60		37,40	35,20	34,20																
56,50	53,60	49,40																				
57,10	56,20	50,10		25,51	25,91	18,18																
				4,59927	4,801023	6,066909																
49,96563	48,36	33,27																				
4,069829	5,024556	9,412831																				

The data shows the maximum, minimum values and the average ($\bar{x}$) and the SD (Standard deviation)

Statistical analysis sheets for both adhesion and wound area

```
Welcome to Minitab®[6]
Analysis of Variance - Adhesion area
Source     DF        SS        MS        F        P
Factor     14   81085.1    5791.8   256.35    0.000
Error     342    7726.9      22.6
Total     356   88812.0
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
C1         32    49.966     4.135                                  (*)
C2         32    48.359     5.105                                 (*)
C3         32    33.272     9.563                       (*)
C5         30    25.507     4.678                  (*)
C6         30    25.913     4.883                  (*)
C7         30    18.183     6.171             (*)
C9         25    14.144     2.878          (*-)
C10        25    14.392     2.979          (-*)
C11        25    10.352     3.253        (*)
C13        22     9.150     2.784       (*)
C14        22     9.359     2.640       (*-)
C15        22     5.809     2.392     (*)
C17        10     5.040     1.049   (-*-)
C18        10     5.080     1.086   (-*-)
C19        10     3.350     0.935  (-*-)
                                   ----------+---------+---------+------
Pooled StDev =    4.753                      15        30        45
```

```
Welcome to Minitab®
Analysis of Variance - Wound area
Factor     14   56067.4    4004.8   273.44    0.000
Error     358    5243.2      14.6
Total     372   61310.6
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  -+---------+---------+---------+-----
C1         35      2.51      0.83  (*)
C2         35      2.39      0.57  (*)
C4         32    17.188     3.176              *)
C5         32    16.703     2.904             (*)
C6         32    48.806     6.970                                   (*
C8         30    12.257     3.570          (*)
C9         30    11.877     3.339          (*)
C10        30    18.274     5.983              (*)
C12        20     9.385     2.254        (*)
C13        20     9.180     2.106        (*)
C14        20    14.614     4.401            (*)
C16        17     7.922     3.916       (*)
C17        17     8.268     4.308       (-*)
C18        17     9.679     4.353        (*-)
C20         6     6.055     2.737     (-*-)
                                   -+---------+---------+---------+-----
Pooled StDev =    3.827             0        15        30        45
```

6 Minitab Inc. Quality Plaza, 1829 Pine Hall Rd, State College PA 16801-3008, USA

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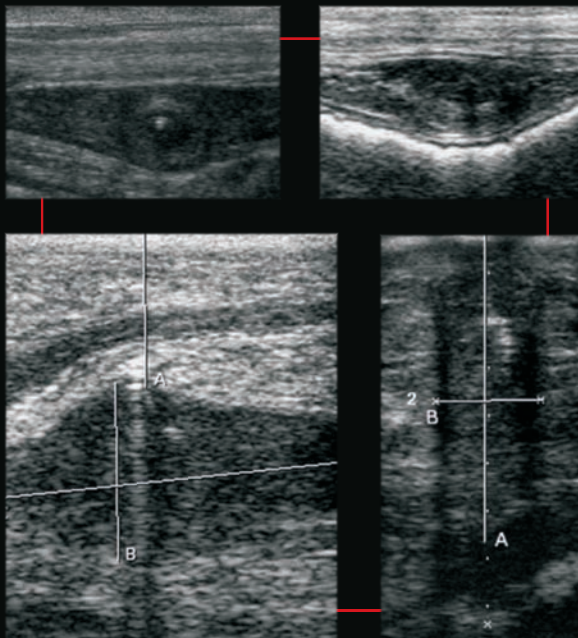
Erklärung

Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichen Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" niedergelegt sind, eingehalten.

Ali Al-Bayati



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