A Resorbable Device for Ligation of Blood Vessels

Development, Assessment of Surgical Procedures and Clinical Evaluation

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Cover: The blood pressure during neutering of a female dog. Two repeated noxious stimuli, the removal of ovaries with a pause in between, resulted in different stress reactions. The results demonstrate the difficulty of using one dog as its own control. Background: The new resorbable device.
Illustration: O. V. Höglund

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Abstract

Maintaining haemostasis during surgery is vital for a successful outcome. The objectives of this thesis were to develop a resorbable device, which would enable safe ligation and less surgical stress than the conventional method. For manufacturing of the device, the resorbable polymer polydioxanone was injection moulded. The device was constructed as a flexible band attached to a self-locking mechanism. The band formed a loop around the tissue, the end of the band was inserted into the locking mechanism and was pulled through until the loop was closed. The design allowed complete closure of the loop and the device resisted ligature slip-off from renal arteries at 10 N. Tissue reactions and surgical stress responses to ligation with the device were studied in healthy dogs subjective to elective neutering. For evaluation of haemostatic efficiency the ovarian pedicles of 14 dogs were ligated bilaterally (9) or unilaterally (5). All pedicles were successfully ligated, but one device first after being further tightened. The dogs were examined by ultrasound for up to twelve months with no adverse observations recorded. In two dogs histological examinations revealed local and transient tissue reactions around the devices. Systolic blood pressure and heart rate were registered during ligation of ovarian pedicles bilaterally in nine dogs and unilaterally in five dogs using the device and in 26 dogs using conventional techniques. In 16 of the 26 dogs laparoscopic and open abdomen techniques were compared and in the remaining 10 dogs ligation of the two ovaries was compared. Plasma vasopressin was analysed in the latter study. The increase in systolic blood pressure was greater in the open abdomen group compared to the laparoscopic group and the device-group. Ligation of the first ovarian pedicle caused a greater cardiovascular response than ligation of the second pedicle. However, plasma vasopressin concentration changed in synchrony with systolic blood pressure and the combination may be useful for evaluation of surgical stress. In conclusion, a resorbable device which efficiently ligated both single vessels and ovarian pedicles was successfully constructed. Whether use of the device enables less surgical stress than a conventional ligation technique remains to be clarified.

Keywords: dog, injection moulding, laparoscopy, ligation, noradrenalin, ovariectomy, ovariohysterectomy, polydioxanone, surgical stress, vasopressin.

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Dedication

To my family

You see things; and you say, 'Why?'But I dream things that never were; and I say, 'Why not?'

George Bernard Shaw

Contents

List of Publications

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

I Odd V. Höglund, Ragnvi Hagman, Kerstin Olsson, Jonas Mindemark, Niklas Borg and Anne-Sofie Lagerstedt (2011). A new resorbable device for ligation of blood vessels - A pilot study. *Acta Veterinaria Scandinavica* 53(47), 1-7.

II Odd V. Höglund, Ragnvi Hagman, Kerstin Olsson, Carolina Carlsson, Fredrik Södersten, Anne-Sofie Lagerstedt (2011). Ligation of the ovarian pedicles in dogs with a resorbable self-locking device – A long-term follow-up study. *Journal of Biomaterials Applications*, e-pub before print.

III Odd V. Höglund, Kerstin Olsson, Ragnvi Hagman, Malin Öhlund, Ulf Olsson, Anne-Sofie Lagerstedt (2011). Comparison of haemodynamic changes during two surgical methods for neutering female dogs. *Research in Veterinary Science* 91, 159-163.

IV Odd V. Höglund, Ragnvi Hagman, Kerstin Olsson, Ulf Olsson, Anne-Sofie Lagerstedt. Intraoperative changes of blood pressure, heart rate, plasma vasopressin and urinary noradrenalin during elective ovariohysterectomy in dogs. Manuscript.

Abbreviations

ACTH	Adrenocorticotropic hormone
BPM	Beats per minute (heart rate)
CRP	C-reactive protein
LOE	Laparoscopic ovariectomy
LSMean	Least square mean
OE	Ovariectomy
OHE	Ovariohysterectomy
PCL	Polycaprolactone
PDO	Polydioxanone
PGA	Polyglycolic acid, polyglycolide
PLLA	Poly-L-lactic acid, poly-L-lactide
SE	Standard error of mean
SEM	Standard error of LSmean
TMC	Trimethylene carbonate

1 Introduction

1.1 Background

Neutering is a frequently performed surgical procedure in small animal practice (Trevejo *et al.*, 2011; Blackshaw & Day, 1994; Manning & Rowan, 1992). The term neutering (from Latin *neuter*, of neither sex) means to desex an animal or to render it sterile by the removal of parts (gonads) of the reproductive organs. In English, neutering of males is generally called castration and neutering of females is referred to as spaying (Bloomberg, 1996).

In surgery, haemostasis to prevent blood loss is vital for a successful outcome. In abdominal surgery in dogs it can be difficult to ligate vessels due to interference of abdominal organs and the depth of the abdominal cavity. One example of such difficulties is the ligation of ovarian pedicles in dogs which is associated with risk of intraoperative or postoperative haemorrhage (Berzon, 1979; Pearson, 1973). Bleeding may originate from the vessels of the ovary, which are imbedded in fat in the suspensory apparatus known as the *mesovarium*. The mesovarium is a double layer of peritoneum which attaches the ovaries to the abdominal wall. It contains nerves, blood and lymph vessels and is popularly referred to as the ovarian pedicle (Adin & Scansen, 2011; Bubalo *et al.*, 2008; Carpenter, 1972).

General and elective surgical skills, such as neutering, are considered by private veterinary practitioners in the USA to be the most important skills required of new graduates (Greenfield *et al.*, 2004). Final year veterinary students were asked which surgical procedure they were most worried about performing and 81 % answered that it was canine ovariohysterectomy and the risk of postoperative haemorrhage (Bowlt *et al.*, 2011). A safer ligation technique, that enables a convenient ligation procedure, could be a significant

improvement on current standard practice and could potentially simplify a relatively common surgical procedure.

The difficulties of achieving sufficient haemostasis and the risk of haemorrhage in abdominal surgery initiated development of an alternative to ligatures with suture. A cable tie is such an alternative. It is typically made of non-resorbable nylon, consists of a flexible band attached to a case containing a locking mechanism. The construction is also known as a tie rap, tie wrap or zip tie. Cable ties have been used for ligation of the ovarian pedicle in dogs (Carpenter, 1973; Carpenter, 1972). They have also been used for ligation of ovarian vessels during ovariectomy in horses (Cokelaere *et al.*, 2005), for partial nephrectomy in pigs (McDougall *et al.*, 1993) and at ovariohysterectomy or splenectomy in cats (Zagraniski, 1980; Zagraniski, 1979; Zagraniski, 1978).

Many of these studies showed that cable ties made surgery quicker and easier (Zagraniski, 1980; Zagraniski, 1979; Zagraniski, 1978; Carpenter, 1973; Carpenter, 1972). However, one limitation with traditional cable ties was that an adequate amount of tissue was necessary inside the loop for the construction to compress tissue and perform well (Zagraniski, 1980). Another limitation was the non-resorbable material. Long-term severe tissue reactions were found and the use of cable ties for ligation purposes was dissuaded (Howe, 2006; Murphy *et al.*, 1998; Werner *et al.*, 1992).

Despite some benefits having been demonstrated with traditional cable ties in surgery, the disadvantages and problems outlined above have limited their use. This initiated the design of a new device, purpose designed for surgery and biocompatible, which is presented in this thesis. The self-locking loop of the new device should close completely and achieve safe haemostasis of large single arteries or of vessels surrounded by fat and cause minimal tissue reactions.

As new surgical techniques are developed, methods for comparison are needed for evaluation purposes. Novel techniques aim to reduce complications, duration of surgery and recovery time. Beside the risk of haemorrhage, ligation and removal of ovaries elicit noxious stimuli, which may cause considerable postoperative pain. This was a further reason for development of a new device. Minimizing postoperative pain is important for reducing recovery time. Pain may be detrimental as it can cause decreased food intake, depression of respiratory function, and cause central hypersensitivity to noxious stimuli and development of chronic pain (Sarrau *et al.*, 2007; Gaynor, 1999; Lascelles *et al.*, 1998).

The assessment of postoperative pain, which is one parameter that could be used for comparison of different surgical methods, has been found to be

difficult (KuKanich, 2011; Hellyer *et al.*, 2007; Vinuela-Fernandez *et al.*, 2007; Holton *et al.*, 2001; Firth & Haldane, 1999). The patient's behaviour in the postoperative period is likely to be affected by its environment and may not be related to the surgery. The extent of tissue trauma inflicted during surgery is related to level of postoperative pain (Goebel *et al.*, 2011; Firth & Haldane, 1999) but evaluation of pain is generally subjective. No direct measurement of pain in a reliable and repeatable way which is consistent across observers is currently possible (Slingsby *et al.*, 2011; Slingsby, 2010; Lascelles *et al.*, 1998). An alternative or complementary objective method may be to use intraoperative stress assessment. A gold standard for assessment of intraoperative stress does not exist but noxious stimuli caused by surgery triggers a stress response characterised by activation of the sympathetic nervous system, endocrine responses as well as immunological and haematological changes. The magnitude of the responses is proportional to surgical injury (Desborough, 2000; Holzer-Petsche & Brodacz, 1999; Chernow *et al.*, 1987; Schmidt & Lee Booker, 1982) and may contribute to development of complications (Myles *et al.*, 2002; Parker *et al.*, 1995).

Objective measurements demonstrated a quicker return to normal activity in the postoperative period in dogs where laparoscopic ovariectomy was performed compared to dogs where traditional open abdomen ovariectomy was performed (Culp *et al.*, 2009). The cause of the shorter recovery has not been explained (Mayhew, 2011). One possible hypothesis is that laparoscopy causes less intraoperative noxious stimuli and less surgical stress. A comparison of the two methods appeared to offer a good model to evaluate arterial blood pressure and heart rate as intraoperative stress indicators. The information could provide an insight into which parts of the surgery that trigger more noxious stimuli and could potentially explain the advantage seen with laparoscopic surgery.

Ligation of the ovarian pedicle is associated with noxious stimuli (Bubalo *et al.*, 2008; Devitt *et al.*, 2005) and was therefore suitable for comparison of new and traditional techniques. In this thesis frequent non-invasive registration of arterial blood pressure and heart rate, complemented with analysis of plasma vasopressin concentration and urinary noradrenalin, were used as intraoperative stress parameters.

The ability to identify differences of noxious stimuli within and between surgical methods is important for the development of surgery with less noxious stimuli and reduction of recovery time. The development and first tests of a device for ligation purposes, with the aim to enable a safe surgery with less noxious stimuli, is presented in this thesis. The biocompatibility of the device was investigated by ultrasound and histology. In parallel, methods to evaluate

intraoperative stress parameters were tested with the goal to compare the use of the device with traditional ligatures.

1.2 Neutering of female dogs

Several surgical approaches have been described for neutering of female dogs (Howe, 2006). Ovariohysterectomy (OHE) means that the ovaries and uterus are removed, whereas in ovariectomy (OE) only the ovaries are removed with the possibility of a shortened incision length (Peeters & Kirpensteijn, 2011). It has been shown that leaving the uterus is not associated with an increased risk of morbidity, provided no adverse uterine pathology exists at time of surgery (Van Goethem *et al.*, 2006; Okkens *et al.*, 2003; Okkens *et al.*, 1997). Ovariohysterectomy or ovariectomy can also be performed with a laparoscopic technique (Culp *et al.*, 2009; Hancock *et al.*, 2005; Davidson *et al.*, 2004; Austin *et al.*, 2003). Ovariohysterectomy is considered the standard surgical procedure for neutering of female dogs in many countries, but the preference for this procedure instead of ovariectomy is currently a matter for debate (Detora & McCarthy, 2011).

1.2.1 Open abdomen surgery - laparotomy

In open abdomen surgery (laparotomy, incision to gain access to the abdominal cavity) for neutering of a female dog, the ovaries are removed through a midline or flank incision into the abdomen. Through the use of sutures, ligatures are tied around the ovarian pedicles after which the tissue is transected. In an open abdomen approach, devices for sealing and cutting of vessels can be used. In general, the ovarian pedicle is stretched for access and the tissue is simultaneously flattened out by the haemostatic forceps.

1.2.2 Laparoscopic surgery

In laparoscopic surgery (minimally invasive surgery; access to the abdomen through one or several small incisions and portals) for neutering a female dog the ovaries are removed with instruments that are inserted through portals (Weisse & Mayhew, 2012). Techniques for using 3, 2 or 1 portal have been described (Case *et al.*, 2011; Dupré *et al.*, 2009). Ovariectomy can also be performed by natural orifice transluminal endoscopic surgery (NOTES) where no portals are used (Freeman *et al.*, 2011; Freeman *et al.*, 2010).

Open versus laparoscopic neutering of female dogs

Different outcome parameters have been compared in neutering of female dogs by either an open abdomen approach or a laparoscopic approach (Freeman *et al.*, 2010; Luntang-Jensen, 2006; Devitt *et al.*, 2005; Hancock *et al.*, 2005; Malm *et al.*, 2005; Davidson *et al.*, 2004). Laparoscopic surgery provides better visualisation of small structures and objective measurements have demonstrated that the laparoscopic approach was associated with a quicker recovery compared to the open abdomen approach (Culp *et al.*, 2009). Additionally, there were less signs of postoperative pain in dogs neutered with a laparoscopic surgery compared to traditional open abdomen surgery (Devitt *et al.*, 2005; Hancock *et al.*, 2005; Davidson *et al.*, 2004). Intraoperative measurements may offer further objective data without interference from environmental and other factors.

1.3 Haemorrhage and haemostasis

Haemostasis and evaluation - background

There are several methods used to achieve haemostasis during surgery such as traditional ligation techniques by use of sutures, clips (resorbable or non-resorbable) and instruments that transfer energy to the tissue (cautery, ultrasound or laser).

Haemostatic efficiency may be investigated by several different methods involving bursting pressure, sealing time and failure rate. Pressure may be applied by infusion of a liquid into the ligated or sealed vessel until leakage is observed. One method is to apply weights to the tested device until slippage is recorded. The time perspective is taken into account in mechanical tests where the devices are pulled to break following different implantation periods that may be performed in vivo or in vitro by dissolution studies (Newcomb *et al.*, 2009; Hsu, 2006; Bubenik *et al.*, 2005; Hay *et al.*, 1988).

1.3.1 Haemorrhage from the ovarian pedicle - open abdomen

In studies of neutering of female dogs where the surgery was performed by students, major intraoperative haemorrhage of the ovarian pedicle was reported in between 4 % and 22 % of the cases (Bowlt *et al.*, 2011; Burrow *et al.*, 2005; Berzon, 1979; Dorn & Swist, 1977).

Large dogs are recognized as more challenging because of their deeper chests and deeper abdomens with a greater distance from skin incision to the tissue to remove, compared to small dogs. Major intraoperative haemorrhage was reported in 2 % of small dogs and in 79 % of large dogs, weighing more than 25 kg (Berzon, 1979). In another study where the surgery was performed

by clinicians in a non-teaching institution, intraoperative bleeding from the ovarian pedicle occurred in 10 % of the dogs (Devitt *et al.*, 2005).

Haemorrhage from the ovarian pedicle in the postoperative period is also reported following neutering of female dogs. The consequence may be that the patient is re-anesthetised, an explorative laparotomy is performed and a new ligature is applied. In a study where the ovariohysterectomy was performed by senior students, haemorrhage from the ovarian pedicle that required reoperation was reported in one of 18 dogs (Davidson *et al.*, 2004).

1.3.2 Haemorrhage from the ovarian pedicle - laparoscopy

New laparoscopic instruments are primarily developed for use in humans. The technique is used in veterinary surgery although cost of laparoscopic equipment may be an obstacle. In veterinary surgery new techniques are challenged with the canine ovarian pedicle and its large amount of adipose tissue. The use of metal clips represents one technique to achieve hemostasis but one study reported bleeding (inconsequential) from the ovarian pedicles in 10 out of 10 dogs. The same study reported no bleeding from the ovarian pedicles where a bipolar vessel-sealer device was used (Mayhew & Brown, 2007). In other studies involving laser technique, mono- and bipolar electrocoagulation, bleeding from the ovarian pedicle was reported in between 2 and 25 % of the cases (Case *et al.*, 2011; Dupré *et al.*, 2009; Van Nimwegen & Kirpensteijn, 2007; Van Nimwegen *et al.*, 2005; Van Goethem *et al.*, 2003).

The use of laser represents a relatively new technique to achieve haemostasis. Bleeding from the ovarian pedicle occurred in 10 - 25 % of dogs when surgical laser was used to achieve hemostasis (Van Nimwegen & Kirpensteijn, 2007; Van Nimwegen *et al.*, 2005). Arterial bleeding from the ovarian pedicle occurred in 13 % of dogs where monopolar electrocoagulation was used (Van Goethem *et al.*, 2003) whereas bipolar electrocoagulation resulted in bleeding from the ovarian pedicle in 8 % of dogs (Van Nimwegen *et al.*, 2005; Van Goethem *et al.*, 2003). A further developed bipolar electrocoagulation system (LigaSure™) was used in two studies investigating a reduced number of portals (Case *et al.*, 2011; Dupré *et al.*, 2009). Bleeding of the ovarian pedicle that required application of the vessel-sealer device occurred in 2.4 % and 5.6 % of all dogs in the two studies.

1.4 Polymers in medicine

A polymer is defined as a large molecule composed of repeating units. Plastics are examples of materials based on synthetic polymers. The introduction of synthetic polymers into modern medicine was often adopted from other areas

of science (Langer & Tirrell, 2004). One such famous example is attributed to Dr Ridley (Apple & Sims, 1996) who was an ophthalmologist working for the Royal Air Force during World War II. The canopy covering the cockpit or gunnery was sometimes shattered when hit by enemy fire and fragments could be lodged in the eyes' of the crew. Dr Ridley observed a minimal tissue reaction to the material, poly-(methyl methacrylate) and suggested its use for an artificial intraocular lens, an idea which was realised during his lifetime.

1.4.1 Non-resorbable polymers

There are several early publications where the use of ligatures of non-resorbable material caused tissue reactions (Borthwick, 1972; Pearson, 1970; Cawley & Archibald, 1958). These reactions may be chronic foreign body reactions and fistulas, and consequently the use of non-resorbable material for ligation was dissuaded (Pearson, 1973; Joshua, 1965). The risk of using non-resorbable material for ligation is also outlined in a more recent paper (Howe, 2006) and in a textbook of surgery (Fossum, 2007). Cable ties were specifically mentioned as an example of a non-resorbable device that may cause pathologic tissue reactions. Consequently these authors suggested that resorbable materials should be used for ligation purposes. (Fossum, 2007; Howe, 2006; Murphy *et al.*, 1998; Werner *et al.*, 1992).

1.4.2 Resorbable polymers

In the 1960s a resorbable polymer, polyglycolic acid, was developed into the first synthetic resorbable suture (Gilding & Reed, 1979). Today, all registered resorbable medical devices such as sutures and clips are based on five monomers and combinations thereof (Roby & Kennedy, 2004; Middleton & Tipton, 2000). These are shown in Figure 1 and Table 1.

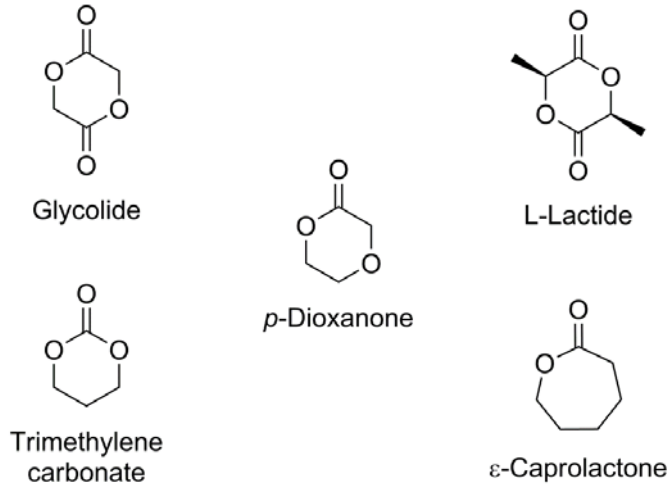

Figure 1. Resorbable medical devices such as sutures are made of polymers based on one or more of five cyclic monomers: glycolide, l-lactide, *p*-dioxanone, trimethylene carbonate and ε-caprolactone. Courtesy J. Mindemark.

The first commercially available synthetic resorbable sutures were Dexon® (Davis & Geck, USA) and Vicryl® (Ethicon, USA). Both sutures were multifilamental and made of glycolide although the Vicryl® suture contained L-lactide. Other materials and blends thereof were subsequently introduced.

Table 1. *Multifilament and monofilament resorbable sutures, adapted from "Biomaterials Science, an introduction to materials in medicine" (Roby & Kennedy, 2004)*

Trade name	Polymer(s)	Composition %	Generic name
Dexon®	PGA		Polyglycolide
Vicryl®	PGA/PLLA	90/10	Polyglactin 910
Polysorb®	PGA/PLLA	90/10	Lactomer
PDS®	PDO		Polydioxanone
Maxon®	PGA/TMC	67/33	Polyglyconate
Monocryl®	PGA/PCL	75/25	Poliglecaprone 25
Biosyn®	PGA/TMC/PDO	60/23/17	Glycomer 631
Caprosyn®	PGA/PCL/PLLA/TMC	68/17/7/7	Polyglytone 6211

Abbreviations on page 9.

Tissue reaction to resorbable polymers

All resorbable materials trigger a tissue reaction. Generally, natural materials degrade by enzymes whereas synthetic resorbable materials degrade through hydrolysis. The materials are broken down and eliminated by tissues, a process referred to as resorption. Biocompatibility refers to the degree of effect of the

material or breakdown products on surrounding tissue and performance of the material (Williams, 2008; Williams, 1987). To be of clinical use, the inflammatory response to the material must be less than the beneficial effects (Roby & Kennedy, 2004; Middleton & Tipton, 2000). In general, the tissue reaction to resorbable polymers is local and transient but there are some exceptions. A resorbable multifilament suture (Panacryl®) with a composition ratio of 97% PLLA and 3% PGA was recalled from the market in 2006 due to reports of foreign body reactions (FDA, Accessed 2011 10 03). Therefore, the fate of a new resorbable device must be fully evaluated. In this thesis ultrasound and histology were used to evaluate the fate of the device.

Polydioxanone

Sutures made of polydioxanone were introduced in 1983 (PDS®, Ethicon). This was the first clinically tested monofilament synthetic resorbable suture with a resorption time of 6 - 12 months (Middleton & Tipton, 2000). Other devices such as clips for ligation of vessels and pins for fracture fixation have also been manufactured from polydioxanone (Atkinson *et al.*, 1998; Michel *et al.*, 1985). Polydioxanone has reasonable strength and flexibility (Roby & Kennedy, 2004) and was therefore considered suitable for the device developed in this thesis.

1.5 Surgical stress

The hormonal and metabolic changes which follow injury or trauma are referred to as a stress response (Desborough, 2000). A systemic catabolic response to injury was originally described in fracture patients (Wilmore, 2002; Cuthbertson, 1930) which later turned attention to stress caused by surgery. Surgical stress is described as the systemic response to surgical injury and is characterized by activation of the sympathetic nervous system, endocrine responses as well as immunological and haematological changes. Endocrine responses are characterised as increased secretion of pituitary hormones, an increased release of catabolic and immunosuppressive hormones (Ledowski *et al.*, 2005), together with decreased insulin secretion and increased insulin resistance (Desborough, 2000). The surgical stress response may also contribute to development of complications (Myles *et al.*, 2002; Parker *et al.*, 1995) and the magnitude of the responses is proportional to the inflicted surgical injury (Desborough, 2000; Holzer-Petsche & Brodacz, 1999; Chernow *et al.*, 1987; Schmidt & Lee Booker, 1982).

In several studies involving dogs subjected to minimally invasive or traditional open abdomen surgery, the postoperative surgical stress responses

were compared. Lower cortisol levels were observed with laparoscopic nephrectomy (Marcovich *et al.*, 2001), distal pancreatectomy (Naitoh *et al.*, 2002) and partial pericardectomy (Walsh *et al.*, 1999) when compared to traditional open surgical techniques.

The acute phase protein C-reactive protein (CRP) has been used for grading surgical trauma by quantitatively reflecting the inflammatory activity induced by the surgery. CRP-levels varied with the degree of surgical trauma in dogs subjected to three standardized surgical procedures; traditional laparotomic OHE, laparoscopic assisted OHE and vasectomy in male dogs. (Kjelgaard-Hansen *et al.*, 2008).

Examples of the difficulties in this area are that different studies on the same biomarker resulted in different results. For example, a postoperative increase in glucose in dogs subjected to an open abdomen OHE procedure was demonstrated in a study but no difference was found in another study (Devitt *et al.*, 2005; Hancock *et al.*, 2005). A postoperative increase in levels of cortisol is associated with a traditional open abdomen OHE in contrast to a laparoscopic procedure (Devitt *et al.*, 2005; Hancock *et al.*, 2005) but other studies reported no difference (Freeman *et al.*, 2010; Luntang-Jensen, 2006) or a higher cortisol level in the laparoscopic group (Malm *et al.*, 2005). One study found that the duration of surgery was associated with a significant effect on cortisol in the postoperative period. Laparoscopic, micro-laparoscopic and hand-assisted laparoscopic nephrectomies were compared. The hand-assisted group was lowest at skin closure, highest at 2 hours post surgery and there was no difference between groups at 4 hours post surgery (Yoder & Wolf, 2005).

One area where the surgical stress response is used for evaluation purposes is when different anaesthetic protocols are used on groups of patients subjected to the same surgical procedure (Goldmann *et al.*, 2008; Ledowski *et al.*, 2005). The anaesthetic protocol will affect the stress response and the protocol with the least stress response is considered the most suitable for that surgery.

Another approach is to use the same anaesthetic protocol for all patients and compare the surgical stress response for different surgical methods. The method that yields the least surgical stress response is assumed to cause the least noxious stimuli. Blood pressure has been shown to increase in relation to and in proportion to traction applied to the mesentery (Holzer-Petsche & Brodacz, 1999). Similarly, a greater force applied to the ovarian pedicle (2-4 Newton), is associated with an increased minimal alveolar concentration (Boscan *et al.*, 2011).

Nociception

Nociception (initiated by nociceptors or sensory receptors) is the neural process of encoding and processing noxious stimuli. Actions that are potentially or actually tissue damaging are defined as noxious stimuli (Loeser & Treede, 2008). Generally, with a greater nociceptive stimuli inflicted, a greater stress response is expected whether it be hormonal, haematological or haemodynamic, provided the response is not blocked by anaesthesia or analgesia.

The magnitude of the surgical stress response is generally proportional to the inflicted injury (Desborough, 2000; Chernow *et al.*, 1987). The surgical stress response, in the form of intraoperative sympathetic activation, leads to release of noradrenalin which may increase blood pressure and heart rate (Guyton & Hall, 2006).

Plasma vasopressin levels have also been seen to increase during the course of abdominal operations (Desborough, 2000; Melville *et al.*, 1985). Activation of nociceptive somatic afferents excites hypothalamic neurosecretory cells, probably in conjunction with the A1 noradrenalin cell group in the ventral medulla (Day & Sibbald, 1990). Intraventricular infusion of noradrenalin inhibits water diuresis in conscious goats indicating interaction between sympathetic nervous system and vasopressin release at the hypothalamic level (Olsson, 1970).

Assessment of noxious stimuli – other methods

There are several other methods available for the measurement of intraoperative noxious stimuli. Electroencephalographic responses to noxious stimuli have been suggested for evaluation of nociception and efficacy of analgesics (Kongara *et al.*, 2010). Bispectral index is a monitor of anesthetic depth derived from the electroencephalogram, which is used in humans to measure level of hypnosis during anaesthesia with the purpose of reducing the risk of intraoperative awareness. One study on pharmacological effects involving dogs subjected to ovariohysterectomy reported no change of bispectral index, whereas blood pressure and heart rate did change during surgery (Belda *et al.*, 2011). There appears to be no clear link between bispectral index and nociceptive stimuli or between the bispectral index and haemodynamic changes during surgery. Bispectral index is therefore not suitable for measurements of surgical stress.

Surgical stress index is a multivariate index based on the normalized pulse beat interval and photoplethysmography and has been shown to correlate well to surgical nociceptive stimuli (Ahonen *et al.*, 2007; Struys *et al.*, 2007). In one study, surgical stress index was better at detecting nociceptive stimuli than

blood pressure or heart rate (Wennervirta *et al.*, 2008). On the contrary, surgical stress index, blood pressure and heart rate changed in response to changes in depth of analgesia before and after a bolus of fentanyl but surgical stress index did not reliably reflect intraoperative changes in plasma stress hormone levels (Ledowski *et al.*, 2010).

1.5.1 Intraoperative surgical stress in neutering of female dogs

Several studies have involved intraoperative measurements of physiological parameters in female dogs subjected to neutering. In some studies the main purpose was to compare different anaesthetic protocols, and blood pressure has been used as a parameter of intraoperative surgical stress (Shih *et al.*, 2008; Väisänen *et al.*, 2002; Benson *et al.*, 2000; Fox *et al.*, 1994). Apart from blood pressure, parameters such as heart rate, adrenalin, noradrenalin, cortisol, ACTH, glucose, insulin and β-endorphin were also used.

Increased frequency of intraoperative blood pressure measurements

In studies that involved neutering of female dogs the patients' haemodynamic reaction was measured with a set time interval (20, 15 or 5 minutes) without correlation to surgical events (Shih *et al.*, 2008; Goyenechea Jaramillo *et al.*, 2006; Acosta *et al.*, 2005; Miyake *et al.*, 2005). Different time intervals for measurements, different anaesthetic protocols as well as methods for statistical comparison are possible reasons for different results in these studies.

Studies have also been carried out where the measurements have instead been guided by specific surgical events. In a study on the effect of administration of medetomidine or acepromazine on the perioperative stress response in dogs that were subjected to OHE, blood pressure was measured at various time points guided by the surgery (Väisänen *et al.*, 2002). The potential anaesthetic sparing effect of local anaesthesia of the ovarian pedicle during OHE was studied (Bubalo *et al.*, 2008) and blood pressure was measured at defined time points as well as continuously during removal of the ovaries.

Advances in monitoring technology allow convenient and frequent measurement of blood pressure as well as storing data for later analysis. In the present thesis blood pressure and heart rate were measured once a minute and for evaluation, recorded data were split into phases guided by events of the surgery.

Biomarkers

A biological marker, or biomarker, is a substance that can be objectively measured and used as an indicator of a biological state or process. Noradrenalin (a neurotransmitter released from varicosities at terminal nerve

endings and adrenal medulla) has been used as an intraoperative biomarker of the sympathetic response to surgery (Väisänen *et al.*, 2002; Benson *et al.*, 2000). Whilst a reuptake of noradrenalin occurs, a proportion diffuses into circulation and is excreted in urine where it is stable at low pH (Roberts *et al.*, 2010). Therefore, urinary noradrenalin concentration during surgery may vary with surgical events and degree of inflicted tissue trauma.

Vasopressin, or antidiuretic hormone, is released from the pituitary into systemic circulation in response to the increase in osmotic pressure of blood plasma at dehydration, or secondary to hypovolemia or acute hypotension. The hormone increases the kidneys' resorption of water but can also cause vasoconstriction and increase the blood pressure. Vasopressin levels increases in response to pain and also during and after surgery, predominantly abdominal surgery (Goldmann *et al.*, 2008; Furuya *et al.*, 1993; Murakawa *et al.*, 1989; Haas & Glick, 1978), and more specifically gut manipulation (Nussey *et al.*, 1988; Melville *et al.*, 1985). Therefore vasopressin was of interest as a biomarker of surgical stress and noxious stimuli during neutering of female dogs. However, the mechanism of the intra- and postoperative release of vasopressin and the influence of blood pressure alterations during surgery is not fully understood (Carvalho *et al.*, 2011).

In a comparison of endovascular and conventional abdominal aortic aneurysm repair, the conventional technique was associated with postoperative increase of vasopressin levels (Kataja *et al.*, 2007). Similarly, in a comparison of different approaches for lumbar spine surgery, an intraoperative increase of vasopressin levels was associated with the method in which more postoperative analgesics were used (Yoo *et al.*, 2009). However, there are few canine studies on vasopressin in the perioperative period (Bergström *et al.*, 2010; Hauptman *et al.*, 2000).

It was hypothesised that intraoperative measurements of noradrenalin and vasopressin would be suitable tools, besides blood pressure and heart rate, to identify differences between surgical methods and to evaluate new techniques.

2 Aims of the thesis

The aim of the work presented in this thesis was to develop a resorbable device for ligation purposes that enables safe ligation with minimized noxious stimuli. An increased understanding of the physiological processes involved during surgery may be of use for comparison and evaluation of new surgical methods and techniques.

The specific aims were to:

- Test the device for ligation of renal arteries and vessels embedded in tissue, the ovarian pedicle.

- Evaluate the biocompatibility of the device used for ligation of the ovarian pedicle of dogs with ultrasound and histology.

- Investigate if frequent measurements of blood pressure and heart rate can detect variations in noxious stimuli related to specific events during surgery and between different surgical methods.

- Investigate if plasma vasopressin levels and urinary noradrenalin/creatinine ratio change in relation to specific events during surgery.

- Investigate if removal of one ovary can be used as a control for removal of the other ovary in the same dog in studies evaluating the surgical stress response.

3 Materials and methods

3.1 Development and design of the device

The device (Figure 2) was designed (paper I) using computer aided design (Solidworks, Dassault Systèmes SolidWorks Corporation, Concord, USA) and consisted of a case with a channel that contained a locking mechanism. A flexible band, in part perforated, which thus formed a ladder structure, was attached to the locking case. The end of the flexible band was fed into and through the channel of the locking case and a loop was formed. At the interface of the flexible band and the locking case there were ridges at each side of the locking case that aligned towards the band surface and connected with tissue caught in the loop. If the flexible band were fully pulled through the locking case, a protrusion at the connection between locking case and flexible band merged with the first hole in the perforated part of the flexible band. Tissue was then engaged at both ridges at each side of the locking case. The protrusion at center pushed the tissue up through the first hole in the perforated band and the tissue was squeezed into a zigzag pattern.

As the perforated part of the band was introduced into the locking case the locking mechanism merged with the perforated parts of the flexible band. The interlocked position prevented motion in the opposite direction.

In a second version of the device, the locking case was reduced from 105 mm^3 to 74 mm^3, the corners were rounded off and the walls were made thinner. In addition, the thickness of the flexible band was increased from 0.55 mm to 0.65 mm.

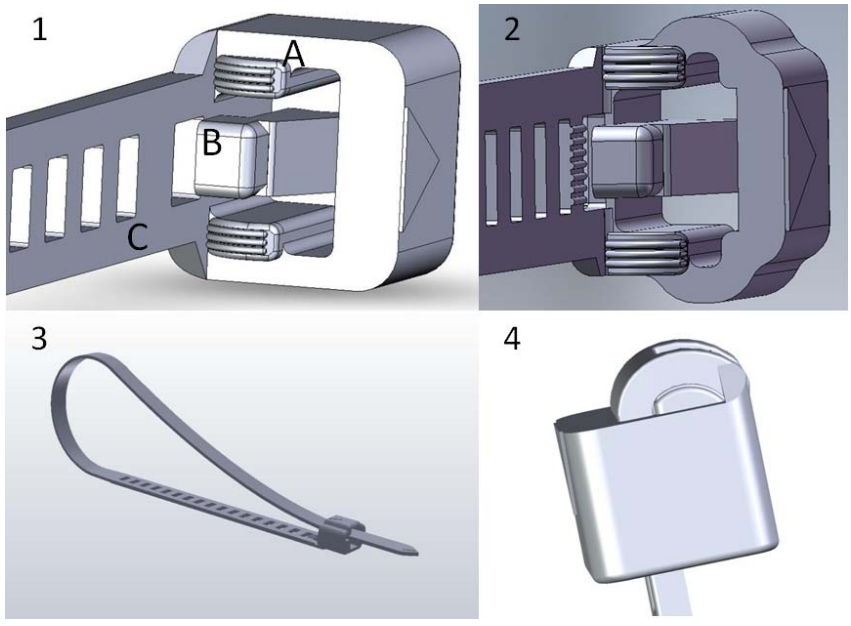

Figure 2. Design of the device used in the study. 1. Version one of the device. 2. Version two of the device with a downsized locking case. 3. The formation of the loop of the device. 4. Complete closure of the loop, the locking case with the flexible band fully pulled through. A: Ridges at each side of the locking case. B: Protrusion at centre. C: The flexible band, in part perforated.

Prototypes

Prototypes of the device were manufactured using rapid prototyping of polyamide-powder (HD SLS technique, high definition selective laser-sintering, PA2200 and Formiga P100 from EOS GmbH Electro Optical Systems, Germany), Figure 3.

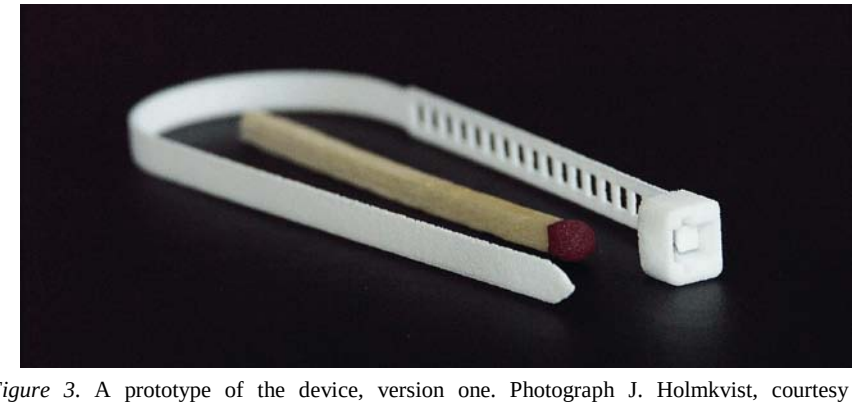

Figure 3. A prototype of the device, version one. Photograph J. Holmkvist, courtesy of Teknikförlaget.

3.2 Moulds and injection moulding

Pilot manufacture of the perforated band

A steel mould was manufactured (Mecdon, Laxå, Sweden) and used for injection moulding of two different dimensions of the flexible perforated band of the device (Figure 4).

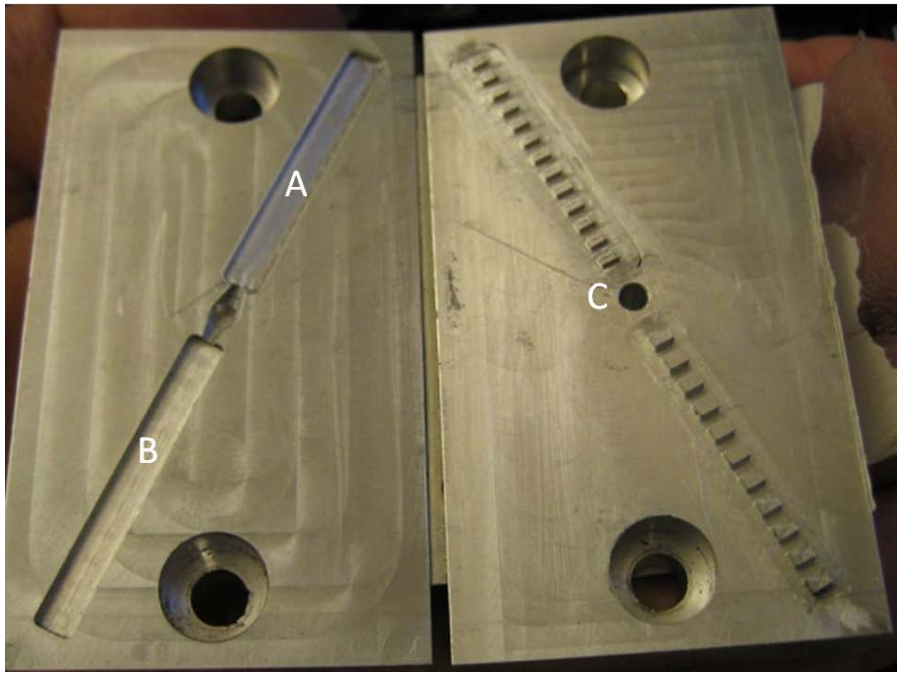

Figure 4. A mould for manufacturing the perforated part of the band in two different dimensions, 0.5 mm and 1 mm. A: Cavity for the thinner perforated band. B: Cavity for the thicker perforated band. C: Inlet into the mould. Photograph by O. Höglund.

Moulds of the device

Steel moulds were manufactured for injection moulding of the first and second versions of the device. The mould for the first version of the device (Figure 5) was equipped with an inset which was manually removed. In the mould for the second version an ejector mechanism at the locking case of device was added to the mould. Both heating rods and water for cooling could be attached to the second version of the mould.

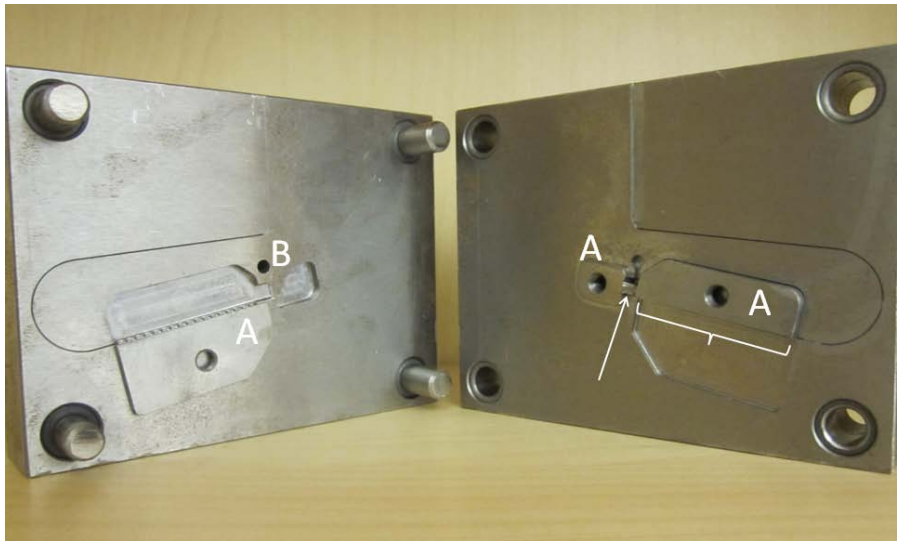

Figure 5. The mould for manufacturing the first version of the device. A: Inset to create the cavity of the device in the mould. B: Inlet of the melted polymer. Brackets: cavity for the perforated band. Arrow: Place for formation of the locking case. Photograph by O. Höglund.

Injection moulding of a resorbable polymer

The resorbable polymer polydioxanone (Resomer® X, Boehringer Ingelheim Pharma GmbH, D-55216 Ingelheim, Germany) was heated above melting temperature and injected into the mould. Batches of low and high inherent viscosities were used. Solidification and crystallization occurred, the mould was opened and the device removed. The devices were placed in aluminum bags, flushed with dry nitrogen gas and sealed.

Tensile testing

The tensile strength of the flexible band (approximately 4 cm) was measured using a 5544 Single Column Testing System (Instron, USA). The samples were pulled to break at a rate of deformation of 40 mm/min. The tensile strength was determined as the maximum failure load. Eleven samples manufactured from each of the polymer batches were tested.

3.3 Animals, ethical permit and informed consent

The protocols of the studies were approved by the Swedish Board of Agriculture and the Uppsala Animal Ethics Committee, Sweden. An informed consent was obtained from each owner of the privately owned dogs prior to inclusion of their dog. The operations were performed at the University Animal Hospital (UDS), Swedish University of Agricultural Sciences (SLU).

Initially, the device was tested in one euthanized dog and six pigs anesthetized for reasons not associated to these studies (paper I).

Forty female dogs were neutered during the course of this thesis. They were all deemed as overtly healthy and neutered at the owners' request. Two of them were research dogs (paper I), owned and kept by the Department of Clinical Sciences, SLU. The other 38 dogs were privately owned.

In nine of the 40 dogs (body weight 3–31 kg) the device was tested for ligation of both ovarian pedicles (paper II). In five of the dogs (body weight 7–29 kg) the device was used for ligation of the ovarian pedicle on one side and traditional suture was used on the other side (unpublished).

Sixteen of the dogs (body weight 10–43 kg) were used to compare the intraoperative surgical stress response during two different surgical techniques for neutering female dogs (paper III). Eight of them were neutered by LOE and eight by OHE where traditional suture was used for ligation.

In ten of the dogs (body weight 14–32 kg) the intraoperative surgical stress response to ligation of each separate ovarian pedicle was studied (paper IV).

3.4 Functionality of the device

3.4.1 Test of the device in a euthanized dog

The device was used for ligation of the ovarian pedicle in a euthanized dog (*post mortem*). The ovarian pedicle, with the device attached to it, was removed and examined histologically (paper I).

3.4.2 Ligation of renal arteries and test of tissue grip

The ability of the device to ligate a single artery was tested on twelve renal arteries in six pigs. The tissue grip and the ability of the device to resist a ligature slip-off were tested by an applied force of 10 N using a dynamometer attached to the device (paper I).

3.4.3 Ligation of the ovarian pedicle in dogs

Surgery

The performed surgery is described in section 3.5.

Bilateral ligation

The device was used for bilateral ligation of the ovarian pedicles in nine dogs (paper II). The larger version (version one) was used in the first two dogs and the smaller (version two) in the other seven dogs.

Unilateral ligation

The device was used for unilateral ligation of the ovarian pedicle in an additional five dogs where traditional suture material (polydioxanone, PDS, Ethicon) was used on the contralateral side (unpublished). The consecutive order of device or suture and left ovarian pedicle or right ovarian pedicle was decided before the experiment started.

3.4.4 Biocompatibility

Follow up by ultrasound

The fate of the device and the local tissue response were repeatedly examined using ultrasound approximately monthly until no acoustic shadowing was visible in any of the 14 dogs (paper II and section 4.2.4).

Histology

Seven and 18 months following surgery, post-mortem examinations were performed in the two research dogs where the first (larger) version of the device was used (paper II). The dogs were euthanized for reasons not related to this thesis. Tissues of the ovarian pedicles were examined by histology (paper II).

3.5 Surgery

3.5.1 Anaesthesia and analgesia of the dogs

The drugs used for premedication of the dogs were acepromazine (sedative), carprofen (analgesic) and methadone hydrochloride (analgesic). Glycopyrrolate (anticholinergic, parasympatholytic, reduces salivary secretion) was used for premedication (papers II and III). Anaesthesia was induced with intravenous propofol (Rapinovet vet, Schering-Plough) and maintained with 2% isoflurane inhaled in a mixture of oxygen and air. All dogs breathed spontaneously and a crystalloid solution containing glucose, sodium chloride and sodium acetate (Rehydrex with glucose, Fresenius Kabi AB) was used for fluid replacement at approximately 10 mL/kg/h.

3.5.2 Ovariohysterectomy – open abdomen surgery

The surgical field was aseptically prepared before surgery and the dog was placed in the Trendelenburg position (Bernstein *et al.*, 1999; Belloni, 1949; Meyer, 1885) on the operating table. The abdomen was opened along the midline (*linea alba*) from slightly cranial of the umbilicus to approximately the midpoint between the umbilicus and the pubis bone.

The uterine horn was lifted at its cranial end and the ovarian pedicle was stretched for access to the ovary and the vessels of the *mesovarium*. A hole was made in the broad ligament close to the ovary and haemostatic forceps were placed across the ovarian pedicle to create a groove where after the haemostatic forceps were moved closer to the ovary.

When the ovarian pedicle was ligated with the device (paper II and unpublished unilateral group), the flexible band of the device was passed half-way through the hole in the broad ligament and its end was introduced through the case with the locking mechanism. The loop formed by the device around the ovarian pedicle was tightened with one hand to compress the tissue and blood vessels within the loop.

The ovarian pedicle was ligated with one ligature (paper III) or two ligatures (paper IV). After transection of the ovarian pedicle it was inspected for haemostasis. In the studies presented in paper II the other ovarian pedicle was subsequently ligated and transected whereas in the studies presented in paper III the mesometrium was ligated before the procedure was repeated at the opposite side.

The cervix was ligated and the uterine arteries were separately ligated where after the cervix was transected. Before closure, the abdomen was inspected to confirm satisfactory haemostasis (paper II-IV).

The *linea alba* and subcutaneous tissues were closed with resorbable sutures. The skin was closed in accordance with the surgeon's preferences.

In paper IV two pauses were introduced. The first pause of eight minutes was introduced after the abdomen was opened. The other pause of 15 minutes was introduced after the first ovarian pedicle was transected and haemostasis was verified. During the pauses the wound was covered with a wet surgical sponge warmed to body temperature.

3.5.3 Ovariectomy - laparoscopic surgery

The dogs were anesthetized, the surgical field was aseptically prepared before surgery and the dogs were placed in the Trendelenburg position. A 2-cm skin incision was made midway between the umbilicus and the pubic bone. The abdomen was insufflated with carbon dioxide to approximately 8 mm Hg pressure (Wiest Laparoflator Electronic 3509) through an inserted Verres' cannula. The Verres' cannula was replaced with a 10-mm-troacar (Storz) and a 10 mm, 30° telescope (Karl Storz endoscopy) was inserted in the troacar. Under laparoscopic guidance, two further portals were established, one caudal and one cranial to the umbilicus along the midline. The telescope was redirected to the middle portal. The dog was tilted sideways to facilitate access to the uterine horn and the ovarian pedicle (*mesovarium*). Two endoscopic

grasping forceps (Karl Storz endoscopy) were used to locate and grasp the ovary. As the uterine horn was held close to the *ligamentum proprium* and was lifted, the ovarian pedicle was stretched for access. A SonoSurg harmonic scalpel (Olympus) or a LigaSure unit (LigaSure Atlas device 10 mm, Tyco, Covidien) sealed and cut the ovarian pedicle and the uterine horn, at the uterotubal junction.

The first ovary with its bursa was placed caudally in the abdomen for later retrieval. The dog was tilted in the opposite direction, the procedure for ovariectomy was repeated at the opposite side and the other ovary was located and detached with the other instrument. The abdomen was inspected to verify haemostasis and the ovaries were removed through the caudal portal. The openings of the portals were closed with 2–0 polydioxanone (PDS, Ethicon) and subcutaneous tissues and skin were intradermally sutured with 3–0 poliglecaprone 25 (Monocryl, Ethicon).

3.6 The intraoperative surgical stress response

3.6.1 Blood pressure and heart rate

Systolic, diastolic, and mean blood pressures and heart rates were registered by an anaesthetic monitoring device (LifeWindow™ 6000, Digicare Biomedical Technology) in all operations. A non-invasive oscillometric method with an automatic pneumatic cuff was used. Data were stored once per minute.

3.6.2 Definition of surgical phases

Guided by surgical events, the recorded data were split into phases to facilitate intraoperative comparison (paper III-IV and data from the nine dogs used in paper II). Phase zero started when the arterial blood pressure and heart rate had stabilized after induction of anaesthesia. Steady state was defined as the sum of the differences between three consecutive measurements being $< \pm 10$ units or four consecutive measurements being $< \pm 15$ units. In paper IV phase zero was defined as 10 minutes prior to skin incision.

Phase one started when the skin was incised and lasted until visualisation of the uterine horn or ovarian suspensory ligament. Phase two started with the manipulation of the first uterine horn to access the ovarian pedicle and ended with cutting the second ovary free (LOE) or removal of the second ovary and severance of the mesometrium (OHE). Phase three included removal of the ovaries from the abdomen (LOE) or ligating and cutting the cervix (OHE) and ended with abdominal closure of *linea alba*. The same definitions were used in the dogs provided with the device in study II and in the dogs with the device implanted unilaterally during OHE.

Two pauses were introduced, one after opening the abdomen and the other after removal of the first ovary (paper IV).

3.6.3 Comparison of the surgical stress response

The systolic pressure and heart rate were used for comparison of the surgical stress response in dogs subjected to elective laparoscopic ovariectomy or open abdomen ovariohysterectomy (paper III). The results were compared to measurements in experiments using the device (paper II, unpublished data). The stress response to opening of the abdomen and removal of the two ovaries were compared (paper IV).

3.6.4 Urinary noradrenalin and creatinine ratio

Seven urine samples for analyses of noradrenalin and creatinine were collected in ten of the dogs. The first urine sample was collected at home by the owners and the other samples were collected before and during surgery (paper IV).

Concentrations of urinary noradrenalin were analyzed using human ELISA assays (IBL International Gmbh, Hamburg, Germany) and of creatinine using another ELISA assay (Assay Designs, Ann Arbor, Michigan, USA) validated for canine urine in the laboratory at the Department of Anatomy, Physiology and Biochemistry, SLU. The noradrenalin concentration in urine (nmol/L) was corrected for differences in glomerular filtration rates by presenting the ratio of noradrenalin concentration and creatinine concentration (mmol/L).

3.6.5 Plasma vasopressin concentration

Seven blood samples were collected, one before anaesthesia and the others during surgery in ten dogs (paper IV). Concentrations of vasopressin were analyzed using an EIA assay (Enzo Life Sciences, Farmingdale, NY 11735, USA).

3.6.6 Statistics

Data are presented as means ± SE or as least square means ± SEM. The SAS package (SAS, 2008), version 9.1, was used for all data analyses. Mixed linear models were used when data involved repeated measurements on the same dogs (Littell *et al.*, 2006; Fitzmaurice *et al.*, 2004).

The dependence between observations over time was modeled with an unstructured covariance matrix. For all dependent variables, the model included the effect of phase, surgical method, and the interaction between these variables. Tests comparing the surgical methods for potential differences in change over time were constructed with linear contrasts in the interaction terms.

Student’s t-test was used for comparison of data of tensile tests (paper I) and ultrasound examinations (paper II). Results of ultrasound examinations are presented as mean number of months (minimum – maximum).

4 Results

4.1 Development and design of the device

Pilot manufacture of the perforated band

Injection moulding of the two different dimensions of the perforated band in PDO showed satisfactory flow distance and device strength at a thickness of 0.5 mm (unpublished). Band thickness of 1 mm resulted in an unacceptable pliability.

Injection moulding of the device and tensile testing

The device, its locking case and the flexible band, was manufactured by injection moulding of PDO. The design of the mould for version two of the device resulted in flexible bands of greater length. A lower inherent viscosity facilitated flow distance in the mould but resulted in devices of lower tensile strength (paper I).

4.2 Functionality of the device

4.2.1 Test of the device in a euthanized dog

The tissue of the ovarian pedicle was compressed by the device in the euthanized dog (Figure 6) and the vessels were compressed as verified by histology (paper I).

4.2.2 Ligation of renal arteries and tissue grip

Twelve renal arteries in six pigs were ligated (Figure 6). Haemostasis was verified for approximately five minutes. At applied force of 10 N no sliding along the vessels was observed (paper I).

Figure 6. Left: The ovarian pedicle of the euthanized dog was ligated and compressed with the first version of the device. Centre and Right: Ligation of the renal arteries in pigs with the second version of the device. Photograph by O. Höglund.

4.2.3 Ligation of the ovarian pedicle in dogs

The device was used for ligation of both ovarian pedicles in nine dogs subjected to OHE (paper II). Haemostasis of the ovarian pedicles was achieved at all pedicles except in one dog in which hemoabdomen was diagnosed six hours post surgery. The dog was re-anesthetized, laparotomy was performed, the loop of the device was further tightened, haemostasis was achieved and the abdomen was closed.

Haemostasis was also achieved of the ovarian pedicles in the five dogs provided with the device on one ovarian pedicle and traditional suture on the other ovarian pedicle (unpublished).

4.2.4 Biocompatibility

Follow-up by ultrasound

Ultrasonography revealed a hyperechoic device that caused acoustic shadowing (Figure 7), which decreased over time thereby indicating gradual resorption. The results of the ultrasound examinations of the nine dogs where the device was used for ovariohysterectomy (paper II) are presented in Table 2, showing the number of months a hyperechoic structure, acoustic shadowing, and fluid were observed from the region of the ovarian pedicle.

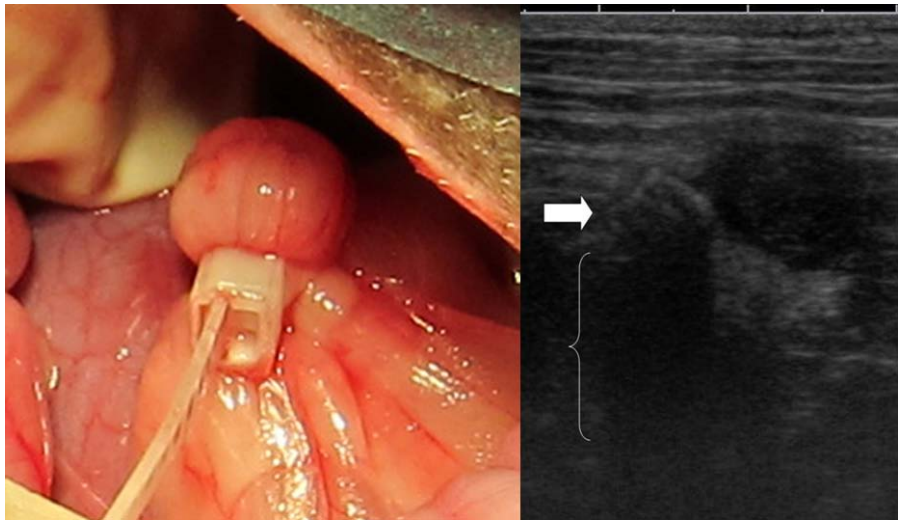

Figure 7. Left: The ovarian pedicle ligated with the second version of the device. Right: Appearance of the device on ultrasound performed eight weeks after surgery. The device (indicated by arrow) was hyperechoic and caused acoustic shadowing (indicated by brace).

Table 2. *The number of months (mean and range) a hyperechoic structure, an acoustic shadow, and fluid were observed by ultrasound from the region of the ovarian pedicle. Data are from examinations of nine ovariohysterectomized dogs (paper II)*

	Hyperechoic structure	Acoustic shadowing	Fluid present	Devices (n)
Device version one	2.5 (1.8 – 3.0)	4.3 (3.8 – 5.3)	2.0 (0.9 – 3.0)	4
Device version two	1.9 (1.0 – 3.4)	2.9 (1.0 – 3.9)	0.3 (0 – 2.9)	14
P-value	*n s*	$P = 0.008$	$P = 0.005$	

n s: non-significant

The results of the ultrasound examinations of five ovariohysterectomized dogs where one ovarian pedicle was ligated with the device and the other was ligated with traditional suture are presented in Table 3.

Table 3. *The number of months (mean and range) a hyperechoic structure, an acoustic shadow, and fluid were observed from the region of the ovarian pedicle on ultrasound examinations in five dogs. One ovarian pedicle was ligated with the device and the other ovarian pedicle was ligated with traditional suture material*

	Hyperechoic structure	Acoustic shadowing	Fluid present
Device	2.5 (0 – 5)	2.4 (0 – 3.9)	0
Ligature	0.9 (0 – 2.4)	2.7 (1.9 – 3.9)	0
P-value	$P = 0.13$	$P = 0.72$	

Post mortem examination

The first two dogs where the device was used (research dogs) were euthanized at seven and 18 months following surgery for reasons not associated to this thesis. The post-mortem examinations demonstrated a transient inflammatory response which was restricted in extent (Figure 8).

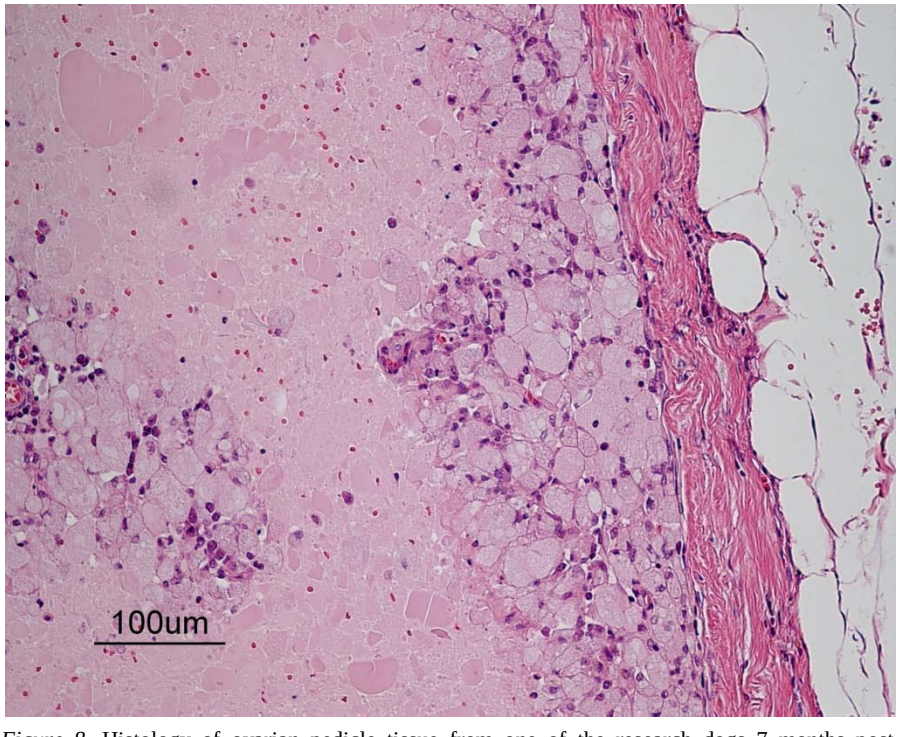

Figure 8. Histology of ovarian pedicle tissue from one of the research dogs 7 months post surgery. The image shows remains of the device and a local tissue response. Left and Centre: Material mixed with cellular debris. Right: Layers of activated macrophages and peripheral fibrous capsule. Far right: Adipose tissue.

4.3 The intraoperative surgical stress response

4.3.1 Blood pressure and heart rate

Blood pressure was measured in all dogs during surgery. In the published paper (II) focus was on functionality and biocompatibility of the device and changes in blood pressure were not reported. The effect on blood pressure in the nine dogs provided with the device is shown in Table 4.

Systolic blood pressure differed between phases during both LOE and OHE (paper III). In the LOE group mean systolic pressure increased from 98 to 105 mm Hg at insertion of the portals. During removal of the ovaries mean systolic pressure increased to 112 mm Hg, which differed significantly from phase zero, but not from insertion of portals. In the OHE group mean systolic pressure was 83 mm Hg and 87 mm Hg at phase zero and at incision of the abdomen (n.s.) but systolic pressure increased to 112 mm Hg during removal of the ovaries ($P < 0.0001$).

In the dogs where the device was applied bilaterally (paper II) mean systolic pressure increased 15 mm Hg when the abdomen was opened (Table 4). The blood pressure did not increase further during removal of ovaries.

The results of blood pressure measurements in the dogs where the device was applied unilaterally are presented in section 4.3.3.

Overall comparison between papers II and III revealed that ligation of ovaries was accompanied by a mean systolic pressure of 110 - 112 mm Hg, regardless of previous values.

The heart rate did not change in the open abdomen group or the laparoscopic group (paper III).

Table 4. *Systolic blood pressure (mm Hg) in nine dogs subjected to laparotomic OHE with ligation of the ovarian pedicle with the device*

Method	Phase zero	Phase one	Phase two	Phase three
DEVICE	84 ± 6	99 ± 7	110 ± 8	96 ± 7
Relative to previous phase		15 ± 3 ($P = 0.02$)	11 ± 4 ($P = 0.35$)	-14 ± 3 ($P = 0.01$)

Data are LSmeans ± SEM.

4.3.2 Comparisons between groups

Linear contrasts were used for comparison of groups. A greater increase in blood pressure was observed during OHE compared to LOE during phase two, when the ovaries were removed (paper III).

A smaller increase in blood pressure at removal of ovaries (phase one to phase two) was observed when the device was used compared to when a

traditional suture was used (P = 0.03). The increase in blood pressure at removal of ovaries did not differ (P = 0.6) between the laparoscopic group (paper III) and dogs where the device was used (Table 4).

4.3.3 Removal of first vs. second ovary

In the five dogs where the device was used on one ovarian pedicle and traditional suture on the other ovarian pedicle, removal of first ovary tended to increase systolic pressure more than removal of the second ovary (unpublished). At phase zero mean systolic pressure was 107 mm Hg and at removal of the first ovary it increased to 143 mm Hg and at removal of the second ovary to 133 mm Hg (P < 0.0001 versus phase zero for both ovaries; P < 0.07 between ovaries).

Two pauses were introduced between phases to investigate if the increase in blood pressure and heart rate were similar at removal of the first and the second ovary (paper IV). After removal of the first ovary followed a 15 min pause. After the pause the blood pressure decreased (P = 0.02) to a level that was higher than before removal of first ovary (P = 0.02). The results showed that removal of the first ovary caused a relatively larger (P < 0.0001) rise in blood pressure (+ 20 mm Hg) compared to removal of the second ovary (+ 7 mm Hg) when increase relative to previous phase was compared. The results were similar for the heart rate; removal of the first ovary caused a relatively larger (P = 0.0002) increase in heart rate (+ 17 bpm) compared to removal of the second ovary (+ 6 bpm).

4.3.4 Urinary noradrenalin and creatinine ratio

The mean urinary noradrenalin/creatinine ratio in samples collected at home by dog owners was lower compared to that seen during anaesthesia (paper IV). The mean urinary noradrenalin/creatinine ratio did not differ between samples collected during surgery.

4.3.5 Plasma vasopressin concentration

Levels of plasma vasopressin showed a ten-fold increase at removal of both ovaries compared to sample zero before anaesthesia. Plasma vasopressin and blood pressure changed in synchrony (paper IV).

4.3.6 Anaesthesia

The same drugs were used for analgesia and anaesthesia in the performed studies with the exception that glycopyrrolate was excluded in the study presented in paper IV. Care was taken to allow sufficient time for blood pressure and heart rate to stabilize before any surgical intervention was started,

but mean systolic blood pressure ranged from 76 (paper IV) to 107 mm Hg (in the dogs with unilaterally applied device).

Generally, anaesthesia was regarded as stabile and only minor adjustments were made on few occasions. Data on intraoperative systolic blood pressure and heart rate are shown in papers III and IV.

Hypotension was observed after induction of anaesthesia and blood pressure and heart rate increased at ligation of ovarian pedicles (paper IV).

5 Discussion

A new resorbable device was developed and manufactured by injection moulding of the polymer polydioxanone. Effective haemostasis and secure ligatures were demonstrated on renal arteries and on ovarian pedicles during elective neutering in female dogs. The device was biocompatible as shown by its gradual resorption.

For evaluation of the intraoperative surgical stress in neutering female dogs, frequent non-invasive registrations of systolic blood pressure in combination with analysis of plasma vasopressin concentration, were the most promising parameters.

5.1 The device

5.1.1 Injection moulding

The resorbable polymer polydioxanone could be injection moulded. Initially the perforated band was manufactured in two different sizes which provided some information concerning possibilities and limitations in the dimensioning of the device and flow distance of polydioxanone in the mould. Thereafter, the device, including its locking case and the flexible band, was manufactured by injection moulding of polydioxanone. Experiences from the use of the mould of the first version of the device initiated further development and resulted in a second mould equipped with a hydraulic ejector. In addition, repositioning of the inlet into the cavity of the mould resulted in devices with longer flexible bands.

A higher temperature during injection moulding and lower inherent viscosity facilitated flow in the mould, but the increased temperature had to be balanced against detrimental change to the polymer as well as the increased time needed for solidification and crystallization. The devices were manufactured in a research and development setting with the consequence that

they were not necessarily identical. In some of the devices an uneven or rough surface was apparent, probably because more time would have been necessary for further solidification and crystallization to occur (Ticona, 2009; Janeschitz-Kriegl, 1992). The time needed for these processes can be shortened by cooling the mould. However, this would result in a slower manufacturing process and be impractical from an industrial perspective. As more experience was gained during manufacturing of the device, it became obvious that there are limitations with polydioxanone. Further development should consider alternative materials, with the aim to improve both simplicity and consistency of the manufacturing processes, as well as ease of tightening the device.

5.1.2 Surgery

The device was tested for ligation of renal arteries in pigs and ovarian pedicles in dogs. Once the flexible band of the device was passed around the tissue and into the locking case, the loop of the device could be tightened with one hand. The other hand was free to safeguard other abdominal tissue.

The design of the resorbable device allowed complete closure of its loop. The renal artery inside the loop was compressed in a zigzag pattern which yielded excellent tissue grip and haemostasis. This was an improvement from traditional cable ties in which the loop did not close completely (Zagraniski, 1980). When applied at the renal artery the closed device restricted slippage at an applied force of 10 N, which was similar to another study of holding power of surgical clips (Hsu, 2006). The device was deemed to exceed clinically relevant forces necessary to resist high intravascular pressure.

As discussed in paper II, an uneven or rough surface of the flexible band may be the result of the injection moulding process. Instead of a smooth passage of the band through the locking case a rough surface may cause friction and the loop may not be tightened completely. This could have been the reason for postoperative haemorrhage from one of the devices. No haemorrhage was observed during the ovariohysterectomy, probably due to hypotension during the operation. After surgery bleeding started and the loop of the device had to be further tightened during a re-operation. This demonstrated the importance of improving the manufacturing process and increasing the device's pliability.

5.1.3 Biocompatibility

Biocompatibility has been referred to as the ability of a material to perform its desired function with respect to a medical therapy. The material should not elicit any undesirable local or systemic effects in the recipient, but optimize the outcome of the therapy (Williams, 2008; Williams, 1987).

Together with the five dogs provided with the device unilaterally, a total of 23 ovarian pedicles in 14 dogs were ligated with the device. Repeated ultrasound examinations of these dogs showed that the device was gradually resorbed. Post-mortem examinations were performed in two dogs where the larger version of the device was used and histopathology demonstrated a transient tissue reaction which was restricted in extent. It appears safe to conclude that the device was biocompatible.

5.2 Surgery and intraoperative stress parameters

Anaesthesia and analgesia

Care was taken to standardize procedures and the same combination of analgesic and sedative drugs were used in the experiments with the exception of paper IV in which glycopyrrolate was excluded. Glycopyrrolate, which is parasympatholytic, is used to avoid bradycardia and it may increase blood pressure (Dyson & James-Davies, 1999). Hypotension is a common perianaesthetic complication and is defined as blood pressure of < 80 mm Hg and/or a mean arterial pressure (MAP) of < 60 mm Hg (Mazzaferro & Wagner, 2001; Waddell, 2000). In experiments with glycopyrrolate the mean systolic pressure was above 80 mm Hg, but without glycopyrrolate it was lower before surgery (paper IV). The heart rate was consistently elevated when glycopyrrolate was used (paper III). These results show the influence of the drugs used for premedication (Ingvast-Larsson *et al.*, 2010; Väisänen *et al.*, 2002). It could be one reason for the wide range in basal levels of blood pressure and heart rate between the studies. In comparison between groups, linear contrast analysis was used for evaluation of the magnitude of the increase at a surgical intervention.

Laparoscopic versus open abdomen surgery

The origin of the pain that predominates following neutering of female dogs has been the subject of debate. It is unclear if the difference in signs of postoperative pain between dogs neutered with a laparoscopic or an open abdomen procedure is due to differences in the size of the incision (Luntang-Jensen, 2006), less traction applied to the ovarian pedicle during LOE, or other unknown parameters (Mayhew, 2011; Epstein *et al.*, 2010; Fitzpatrick *et al.*, 2010).

The cardiovascular response during anaesthesia is known to be related to noxious surgical interference (Bubalo *et al.*, 2008). Systolic pressure and heart rate were used in this thesis for comparison between an open abdomen ovariohysterectomy and laparoscopic ovariectomy. A greater rise in blood

pressure was found at removal of the ovaries during open abdomen ovariohysterectomy with traditional ligatures compared with laparoscopic ovariectomy (paper III), which indicates that laparoscopic ovariectomy caused less noxious stimuli. The results may explain the previously unknown mechanism behind the quicker recovery after LOE compared with OHE (Mayhew, 2011). Earlier studies support that a faster recovery (Culp *et al.*, 2009) and less signs of postoperative pain (Devitt *et al.*, 2005; Hancock *et al.*, 2005; Davidson *et al.*, 2004) are associated with the laparoscopic procedure compared with the open abdomen approach for neutering female dogs.

Furthermore, the increase in blood pressure was greater at removal of ovaries compared to opening of the abdomen during OHE. This was confirmed in paper IV. Therefore, it appears that the incision during OHE is not the main contributor to postoperative pain. Likewise, there was no difference in postoperative pain between the one and three portal groups (Case *et al.*, 2011). An interpretation of these results could be that the surgeon should not hesitate to make a larger incision if it enables easier access to the ovary and thereby less traction to the ovarian pedicle. An indication supporting that suggestion is a study where no difference in postoperative pain was demonstrated between groups of shorter versus longer incision in dogs subjected to ovariectomy or ovariohysterectomy (Peeters & Kirpensteijn, 2011).

The device and noxious stimuli

After function and biocompatibility of the device had been tested it was investigated if it enabled a reduced noxious stimulus which is important for postoperative pain and recovery time. The increase in systolic blood pressure was less when the device was used for bilateral ligation compared to ligation with suture. It is an indication that the use of the device enabled less noxious stimuli at ligation of the ovarian pedicle compared with suture ligation. A comparison of device and suture in the unilateral group was difficult since the first ligation caused a greater increase in systolic pressure compared to the second. Further studies, with a larger number of patients, would be required for definitive conclusions to be drawn concerning the use of the device and elicited noxious stimuli. A prospective randomized comparative study is yet to be performed.

It would appear that local anaesthesia could possibility overcome the problems of noxious stimuli both from skin incisions and removal of ovaries. A study on postoperative pain in dogs subjected to ovariohysterectomy showed no additional analgesic benefit on postoperative pain of local anaesthesia at the incision (Fitzpatrick *et al.*, 2010). However, signs of postoperative pain were reduced by intraperitoneal administration of bupivacaine in dogs subjected to

laparoscopic ovariectomy (Kim *et al.*, 2011). The results support the suggestion that there is less involvement of the incision wound in postoperative pain compared to removal of the ovaries.

Surprisingly, in a study on intraoperative infiltration of the mesovarium with lidocaine prior to removal of ovaries, no anaesthetic sparing effect or effect on autonomic response could be seen. The study offers no data on potential postoperative analgesic benefit (Bubalo *et al.*, 2008). Data revealed that the blood pressure increased already at injection. The next increase in blood pressure during the subsequent removal of ovaries did not differ between the groups receiving local anaesthesia and the control group. In view of the results in paper IV it cannot be excluded that the injections affected the following response.

There are human studies that demonstrate a positive effect of incisional local anaesthetic infiltration (Moiniche *et al.*, 1998). Incisional local analgesia should not be overlooked or neglected despite conflicting or negative results in several studies.

One dog as its own control

The investigations presented in paper III showed that blood pressure reacted to each surgical intervention, and therefore was useful for evaluation of noxious stimuli caused at use of the device. However, using the increase in blood pressure to compare ligation of the ovarian pedicles with a device on one side and suture on the other did not seem appropriate. It turned out that the greatest response was obtained at ligation and removal of the first ovary.

In paper III the second ovary was removed immediately after the first and the respective responses in blood pressure was not discussed. In retrospect, it would appear from the data in paper III that the ligation and removal of the first ovary caused a greater blood pressure increase than the second ovary. The same pattern was seen in paper IV although a 15 min pause was introduced between removals of the two ovaries. If that pause is neglected the blood pressures curves in paper III and paper IV are similar.

In paper IV plasma vasopressin and urinary noradrenalin concentrations were included to get further information on the physiological reactions to noxious stimuli.

The urinary noradrenalin/creatinine ratio was low before surgery and increased after induction of anaesthesia but did not change during surgery. This is in agreement with previous studies on neutering of female dogs (Väisänen *et al.*, 2002; Benson *et al.*, 2000). It may be that, due to the low blood pressure and release of vasopressin, which concentrates urine, there are limitations concerning intraoperative measurements of biomarkers in urine.

Previously, an increase in vasopressin levels after 1 and 2 hours of surgery, involving both soft tissue and orthopedic procedures, was reported. In that study blood pressure was measured at time of collection of samples and a decrease in blood pressure was recorded. It was speculated that the increase in the levels of vasopressin was related to hypotension or surgical manipulation. Sampling was not correlated to surgical stimuli (Hauptman *et al.*, 2000). However, vasopressin increase in relation to manipulation of the uterus has been implicated. A research study reported an increase in vasopressin levels at delivery of the first puppy in caesarian sections of dogs (Bergström *et al.*, 2010). The manipulation of the uterus is associated with stretching and traction of the ovarian pedicles, and the results are therefore in agreement with the present study.

The plasma vasopressin levels increased concomitantly with the increase in blood pressure at removal of the ovaries. The z-scores of vasopressin levels and blood pressure changed in synchrony.

Study limitations

Comparisons of the increase in blood pressure in papers II, III, and IV must be interpreted cautiously as there were differences between procedures and the studies involved a limited number of dogs.

From the initiation of surgery, the trend was for blood pressure to increase, as did the urinary noradrenalin/creatinine ratio (paper IV). The affected physiological processes may have a profound effect on measurements taken later. Another aspect with respect to time was the carry over effect of one surgical event to another. Blood pressure and plasma vasopressin did not return to base levels although a comparatively long pause, without surgical intervention, was introduced. As with postoperative evaluation, there are also limitations regarding intraoperative measurements and comparisons.

5.3 Conflict of interest

In the present thesis, research, development and clinical tests of a new resorbable device were performed. The author is the inventor and owner of the patented design. Similar early development work performed within industry is rarely published. Hopefully the work presented here will offer insights in the development processes and potential of resorbable medical devices.

6 Conclusions

- A new device, consisting of a flexible band and a case with a locking mechanism could be manufactured in a resorbable material, polydioxanone.

- The device was used for ligation of free-dissected renal arteries and the ovarian pedicle. The ovarian pedicle could resorb the device; the tissue reaction was transient and restricted in its extent.

- The design of the device provided a good tissue grip.

- Differences in the surgical stress response, both within a surgical procedure and between different methods, were detected using frequent measurements of blood pressure and heart rate.

- The smaller rise in systolic blood pressure during removal of ovaries with laparoscopy compared with open abdomen technique could be due to less traction of pedicles and thereby less noxious stimuli and faster recovery.

- The increase in blood pressure and heart rate was greater at removal of the first ovary than at removal of the second ovary. This made comparison of suture and device within the same animal difficult.

- The urinary noradrenalin/creatinine ratio was higher during surgery than it was for urine collected at home. However, the ratio did not differ before and after the removal of an ovary.

- Plasma vasopressin concentration increased at the removal of both ovaries.

- A combination of frequent blood pressure and heart rate registrations with analysis of plasma vasopressin concentration may improve possibilities to compare noxious stimuli elicited by different surgical techniques.

7 Future perspectives

Future studies involving other resorbable polymers or blends thereof are close at hand. A blend of two resorbable polymers is presently being investigated for manufacturing of the device. Preliminary results and subjective comparison suggest the blend is easier to process (injection moulding), the device is more pliable and the surface is consistently smooth which is important for safety and ease of use.

An investigation concerning the potential time saving and subjective ease of use of the device would also be interesting to perform. Likewise, a comparative randomised study of the potential reduction of surgical stress in patients where the device is used compared to where a traditional ligation technique or a laparoscopic method is used is motivated.

Further research of urinary noradrenalin and plasma vasopressin as perioperative stress markers is motivated. Sampling protocols need to be compared; potential differences between anaesthetic protocols and between species and breeds should be further investigated.

8 Populärvetenskaplig sammanfattning

I många länder utförs kirurgisk kastration av tikar rutinmässigt. Vid all kirurgi är förhindrande av blödning viktigt. För att förhindra blödning kan en kirurgisk tråd knytas runt vävnadens blodkärl, en ligatur. Vid avlägsnandet av äggstockarna finns risk för blödning, både under och efter operationen. Efter kirurgiska ingrepp i bukhålan kan en blödning vara svår att upptäcka med allvarliga konsekvenser som följd. Det är visat att risken för blödning i samband med kastration är större på stora hundar, där avståndet från hudsnitt till äggstockarnas kärl är längre än hos små hundar.

Kirurgiska ingrepp är även förknippade med sensoriska smärtstimuli. Att ligera (knyta av) äggstockarnas blodkärl på ett säkert sätt, med en metod som möjliggör minimal vävnadshantering och därmed mindre sensoriska smärtstimuli, vore fördelaktigt.

Syftet med studierna i denna avhandling var dels att tillverka en resorberbar medicinteknisk produkt för en säker ligering av blodkärl, dels att utveckla metoder att utvärdera och jämföra olika kirurgiska tekniker.

8.1 Utveckling av en ny medicinteknisk produkt

Buntband har tidigare prövats vid ligering av kärl. Ett buntband är ett böjbart band som i sin ena ände har en låsmekanism. När det böjbara bandet förs in i låsmekanismen bildas en självlåsande loop. Det är visat att när man använde buntband för att ligera vävnaden vid äggstockarna på hundar så gick operationen snabbare och man upplevde att ingreppet förenklades. En begränsning med buntband var att det krävdes en viss mängd vävnad i loopen för att kunna åstadkomma tillräcklig kompression för att förhindra blödning. En annan begränsning var att buntbanden var tillverkade av nylon som inte bryts ner i kroppen. En del hundar utvecklade därför kroniska vävnadsreaktioner på grund av materialet vilket resulterade i att banden slutade att användas.

Den första delen av den här avhandlingen beskriver utvecklandet av en ny medicinteknisk produkt, ett modifierat buntband som är tillverkat av ett resorberbart material och tillåter fullständig slutning av loopen. När bandet designades togs också hänsyn till att bandet skulle ha ett bra vävnadsgrepp. I ett första skede ritades konstruktionen och 3D-prototyper framställdes. Den perforerade delen av bandet tillverkades i ett par olika dimensioner i ett välkänt resorberbart material, PDO (polydioxanone). Tillverkningen utfördes via formsprutning vilket i korthet innebar att polymeren smältes och injicerades under tryck in i en gjutform (verktyg). Gjutformen hade en lägre temperatur än polymeren varvid materialet stelnade och kunde därefter tas ut ur formen. Flödesdistansen i gjutformen (hur materialet fyller gjutformen vid formsprutning) bedömdes som tillfredställande vid en tjocklek av bandet på 0.5 mm, medan en tjocklek av bandet på 1 mm bedömdes resultera i ett band som var alltför styvt och inte tillräckligt böjbart.

En första version av produkten, med låsmekanism och perforerat band, testades i vävnaden på en hund som avlivats av andra orsaker. Därefter testades bandet på njurartärerna på grisar som sövts för kirurgisk undervisning. Produkten fungerade så tillvida att låsmekanismen fungerade väl men tillverkningen resulterade i band som blev för korta för praktisk hantering. Dessutom bedömdes att höljet som innehöll låsmekanismen var överdimensionerat. En ny gjutform för tillverkning av ett modifierat band togs fram. Placeringen av intaget i gjutformen ändrades, bandets tjocklek ökades något och en hydraulisk mekanism konstruerades för att enklare få ut produkten ur gjutformen. Formsprutningen i denna gjutform resulterade i längre band vars låsmekanism och hölje innehöll mindre material. Det valda materialet (PDO) visade sig dock vara relativt svårt att formspruta. Om gjutformen öppnades innan materialet stelnat helt kunde produkten till viss del expandera vilket resulterade i ojämnheter av bandet. Råvarans molekylvikt, dimensioneringen av kanalerna i gjutformen liksom temperaturen i formsprutan behövde balanseras i processen. Lägre molekylvikt av råvaran gav bättre flöde i gjutformen medan högre molekylvikt gav bättre mekaniska egenskaper av bandet.

Nästa steg var att använda bandet för att ligera äggstockarnas kärl på tikar som skulle kastreras. Initialt användes den första versionen av bandet på två försökshundar. Därefter, när den förbättrade versionen av produkten hade tillverkats, kastrerades ytterligare sju tikar. På dessa tikar användes bandet vid ligering av båda äggstockarna. En av tikarna började blöda sex timmar efter operationen. Tiken fick sövas igen och vid öppning av buken sågs att buntbandets loop inte var tillräckligt åtdragen vid den ena äggstocken. Loopen drogs åt ytterligare ett steg, blödningen upphörde och buken syddes ihop. En

orsak till den otillräckliga komprimeringen av vävnaden i loopen kan ha varit ojämnheter i produkten som orsakat friktion vilket försvårat genomdragningen av bandet genom låsmekanismen. På ytterligare fem tikar som kastrerades användes bandet som ligatur. Hos dessa hundar ligerades den ena äggstocken med bandet och den andra med traditionell tråd (i dessa fem fall granskades banden noga med avseende på eventuella ojämnheter).

Alla hundar följdes upp med ultraljud av buken ungefär en gång i månaden tills bandet inte längre kunde ses. Resultaten från ultraljudundersökningarna visade att bandet till en början syntes tydligt i form av ett eko och ekoskugga som efter hand avtog. Den något större första versionen av bandet kunde följas under cirka fyra månader jämfört med den mindre versionen som kunde följas i cirka tre månader. Detta antyder en kortare tid för resorption av den mindre versionen. De två första tikarna (försökshundarna) avlivades (av andra skäl än den här studien) sju och arton månader efter operationen. Histologisk vävnadsundersökning visade en välavgränsad och övergående vävnadsreaktion vid platsen för bandet.

Sammanvägt bedömdes att den mängd material som bandet bestod av resorberades av vävnaden vid äggstockarna vilket innebar att produkten bedömdes vara vävnadsvänlig (biokompatibel, tas upp av kroppen utan skadlig vävnadspåverkan).

8.2 Kirurgisk stress

I och med att nya kirurgiska tekniker utvecklas behövs metoder för utvärdering. Jämförelse av smärtyttringar efter en operation är en metod som använts men smärta är svårt att bedöma. Smärta efter operationer har också mätts indirekt genom att jämföra effekter på olika biomarkörer (en biologisk variabel, exempelvis ett hormon som speglar en fysiologisk förändring). Rapporter som beskriver tikars smärta efter kastrering visar motsägelsefulla resultat, både vid bedömning av smärta och också vid användandet av biomarkörer.

Den andra delen av avhandlingen beskriver en metod att mäta smärta under pågående operationer. Vår hypotes var att det redan intraoperativt skulle vara möjligt att identifiera skillnader i kroppens fysiologiska stressförsvar mellan operationer beroende på ingreppets art. Även hos sövda djur har man visat att vävnadshanteringen medför aktivering av sympatiska nervsystemet och olika hormoner. Generellt mobiliseras kroppens försvarsmekanismer i proportion till de sensoriska stimuli och den vävnadshantering som kroppen utsätts för. I våra studier registrerades blodtryck och hjärtfrekvens med täta mellanrum under operationerna och dessutom togs blod- och urinprover vid förutbestämda tidpunkter.

En jämförande studie mellan två olika kirurgiska tekniker för kastration av tik genomfördes. Den ena tekniken innebar att äggstockar och livmoder togs bort via öppen bukkirurgi (ligering med kirurgisk tråd). Den andra tekniken innebar att operationerna utfördes med laparoskopisk teknik (titthålskirurgi). Skillnader kunde registreras både inom ett operationsförlopp och mellan de båda metoderna. Resultaten visade att ligering av äggstockarnas kärl vid den öppna buktekniken medförde en större ökning av blodtrycket jämfört med själva öppnandet av buken. I jämförelse mellan teknikerna sågs en mindre ökning av blodtrycket då äggstockarna avlägsnades med laparoskopisk teknik. Detta tolkades som mindre sensorisk stimulering vid hanteringen av äggstockskrösen hos hundar som kastrerades med laparoskopisk teknik jämfört med öppen bukkirurgi. Försöket visade att förändringar i blodtrycket borde kunna ge vägledning om graden av sensoriskt stimuli som olika kirurgiska metoder medför. I en fortsatt delstudie jämfördes ökningen av blodtrycket då tråd användes för att ligera vävnad vid den ena äggstocken och det nya buntbandet vid den andra äggstocken. Oavsett metod så ökade blodtrycket mer vid ligering av den första äggstocken jämfört med den andra på samma individ. Pilotförsöket avbröts för att utreda om det skulle vara möjligt att jämföra två ingrepp utförda efter varandra på samma hund. Därmed initierades den fjärde studien i avhandlingen med frågeställningen om stressreaktionen som initieras vid ligering av den ena äggstocken motsvarar den reaktion som ses vid ligering av den andra om samma teknik används. Om så vore fallet skulle stressreaktioner, som initieras av två olika metoder för ligering av äggstockarnas kärl, kunna jämföras hos en och samma hund. Det vore av värde eftersom det alltid finns skillnader mellan olika individer och raser.

I den fjärde delstudien sågs återigen en större ökning av blodtryck och hjärtfrekvens vid ligering av den första äggstocken jämfört med den andra. Kvoten av noradrenalin och kreatinin i urin var högre efter sövning jämfört med urinprov taget i hemmiljö men ändrades inte under operationen. Förändring av blodplasmans koncentration av vasopressin överensstämde väl med förändring av blodtrycket och ökade vid ligering av äggstockarna .

En möjlig tolkning av resultaten är att kroppens reflexer reagerade starkast vid det första större kirurgiska delmomentet under operationen och att reflexerna därefter blev svagare. Ytterligare en möjlig tolkning är att anledningen till mindre tecken på smärta efter kastration med laparoskopisk metod berodde på mindre manipulation av vävnaderna vid avlägsnandet av äggstockarna.

Sammantaget gick det att tillverka det nya buntbandet, designat för kirurgi, i ett resorberbart material. Den nya produkten kunde användas vid ligering av äggstockarna hos tik och det gav ett bra vävnadsgrepp. Materialet gav inte

upphov till några postoperativa komplikationer innan det resorberats. Frekventa mätningar av blodtryck och puls, i kombination med blodplasmans koncentration av vasopressin, bedömdes vara lovande metoder för att mäta kirurgisk stress i syfte att utvärdera och jämföra olika kirurgiska ingrepp.

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