Prepubertal gonadectomy in shelter cats:

Anaesthesia, surgery and effect of age at time of gonadectomy on health and behaviour

Nathalie Porters

Dissertation submitted in the fulfillment of the requirements for the degree of Doctor in
Veterinary Sciences (PhD), Faculty of Veterinary Medicine

Ghent University

2014

Promoter

Prof. Dr. Hilde de Rooster

Co-promoters

Prof. Dr. Ingeborgh Polis

Dr. Christel Moons

Department of Medicine and Clinical Biology of Small Animals

Faculty of Veterinary Medicine

Ghent University

Table of Contents
_____

List of Abbreviations

4AVet	multidimensional pain scoring system
AUC	area under the plasma concentration-time curve
BCS	body condition score
C_{max}	maximal plasma concentration
COX	cyclo-oxygenase
DB	dexmedetomidine-buprenorphine
DIVAS	dynamic and interactive visual analogue scale
FPS	federal public service
FLUTD	feline lower urinary tract disease
HPLC	high-performance liquid chromatography
HR	heart rate
IM	intramuscular
IV	intravenous
K_{10}	elimination rate constant
K_{12}	elimination rate constant from central to peripheral compartment
K_{21}	elimination rate constant from peripheral to central compartment
K_a	absorption rate constant
LC-MS/MS	liquid chromatography coupled to tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MBK	medetomidine-buprenorphine-ketamine
MNT	mechanical nociceptive threshold
MRT	mean residence time
N	Newton
NRS	numerical rating scale
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
OTM	oral transmucosal
PPG	prepubertal gonadectomy
REF	reference range
RIA	radio immuno assay
RR	respiration rate

SC	subcutaneous
SD	standard deviation
SEM	standard error of the mean
SPO_2	peripheral arterial haemoglobin oxygen saturation
T	rectal temperature
$t_{1/2a}$	absorption half-life
$t_{1/2e}$	elimination half-life (one-compartmental model)
$t_{1/2\alpha}$	elimination half-life (two-compartmental model; first slope)
$t_{1/2\beta}$	elimination half-life (two-compartmental model; second slope)
TAG	traditional age gonadectomy
T_{max}	time to maximal plasma concentration
UPLC	ultra performance liquid chromatography

GENERAL INTRODUCTION

1. Introduction

In today's Western society of working couples and urban dwellers, cats are deemed to be ideal pets offering affection and companionship while needing less care and commitment than dogs (Mertens & Schär 1986; Olm & Houpt 1988; Heath 2007; Casey & Bradshaw 2008). In most European countries, cats even outnumber dogs as companion animals (Batson 2008). According to a report of the Federal Public Services (FPS) Economy, Statistics, there were 1,974,000 cats compared to 1,167,000 dogs in Belgium in 2008. The real numbers will undoubtedly be higher since unowned cats and dogs (in shelters or stray) were not included.

Despite their popularity, cats are relinquished in large numbers to animal shelters. In 2012, 31,434 cats, including 21,175 stray animals, were admitted to 79 shelters in Belgium, of which 16,180 were adopted, 1,193 were reunited with their owner and 11,208 were euthanized due to illness, feral status, lack of shelter capacity, … (FPS 2012).

According to the literature, various reasons are given by owners for relinquishing cats to shelters, the main ones being owner circumstances (e.g. moving house, divorce, owner illness or death, …), owner allergy, and cat behaviour and/or medical problems (Luke 1996; New et al. 1999; Neidhart & Boyd 2002; Shore et al. 2003; Casey et al. 2009). A significant proportion of shelter cats, however, consists of stray adults and, more importantly, kittens (Luke 1996; Casey et al. 2009). The latter emphasizes the need for a neutering policy regarding cats and for educating owners about feline reproduction and the avoidance of accidental and/or unwanted litters.

Cats are prolific breeders due to sexual maturity occurring as early as 4 months of age (Jemmett & Evans 1977; Jackson 1984), a seasonally polyestrous breeding structure (Gorman et al. 2002; Kutzler 2007), an induced ovulation following single or multiple copulations (Concannon et al. 1980; Kutzler 2007), a relatively short gestation length of 67 days (Root et al. 1995) and a highly adaptable nature to survive (Levy 2011). It is estimated that free-roaming cats can produce 1.4 litters per year with a median of 3 kittens in one litter (range, 1 to 6) (Nutter et al. 2004).

Non-surgical (Kutzler & Wood 2006) and surgical methods (Howe 2006) can be used for contraception in cats. Hormonal approaches resulted in the progestin-based 'Pills for pets' which were developed in Europe in 1963 (ACC&D 2013). Their long-term use in cats is limited due to life-altering side effects (higher increase in mammary tumours and endometrial hyperplasia) (Munson 2006). Moreover, the limited duration of contraception makes this

approach less reliable in shelter medicine, but also in privately owned cats when owners do not administer the drugs consistently. Other avenues for non-surgical pet contraception are being explored: chemical castration methods based on an injection of zinc gluconate (Oliveira et al. 2013) or calcium chloride in the testes (Jana & Samanta 2011) and contraceptive vaccines for male and female animals (Levy 2011; Levy et al. 2011). To date, however, surgical gonadectomy (i.e. the removal of the ovaries in females and testes in males) remains the most reliable and the only permanent method with rapid onset for reproduction control in cats (ACC&D 2013). Furthermore, other potential benefits have been associated with gonadectomy. For example, neutered cats are less likely to display sex hormone-related behaviours (e.g. urine spraying and fighting, unwanted heat) that are often undesirable to owners (Hart & Barrett 1973; Hart & Cooper 1984; Patronek et al. 1996). Next, substantial health benefits of gonadectomy include a decreased transmission risk of infectious diseases (e.g. feline immunodeficiency virus and feline leukaemia virus) because of reduced copulation and inter-male fighting (Little et al. 2009), and a smaller risk for female cats to develop mammary gland tumors later in life (Overley et al. 2005).

Notwithstanding the advantages of gonadectomy, some pet owners may choose to not have their cat neutered because of the cost of the procedure, concerns about detrimental effects on health (anaesthetic or surgical complications, obesity, …), the desire to breed their animal at some point or, because of personal beliefs (Trevejo et al. 2011). According to household surveys done in the United States of America (USA) and the United Kingdom (UK), out of 850 and 1,234 cats, 680 (80%) and 1,067 (86%) were neutered, respectively (Chu et al. 2009; Murray et al. 2009). More importantly, owners of 622 female cats in the UK (Murray et al. 2009) reported a total of 110 out of 156 litters as unplanned. Such litters are commonly relinquished to shelters or added to the existing stray population by dumping them in public places (Casey et al. 2009; Aurich & Becher 2013).

The welfare of the unwanted cat population in shelters or as strays is, to a certain extent, compromised (Levy et al. 2003; Jessup 2004; Stavisky et al. 2012; Sparkes et al. 2013). Many cats admitted to animal shelters spend a period of time in rather stressful conditions and might be euthanized in the end because a home cannot be found for them (Levy & Crawford 2004; McNeil & Constandy 2006). Free-roaming cats may not have sufficient food available, they can become ill while not being treated, they can be hurt or killed in road

traffic accidents or they are at risk of being culled by local or governmental authorities (Levy & Crawford 2004).

As stated above, gonadectomy is a guaranteed method to stop cats from reproducing and from adding kittens to the unwanted cat population, but owners often do not comply. Consequently, gonadectomy prior to adoption is preferable (Aronsohn & Faggella 1993; Bushby & Griffin 2011). In addition, neutering cats at the age at which this is traditionally performed (6-9 months) does not guarantee that no litters are produced prior to the gonadectomy, as many cats have entered puberty before this age. Performing gonadectomy at an earlier age would deal with these issues.

Prepubertal gonadectomy (PPG) or early age neutering is defined as gonadectomy performed before 4 months of age or sexual maturity (Jemmett & Evans 1977; Root Kustritz 2013; Sparkes et al. 2013). This approach has been promoted for shelters to manage cat population, especially in the US during the last decades (Lieberman 1987; Theran 1993; Stubbs et al. 1995; Howe et al. 2000; Root Kustritz 2002; Looney et al. 2008a), and in the UK the past few years (The Cat Group 2011; Joyce & Yates 2011; Sparkes 2011; Sparkes et al. 2013). There are, however, many veterinary surgeons who insist on waiting until after the cat is 6 months of age due to concerns about the feasibility and safety of the anaesthesia and surgery, and about the physical and behavioural development of those kittens due to the role of gonadal hormones on multiple organ systems (Jackson 1984; Spain et al. 2002; Murray et al. 2008).

2. Anaesthesia and surgery

2.1. The paediatric patient: anaesthesia- and surgery-related concerns

A paediatric patient requires special attention concerning anaesthetic and surgical matters due to some differences in anatomy and physiology of kittens compared to adult cats (Table 1). Specific concerns that should be taken into account when anaesthetizing kittens are an appropriate use of anaesthetics and analgesics, hypoglycaemia, and hypothermia (Root Kustritz 1999; Joyce & Yates 2011).

Hepatic and renal functions in kittens might not be fully developed and kittens may have lower plasma albumin concentrations (Hosgood et al. 1998; Root Kustritz 1999; Holden 2007a; Meyer 2007). Consequently, drug distribution, metabolism and excretion might be altered and the anaesthetic protocol for kittens should include lower or equal dosages of

drugs compared to adult cats (Faggella & Aronsohn 1994; Howe 1997; Hosgood et al. 1998; Root Kustritz 2002; Holden 2007a).

In addition to the recommendations displayed in Table 1, body weight should be determined with an accuracy of 0.1 kg to use appropriate dosages of drugs (Looney et al. 2008a). To minimize excessive heat loss, minimal clipping of the fur and the use of limited amounts of non-alcoholic products (e.g. based on povidone iodine) for skin disinfection are recommended for surgical preparation (Chalifoux et al. 1981; Looney et al. 2008b; Joyce & Yates 2011). Hypothermia might induce bradycardia (Hosgood et al. 1998; Hoskins 1999; Robertson 2005) and a reduced metabolism which is associated with a prolonged recovery (Robertson 2005; Meyer 2007; Little 2008).

Table 1: Physiological differences between kittens and adult cats and their implications on perioperative protocols (Faggella & Aronsohn 1993; Stubbs & Bloomberg 1995; Hoskins 1999; Root Kustritz 2002; Holden 2007b).

Physiological difference in kittens	Concern	Action
Smaller glycogen store	Hypoglycaemia	Small meal 2 to 4 hours before anaesthesia, maximum 4 hours fasting
Little body fat Greater total body fluid High surface area to volume ratio	Different distribution volume, faster redistribution (water-soluble drugs) and limited accumulation (lipid-soluble drugs)	Greater initial dosage
	Hypothermia	Heat sources and body temperature monitoring Minimal clipping Use of non-alcoholic products
Cardiac output dependent on heart rate Higher proportion of non-contractile cardiac tissue Limited ventricular compliance Immature sympathic nervous system	Bradycardia, hypotension	Monitoring heart rate
Greater respiratory rate and tissue oxygen consumption	Hypoxaemia	Monitoring respiratory rate and peripheral arterial haemoglobin oxygen saturation

2.2.   Criteria for anaesthetic and surgical protocols in shelter medicine

In view of shelter medicine, it is essential that anaesthetic and surgical protocols guarantee safety and animal welfare while being suitable for high volume neutering within a short period of time under less sophisticated conditions than an operating theatre in a well-equipped veterinary practice (Looney et al. 2008a; Ko & Berman 2010; Polson et al. 2012).

2.2.1. Anaesthetic protocols

Generally, an efficient, humane and safe anaesthetic protocol should fulfill four criteria, namely anxiolysis (stress reduction), sedation (muscle immobilization), analgesia (lack of pain) and a reversible depression of the central nervous system to induce temporary unconsciousness (Looney et al. 2008a).

Anxiolytic, sedative and analgesic effects are primarily obtained through the administration of premedication (Pascoe 2000b). Preferentially, a sedative agent is combined with an opioid. The alpha2-agonist (dex)medetomidine is widely used for feline sedation and premedication (Ansah et al. 1998; Selmi et al. 2003; Slingsby & Taylor 2008). Buprenorphine, a partial μ-opioid agonist, combined with a low dose of dexmedetomidine, results in anaesthetic-sparing and analgesic effects superior to that obtained with an opioid alone (Lamont 2008; Grint et al. 2009). This sedative-analgesic combination is an excellent premedication and can be used safely in healthy cats as well as in patients with very mild systemic disease (Murrell 2007).

For optimal pain relief, a non-steroidal anti-inflammatory drug (NSAID) can additionally be used (Pascoe 2000; Corletto 2007; Murrell 2007). The rationale of administration of an NSAID as part of the premedication is that NSAID's act peripherally by reducing prostaglandin synthesis through inhibition of cyclo-oxygenase (COX) (Wright 2002; Robertson & Taylor 2004). Whereas COX-1 is associated with normal metabolic function, COX-2 is involved in the inflammatory reaction to tissue injury (Wright 2002; Robertson & Taylor 2004). Therefore, drugs such as carprofen or meloxicam, that preferentially block the COX-2 receptor, are preferred (Wright 2002).

The combination of buprenorphine, dexmedetomidine, and an NSAID is likely to provide sufficient analgesia to cover mild or moderate postoperative pain (Pascoe 2000), which can be expected after gonadectomy (Wright 2002; Vaisanen, Tuomikoski et al. 2007; Dyson 2008). This combination is based on a multimodal and pre-emptive analgesic approach. Multimodal analgesia involves the administration of analgesic drugs with a different mechanism of action targeting pain at different levels (perception, transmission and

modulation of pain) (Slingsby & Waterman-Pearson 2000; Lamont 2002; Corletto 2007). By combining analgesic agents, additive or synergetic effects are obtained with fewer adverse side effects (Lamont 2002; Hellyer et al. 2007). Pre-emptive refers to the administration of the analgesic drugs prior to the onset of a painful stimulus (i.e. the surgical procedure) to prevent central hypersensitivity and thus, surgically induced hyperalgesia (Woolf & Chong 1993; Lascelles et al. 1995; Lamont 2002).

To produce short-term anaesthesia in cats, injectable (e.g. ketamine) or inhalant (e.g. isoflurane) protocols can be used (Lin 2007; Wiese & Muir 2007). Inhalant anaesthesia provides control of anaesthetic depth, but requires special equipment. Injectable anaesthetic drugs can also provide surgical planes of anaesthesia while requiring minimal resources (syringes and needles). However, once administered, the drug effect cannot be controlled. Alongside the relatively easily administration, ketamine possesses analgesic properties (Slingsby et al. 1998; Robertson 2008) and has been used successfully in combination with (dex)medetomidine and/or an opioid for gonadectomy in cats (Slingsby et al. 1998; Wiese & Muir 2007; Harrison et al. 2011; Ko et al. 2011).

Previous studies in kittens have described anaesthetic protocols (Faggella & Aronsohn 1993; Theran 1993; Howe 1997; Howe 1999; Robertson et al. 2003a); however, most protocols were based on induction and/or maintenance by intravenous drug administration or inhalation anaesthesia. Due to the small size and agility of kittens, intravenous catheter placement might be challenging, time consuming and less practical (Holden 2007b). Additionally, catheter placement might be stressful for the animal and animal handlers (Murrell 2007). When large number of shelter kittens have to be neutered in a short period of time, an injectable anaesthetic protocol is ideal since it does not require dedication of an anaesthesia machine to every animal (Robertson 2005). Moreover, practitioners are familiar with injectable anaesthetics for short surgical procedures in cats (Ko et al. 2011). For all the reasons listed above, injectable anaesthetic protocols are deemed to be a better alternative to anaesthetise shelter kittens.

Intramuscular and subcutaneous injections are most commonly used routes for drug administration in cats. Recently, the oral transmucosal (OTM) administration (Figure 1) has been proposed as a painless and practical alternative method in cats to administer sedative-analgesic drugs such as (dex)medetomidine (Ansah 2004; Slingsby et al. 2006; Slingsby et al. 2009), buprenorphine (Robertson et al. 2003b; Robertson et al. 2005; Hedges et al. 2013a) or a combination of dexmedetomidine and buprenorphine (Santos et al. 2010).

Weak bases such as dexmedetomidine and buprenorphine (pKa = 7.1 and pKa = 8.24, respectively) (Salonen 1989; Mendelson et al. 1997; Rosen & Daume 2006; Slingsby et al. 2009) are considered to be excellent candidates for OTM administration. They are lipid-soluble drugs with a low degree of ionization, which is favourable for maximal absorption by the oral mucosa (Weinberg et al. 1988). Additionally, the unionized lipophilic form of the drug will predominate at a pH above the pKa of the drug.

Figure 1. Oral transmucosal administration is performed by inserting the nozzle of a 1mL syringe into the buccal cavity of the cat's mouth and gently squirting the content into the cheek area.

The pH of feline saliva in the buccal cavity is between 8 and 9 (Robertson et al. 2003b; Slingsby et al. 2009; Ferreira et al. 2011), which will increase the amount of unionized molecules and thus the extent of absorption of the drug (Weinberg et al. 1988; Mendelson et al. 1997; Abbo et al. 2008; McInnes et al. 2008). In addition, the oral cavity provides direct entry into the systemic circulation thereby avoiding the hepatic first pass effect (Rathbone et al. 1994; Madhav et al. 2009).

For buprenorphine, pharmacokinetic studies found a similar pharmacokinetic profile after OTM, intramuscular (IM) and intravenous (IV) administration suggesting a large bioavailability of buprenorphine after OTM administration (Robertson et al. 2003b; Robertson et al. 2005). In contrast, a lower bioavailability of buprenorphine after OTM than after IV administration in cats has recently been reported (Hedges et al. 2013a). Only one study investigated the pharmacokinetic profile of OTM administered dexmedetomidine and reported comparable plasma concentrations of medetomidine after spraying it into the cat's mouth compared to IM injection, but a slower absorption after oral administration was observed (Ansah 2004).

Antinociceptive effect by means of thermal threshold testing (i.e. by applying a mild, transient heat stimulus to elicit pain) were similar for dexmedetomidine (40 µg/kg) (Slingsby et al. 2009) and buprenorphine (20 µg/kg) (Robertson et al. 2005) following IM and OTM administration. Hedges and colleagues (2013), however, observed a slightly smaller and

shorter lasting increase in thermal threshold following OTM administration compared to IV administration of buprenorphine.

2.2.2. Surgical protocols

In order to meet the demand in shelters for safe and efficient neutering of large numbers of cats within a short period of time, the surgical technique should be practical, fast and cost-effective. It is evident that the general principles of aseptic surgery with minimal tissue handling and trauma, good haemostasis and minimal loss of body temperature should be taken into account. Aside from the abovementioned practical considerations related to the anaesthesia and/or the surgery, it is important to emphasize that kittens have a smaller blood volume compared to adult cats and that, consequently, a small amount of blood loss can have a considerable impact on the animal (Aronsohn & Faggella 1993).

Gonadectomy is defined as removal of the gonads: testes in males (orchidectomy) and ovaries in females (ovariectomy). It has been stated that gonadectomy in kittens is similar to that in adult animals, although paediatric tissues are more fragile and delicate and, as such, should be handled gently to avoid excess traction and tearing of tissues (Aronsohn & Faggella 1993; Theran 1993; Howe 1997). The use of a Snook ovariohysterectomy hook for ovarian identification in female kittens should be avoided (Howe 2006).

Most reports in the veterinarian literature describe an ovariohysterectomy (i.e. removal of the ovaries and uterus) whereby the ovarian vessels and the uterine body are ligated with suture material (Goeree 1998b; Bushby 2010) or with vascular clips (Figure 2) (Aronsohn & Faggella 1993). Studies comparing ovariohysterectomy and ovariectomy in cats or kittens are lacking, but there is currently no scientific evidence to prefer ovariohysterectomy instead of ovariectomy in cats and dogs (DeTora & McCarthy 2011).
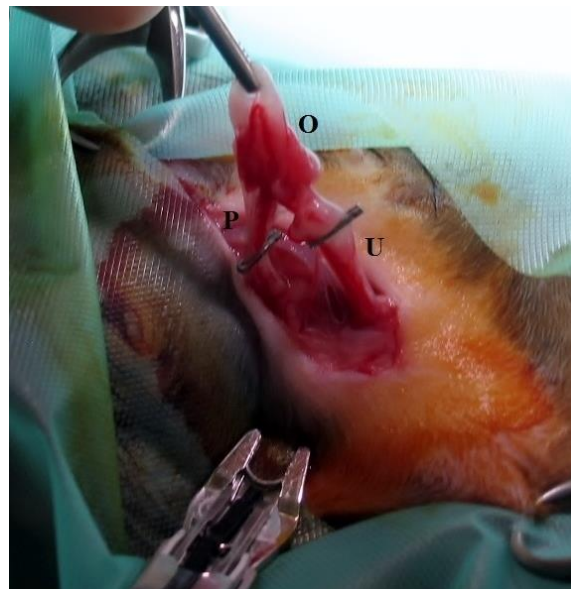
Figure 2. Removal of an ovary (O) in female kittens by placing a vascular clip on the ovarian pedicle (P) and on the cranial part of the uterine horn (U).

It can be assumed that ovariectomy for routine neutering in healthy cats is preferable because it is less invasive, faster and results in a smaller incision site, as has been described in dogs (van Goethem et al. 2006).

Beside the discussion about ovariectomy versus ovariohysterectomy, two approaches for a gonadectomy in female cats be used: the midline or the flank approach. The midline approach is most commonly used in Europe, except for England where the flank approach is preferred (Coe et al. 2006). However, wound tenderness, postoperative pain and wound complications (discharge, swelling, redness) were less after a midline incision compared to a flank incision (Burrow et al. 2006; Coe et al. 2006; Grint et al. 2006). In addition, a better overview of the surgical field is obtained by a midline approach to observe bleeding quicker compared to a flank approach (Hedlund 2002; Burrow et al. 2006).

In pediatric males, the small and mobile testes of the kitten might be difficult to stabilize in the scrotal region before incision (Howe 2006). An open or closed castration (with or without opening the vaginal tunic, respectively) can be performed in kittens since the tunic is not yet dense (Figure 3). Both have been reported for PPG (Aronsohn & Faggella 1993; Howe 1997; Griffin & Brestle 2010; Bushby & Griffin 2011; Joyce & Yates 2011). The testes should not be exteriorized to the same distance as in the adult cat (Howe 2006). The spermatic cord can be tied on itself (Figure 4) (Howe 1997) or vascular clips (Aronsohn & Faggella 1993) or ligatures (Goeree 1998b) can be placed on the spermatic cord.

Figure 3. Transparant vaginal tunic in male kittens.

Figure 4. Gonadectomy in male kittens by tying the spermatic cord onto itself.

3. Gonadal hormones, gonadectomy and physical development

Another consideration for PPG is the concern that drop of gonadal hormones would result in more urogenital disorders, stunted growth, obesity, and immune-mediated diseases.

3.1.   Urogenital disorders

Previously, it has been demonstrated that gonadal hormones are responsible for the normal development of the external genitalia in cats (Root et al. 1996a; Stubbs et al. 1996). Female cats gonadectomised at 7 weeks or 7 months had a smaller vulva compared to sexually intact cats at 12 months of age (Stubbs et al. 1996). It is well known that penile spines and the balanopreputial fold, a tissue connecting the penile and preputial mucosa in male cats, are sensitive to androgens (Aronson & Cooper 1967). Aronson and colleagues (1967) found that penile spines developed at about 8 weeks of age, reached their full size at 6 to 7 months of age and decreased in size as the androgen level fell. These penile spines contribute to genital sensitivity during intromission and help thereby sexual arousal (Aronson & Cooper 1967). In male cats gonadectomised at 7 weeks or 7 months, penile spines did not develop or atrophied, respectively (Stubbs et al. 1996). Complete penile extrusion was impossible in cats gonadectomised at 7 weeks, and possible in only 60% of cats gonadectomised at 7 months whereas the penis could be extruded in all sexually intact cats (Root et al. 1996a). The clinical significance of this decreased ability to extrude the penis in (early age) neutered cats is unknown (Root Kustritz 2007), although it could increase the difficulty of catheterizing animals that require such a procedure (Howe 2006). Likely to be of greater concern is that (prepubertal) gonadectomy may also influence urethral development in tomcats, resulting in a smaller urethra and thus, a potential increased risk for urethral obstruction (Herron 1972; Spain et al. 2002).

Experimental studies failed to find evidence for a narrowing of the urethra in male cats following gonadectomy at different ages. In one study investigating the penile urethra histologically, no differences were observed in the penile urethral circumference between male cats castrated at 5 months of age with or without testosterone treatment or left sexually intact at 10 months of age (Herron 1972). In the same study, histological findings revealed that castrated cats had a significantly lower mean epithelial height and greater fibrocyte density in the penile urethra probably responsible for the overall reduction in size of the penis following castration. In another experimental study, gonadectomy (7 weeks or 7 months) did not lead to a reduction in urethral diameter in male cats assessed at 22 months by means of

contrast cystourethrography, a technique whereby the bladder was filled with contrast fluids and radiographs were taken while the cat was urinating (Root et al. 1996a). In the same study, however, female cats gonadectomised at 7 weeks had a significantly smaller pre-pelvic urethral diameter than sexually intact cats. Root et al. (1996) suggested that the observed change in the female urethra was unlikely to have any clinical relevance.

Clinical relevance of anatomic differences in the external genitalia remains unclear. Nevertheless, gonadectomy at any age in both male and female cats has been described repeatedly as one of the potential risk factors for feline lower urinary tract disease (FLUTD) (Fennell 1975; Willeberg & Priester 1976; Walker et al. 1977; Lekcharoensuk et al. 2001). FLUTD is defined as a syndrome affecting the bladder and/or urethra including signs as dysuria, hematuria, urethral obstruction and/or urolithiasis in the cat (Root et al. 1996a; Gunn-Moore 2003).

Two clinical long-term studies have addressed the effect of age at time of gonadectomy on health issues, including FLUTD. No correlation was found between age at time of gonadectomy (< 22 weeks of age or older) and the incidence of FLUTD in cats of both sexes, or urethral obstruction in tomcats (Spain et al. 2004). Howe and colleagues (2000), on the other hand, suggested that gonadectomy at an early age (< 24 weeks of age) may result in some unidentified protective effect on the urinary tract since FLUTD was less commonly reported by owners of cats neutered at an early age (< 24 weeks of age) than by owners of cats neutered later (≥ 24 weeks of age). For urethral obstruction in male cats, no significant effects of age at gonadectomy were found (Howe et al. 2000).

3.2. Growth-related problems

Longitudinal bone growth in humans and animals occurs at the growth plate of physis through a process called endochondral ossification: cartilage is first formed and remodeled into bone tissue (Ohlsson et al. 1993; Nilsson et al. 2005; Murray & Clayton 2013). This process is regulated by different endocrine signals, including growth hormone, insulin-like growth factor 1, glucocorticoid, thyroid hormone, vitamin D, leptin; during puberty, the gonadal hormones contribute a great deal to this process (Nilsson et al. 2005; Murray & Clayton 2013).

The exact mechanisms and factors involved in induction of endochondral ossification are not yet established. It has been suggested that testosterone stimulates chondrocytes proliferation

and matrix production whereas estrogen reduce chondrocyte proliferation and accelerates growth plate senescence by activating the estrogen receptors in the growth plate (Nilsson et al. 2005). In particular estrogens appear to be involved in epiphyseal closure in males as well as in females (Nilsson et al. 2005). In males, the aromatase enzyme is responsible for conversion of testosterone in estrogens (Juul 2001).

Physeal closure in sexually intact cats begins as early as 4 months of age and is completed around 20 months of age (Smith 1969). According to anatomical and radiographic physeal closure times (Smith 1969), physes can be assigned to one of three groups (first, middle, or last) (May et al. 1991) (Table 2). When comparing the average age at onset of puberty (i.e. between 6 to 12 months of age) and physeal closure times as depicted in Table 2, it is clear that most physes close postpubertally.

Table 2: Closure time of physes in cats can be divided into three groups (first, middle or last) (May et al. 1991), based upon anatomical and radiographic findings (Smith 1969).

Group	Age (months)	Physes
First	4 – 8	Distal and middle phalanges
		Scapular tuberosity
		Accessory carpal bone
		Distal humerus
Middle	8 – 14	Proximal femur
		Metacarpal bones
		Metatarsal bones
		Proximal ulna
		Proximal radius
		Distal tibia
		Distal fibula
Last	14 – 24	Proximal tibia
		Proximal fibula
		Distal femur
		Distal radius
		Distal ulna
		Proximal humerus

Previously, several investigators discovered that fractures in adult cats (12 to 42 months of age) occurred through physes that had not closed yet (McNicholas et al. 2002) and that the practice of (early age) gonadectomy might be responsible for this delayed physeal closure time (May et al. 1991; Houlton & McGlennon 1992). Delayed closure as a result of

(pre)pubertal gonadectomy may have clinical implications: kittens neutered at early age might have an increased risk for Salter Harris fractures (type I: involving the physis) or they might simply become taller. In the past no evidence of continued growth was found in male cats gonadectomised at 7 months compared to sexually intact male cats, although the distal radial physes of castrated males were still radiographically open at the end of the study (at 20 weeks of age) (Houlton & McGlennon 1992). Similar results were found in a 12-month experimental study in which both male and female cats were either neutered at 7 weeks, 7 months or left intact: whereas closure of the distal radial physis was delayed in all gonadectomised cats, mature radial length did not differ among groups (Stubbs et al. 1996). However, another study countered these findings partially, as radial length at 24 months of age of males and females gonadectomised at 7 weeks or 7 months was found to be significantly longer (13% and 9% respectively) than intact males and females (Root et al. 1997). In addition, proximal radial physeal closure times were delayed in cats neutered at 7 weeks of age compared to those gonadectomised at 7 months or left sexually intact (Root et al. 1997).

Retrospective studies investigating medical records of cats with femoral capital physeal fractures (McNicholas et al. 2002; Fischer et al. 2004) or with slipped capital femoral epiphysis (Craig 2001) identified being male and being neutered as two out of four risk factors for exhibiting femoral physeal dysplasia. Not unimportantly, in the study of Craig (2001) one 24-month old intact cat had a bilateral femoral dysplasia, which can thus not be explained by a prolonged growth phase caused by (early age) gonadectomy.

3.3. Overweight

Obesity has been defined as a condition of positive energy balance resulting in excess body fat (Burkholder & Toll 2000; Zoran 2010). Companion animals are considered overweight once their body weight exceeds 10 to 19% of the optimal weight and obese when they are 20% or more above the optimal body weight (Burkholder & Toll 2000).

In cats, gonadectomy is considered a risk factor to become overweight and/or to develop obesity (Fettman et al. 1997; Robertson 1999; Allan et al. 2000; Russell et al. 2000; Martin et al. 2001; Colliard et al. 2009; Courcier et al. 2010; Cave et al. 2012; Courcier et al. 2012). It has been shown that both male (Kanchuk et al. 2002) and female (Harper et al. 2001) cats gain weight after neutering (Fettman et al. 1997; Martin et al. 2001). Firstly, this weight gain might be a result of increased food intake and might occur primarily as an increase in body

fat (Fettman et al. 1997; Harper et al. 2001; Kanchuk et al. 2002; Belsito et al. 2009). In neutered male and female cats, this increase in fat is accompanied with an increase in circulating leptin (Martin et al. 2001; Kanchuk et al. 2002; Martin et al. 2006). Leptin, a protein mainly secreted by adipocytes, regulates body fat storage, appetite and energy expenditure (Martin et al. 2001; Hoenig & Ferguson 2002). Secondly, a decrease in metabolic rate in gonadectomised cats compared to sexually intact cats may further promote weight gain after neutering (Root et al. 1996b; Martin et al. 2001). Results suggested that neutered male and female cats require an intake of fewer calories (28% and 33% less, respectively) compared to sexually intact cats of the same age (Root et al. 1996b). Thirdly, compared to the activity level before gonadectomy, a dramatic decrease in physical activity in female cats following gonadectomy has been measured with an activity collar which allows continuous monitoring over several days during dark and light periods and without human interference (Belsito et al. 2009). Unfortunately, a control group of sexually intact cats was not included.

In contrast to the variety of studies on the effect of gonadectomy, few studies have investigated the effect of age at time of gonadectomy on the development of obesity. Root and colleagues (1996) compared in their study heat production, as measure of metabolic rate, with the indirect calorimetry in cats gonadectomised at 7 weeks, 7 months or left intact. Heat coefficients were significantly higher in sexually intact male and female cats at 12, 18 and 24 months of age, than in cats gonadectomised at 7 weeks or 7 months. These findings suggested that metabolic rate decreases after gonadectomy, irrespective of age at time of gonadectomy. In another experimental study (Stubbs et al. 1996), cats gonadectomised at 7 weeks or 7 months were heavier than sexually intact cats at 12 months of age; however, significance was only reached when comparing cats gonadectomised at 7 months with sexually intact cats. In the same study, all gonadectomised cats (7 weeks or 7 months) had a significantly greater falciform fat thickness on radiographic assessment than sexually intact cats at 12 months of age (Stubbs et al. 1996). In two clinical long-term studies, no correlation was found between age at time of gonadectomy (< 22 or < 24 weeks of age and older) and obesity based on body condition score assessments of the cat by the owner (Spain et al. 2004) or based on the owner's perception on body weight (Howe et al. 2000). It is known that owner's perception of body weight may be suspect (Howe et al. 2000) and owner's underestimation of body condition score of their cat is most common in overweight and obese cats (Allan et al. 2000; Colliard et al. 2009). Hence, the conclusion about the lack of correlation between age at

gonadectomy and obesity in both clinical long-term studies is questionable. More recently, it was demonstrated in an experimental study that female cats neutered prepubertally (19 weeks of age) had a 24% higher body weight and a 16% higher body condition score at 12 months of age compared to their sexually intact counterparts (Alexander et al. 2011). Surprisingly, the expected differences in body and lean mass between gonadectomised and sexually intact cats were not confirmed by means of dual-energy X-ray absorptiometry at 12 months of age (Alexander et al. 2011).

3.4.   Immune regulation

Gender dimorphism in the immune response has been observed in humans and in animal models (Gaillard & Spinedi 1998; Verthelyi 2001). Adult female mammals have greater humeral and cell-mediated immunity than their males matches, resulting in a higher susceptibility to autoimmune diseases and allergies and in the development of higher titers of antibodies in response to immunization in females (Grossman 1985; Gaillard & Spinedi 1998). In cats, the sexual dimorphic effects and effects following castration, were reflected in the results of a large-scale retrospective study (Moore et al. 2007). In the latter, hypersensitivity reactions following vaccination were more common in female cats compared to male cats, and also more in neutered compared to sexually intact cats of both sexes. Little is known about the development of the immune system of cats within the first 6 months of life (Day 2007).

It has been demonstrated in rodents that (prepubertal) gonadectomy reversed thymic involution and thus, induced growth of the thymus and other lymphoid organs (Windmill et al. 1993; Windmill & Lee 1998; Windmill & Lee 1999). These observations suggest an enhanced immune response following gonadectomy, leading to an increased ability of lymphocyte reactivity to antigen stimulation. This response is probably the result of an elevated number of immature thymocytes in the thymus which undergo maturation and consequently produce an increase in peripheral T and B cells (Eidinger & Garrett 1972; Windmill & Lee 1998).

Studies investigating the effect of early age neutering on the immune system in cats are extremely rare. Only Spain and colleagues (2004) investigated the incidence of asthma and gingivitis between early age (< 22 weeks of age) gonadectomy compared to gonadectomy on a later age. In the latter, these inflammatory conditions were less frequently reported.

4. *Feline behaviour and gonadectomy*

4.1. Feline biology and social organization

Cats are carnivorous predators that were presumably domesticated from the wild cat (*Felis silvestris*) in Egypt some four to five thousand years ago (Serpell 1986). Cats spend up to 19 hours per day sleeping, but are usually quite active during the awake hours (Rochlitz 1999). For example, they are agile and proficient at climbing. Cats climb when hunting or to reach elevated resting places from which they have an overview of the environment without being exposed to potential threats (Mertens & Schär 1986; Landsberg 1996).

Free-roaming cats can live solitary, but they form social groups whenever there are enough food resources to support a group (Liberg & Sandell 1986; Crowell-Davis et al. 2004). Females and their kittens form the core of the group, whereas male cats are more likely to loosely attach to such groups and roam between them (Liberg & Sandell 1986).

Within a social group, adult cats teach appropriate social and hunting skills to kittens and juveniles. Furthermore, members of a social group will form close relationships with particular conspecifics in this group (i.e. preferred associates), to whom they will display less aggression and more affiliative behaviour like allogrooming, allorubbing, nose touching, playing and resting together (Crowell-Davis et al. 2004; Crowell-Davis 2007).

Individual cats or social groups of cats reside in home ranges that overlap with home ranges of other individuals or groups, but each home range generally has a core area containing several resources that is defended against other cats, often those belonging to another social group (Liberg & Sandell 1986; Mertens & Schär 1986; Crowell-Davis 2007). Conflict arises when cats from different social groups are forced to be in close proximity, for example when limited resources have to be shared. Communication facilitating avoidance between groups occurs via visual and olfactory signals. Cats leave marks by depositing faeces and/or urine and by creating scratching sites within their home range, probably as 'landmarks' mostly along used travelling routes (Mertens & Schär 1986; Crowell-Davis 2007). When spraying, the cat sprays small volumes of urine horizontally onto vertical surfaces (Olm & Houpt 1988; Overall et al. 2005). Spraying probably gives information about the reproductive status, about which cat was at a given location at a given time, as well as about the emotional state (Crowell-Davis 2007).

Nowadays, cats are very popular companion animals and many humans enjoy the affiliative social behaviour of cats (rubbing, purring, …). These friendly behaviours form the basis for

humans becoming attached to cats, caring for them and keeping them in the household (Crowell-Davis 2007). Social species are born with the ability to learn species-specific social skills (Crowell-Davis et al. 2004) and to direct social behaviour to humans, a learning process about interspecific social behaviour is essential. This learning process takes place during kitten development (McCune 1995; Lowe & Bradshaw 2002; Casey & Bradshaw 2005; Casey & Bradshaw 2008).

4.2. Feline developmental stages and socialization

Kitten development is characterized by different developmental stages (Table 3), which are influenced by genetic and environmental factors, health of the queen, health and social interactions of the kitten, learning processes, play and socialization (Landsberg et al. 1997; Crowell-Davis 2002; Radosta 2011). The neonatal and transitional period are characterized by a dependence of the kitten on the mother and by neurological and sensorimotor development (Martin & Bateson 1986; Landsberg et al. 1997; Radosta 2011). Of particular importance for the capacity to develop social relationships with conspecifics and other species, is the socialization period (Landsberg 1996; Casey & Bradshaw 2008). According to the literature, this sensitive period starts between 2 to 3 weeks of age and comes to an end between the 7th and the 10th week of life (Karsh & Turner 1986; McCune 1995; Landsberg 1996; Bradshaw et al. 1999; Adamelli et al. 2005; Overall et al. 2005; Casey & Bradshaw 2008). Typically, an infant animal first relates to its parents (usually mother), then to littermates or siblings, next to peers, and finally to other members of its species to develop appropriate social behaviour towards conspecifics (Karsh & Turner 1986). As the queen is the first cat with which the kitten experiences affiliative social interactions, she is critical in learning social behaviour and should be calm in the presence of humans in order for kittens not to become afraid of people (Crowell-Davis et al. 2004; Overall et al. 2005).

Over recent years, considerable attention has been given to the subject of handling kittens during the sensitive socialization period on the subsequent behaviour of the kitten in adulthood (McCune 1995; Lowe & Bradshaw 2002; Casey & Bradshaw 2005; Casey & Bradshaw 2008). Kittens with a lack of human contact and exposure to environmental stimuli during their socialization period may have an increased risk to display fear and stress-related behaviours in adulthood (McCune 1995; Overall et al. 2005; Casey & Bradshaw 2008). McCune and colleagues (1995) also demonstrated an impact of paternity on the friendliness

towards people at 12 months of age, and thus, a potential genetic influence. It has also been suggested that the kitten's experience in the third and fourth month of life may additionally contribute to its subsequent behaviour towards people (Lowe & Bradshaw 2002), especially if socialization to humans starts before the end of the socialization period (Casey & Bradshaw 2008). Moreover, social play, which is important for future social interactions, peaks in the 3rd and 4th month of life (West 1974; Crowell-Davis 2007). Aside from proper contact during the socialization period, some authors state that contact with humans should be maintained until 16 weeks of life (Overall et al. 2005).

As stated before, cats generally reach their sexual maturity between 6 to 12 months of age, but it has been found to occur as early as 4 months of age (Jemmett & Evans 1977). In general, the phase of social maturity starts at around 2 years of age (Horwitz 2001; Overall et al. 2005).

Table 3. In cats, four consecutive, interrelated phases of behavioural development have been identified (Martin & Bateson 1986; Horwitz 2001; Beaver 2003b; Beaver 2003a; Landsberg et al. 2003; Radosta 2011).

Phases	Age of the kitten (weeks)	Development
Neonatal	0 – 1	Nursing and sleep, fully dependent on the queen (orientation to nest tactile/olfactory/thermal)
Transitional	1 – 2	Locomotion and sensory development (eyes open, orientation to sounds) Start of independence
Socialization	2 – 9	Further locomotion, sensory, thermoregulatory development Weaning, Voluntary elimination Exploration, predatory (object play) Development of locomotor function Formation of social relationship, social play Sensitive period for socialization to humans, and other new stimuli
Juvenile	9 – 24 to 52	Sexual behaviour (16 weeks[a]) before sexual maturity (16[b] to 52 weeks of age)
Adult	104 – 208	Social maturity

[a] male cats: mounting, neck biting, pelvic thrusts; [b] heat cycles can be seen at 16 weeks of age, usually around 20 to 36 weeks of age, spermatogenesis at 20 weeks of age, but copulation at 36 to 52 weeks of age

4.3.   Behaviour problems in cats

Data from surveys administered to veterinary practitioners (Fatjo et al. 2006) or cat owners (Heidenberger 1997) as well as behavioural case studies (referral cases) indicate that inappropriate elimination, aggression, scratching, states of anxiety and feeding problems are quite commonly occurring behaviour problems in cats.

Feline behaviour problems may not only be an issue for the cat displaying them, but their occurrence can also erode the human-animal bond, which ultimately results in animal relinquishment (Patronek et al. 1996; Shore et al. 2003; Overall et al. 2005; Casey et al. 2009). In the literature, destructive behaviour, cat spraying, house soiling and aggression towards other cats or humans are commonly reported by owners as reasons for relinquishment (Patronek et al. 1996; Shore et al. 2003; Casey et al. 2009) or for failure of adoption and subsequent return to the shelter (Shore 2005).

Many behaviour problems (e.g., spraying urine, scratching furniture, predation, climbing, chewing, nocturnal activity, vocalization and mating behaviour) are in fact part of the natural behavioural repertoire of the cat, but when they are performed inside the owner's house, they are considered undesirable (Landsberg 1996; Jongman 2007). Consequently, these behaviours are not problematic to the cat, but rather to the owner (Mertens & Schär 1986).

Other behaviour problems may be associated with underlying anxiety or fear, two emotions that are distinct, yet closely related and often not easy to separate by owners (Casey 2002). Fear, an adaptive response, is elicited by a danger, a threat or an aversive situation and consequently subsides when the aversion diminishes (Odendaal 1997; Rosen & Schulkin 1998; Casey 2002). Anxiety, on the other hand, is an emotional response to stimuli associated with potential or more ambiguous danger and may result from the inability to remove itself from, or to control fearful stimuli (Odendaal 1997; Casey 2002; Levine 2008).

When faced with a stressor, cats will try to cope by hiding, as a means to regain control over their environment (Weiss 1972; Rochlitz 2005; Heath 2007). However, many domestic cats live in environments that fail to provide any opportunity to hide. If hiding is not possible, the cat is likely to experience, depending on the type of stressor, environmental or social stress, often resulting in inappropriate elimination or aggression (Levine 2008).

Whether or not behaviour problems are associated with fear and/or anxiety affecting animal welfare directly (Landsberg 1996; Levine 2008), undesirable behaviours are associated with an increased risk for relinquishment and thus also - albeit indirectly - with animal welfare.

4.4.   Gonadectomy and cat behaviour

Behaviours driven by a sexual motivation are likely to be at least partially mediated by gonadal hormones (Root Kustritz 2012). Consequently, they are also most likely to be affected by gonadectomy. In male cats, for example, a decrease in sexually motivated roaming, fighting, and urine spraying has been observed after castration (Hart & Barrett 1973; Hart & Cooper 1984).

Other behaviours are less likely to be influenced by gonadectomy. However, there is evidence that male cats are likely to become more docile towards people (Hart & Barrett 1973). Furthermore, gonadectomised male and female cats have been associated with an increased risk of fear or hostility to other pets in the household (Adamelli et al. 2005) and an increased risk of aggression when startled (Ramos & Mills 2009).

When performing postpubertal gonadectomy, the age at which the surgery occurs, does not appear to have differential effects on behaviour. In case of urine spraying and fighting, for example, gonadectomy ranging between 6 to 10 months of age in male and female cats seemed no more effective in preventing those behaviours compared to gonadectomy at an older age (Hart & Cooper 1984).

Age may become a determining factor for behaviour, however, when the gonadectomy is prepubertal. Concerns exist that prepubertal gonadectomy, a somewhat unpleasant and potentially stressful experience during the early stages of life might have lasting adverse behavioural consequences, such as the development of an anxiety disorder (Theran 1993; Root Kustritz 1999). An anecdotal report suggests that cats gonadectomised before puberty are more likely to retain juvenile behaviours, become calmer and less aggressive, and that they are less likely to roam compared to cats gonadectomised after puberty (Lieberman 1987). Unfortunately, few studies have addressed the influence of age at time of gonadectomy on the behavioural development in cats. In the experimental study of Stubbs (1996) investigating physical manifestations following (prepubertal) gonadectomy, behavioural manifestations were also assessed at 12 months of age: sexually intact cats displayed greater intraspecies aggression and less affection towards the human observer than did cats neutered at 7 weeks or 7 months. In one long-term follow-up study in which owners were contacted once by telephone no earlier than 30 months after surgery, no differences were detected in age groups at gonadectomy (< 24 weeks of age and ≥ 24 weeks of age) for overall behaviour problems, destructive behaviour, inappropriate elimination and other miscellaneous behaviour problems (Howe et al. 2000). Spain et al. (2004), on the other hand,

reported that early age neutering was associated with shyness in the presence of strangers in both sexes and increased hiding in male cats. In a more recent study, investigating the prevalence of house soiling and aggression in shelter kittens during the first year after adoption, no significant association between age at time of gonadectomy (PPG and TAG) and the behaviours of interest was found (Wright & Amoss 2004).

5. *Conclusion*

Gonadectomy in cats, primarily for population control, is a routine procedure in veterinary practice and yet there are marked differences in how and when it is done. In Belgium, cats are traditionally neutered at 6 to 8 months of age, but there does not appear to be any scientific evidence to document that this is the optimal age. Especially in the context of shelter medicine, early age neutering (as soon as 8 weeks of age) is promoted.

Many veterinary practitioners are reluctant towards adopting the practice of early age neutering because of alleged potential short- or long-term health and behaviour issues. Few studies have directly addressed the effect of age at gonadectomy, and consequently, the clinical effects of early age neutering on health and behaviour remain unclear. Whether cats castrated well before puberty will differ from those castrated at 6 to 8 months should be the subject of a large-scale prospective clinical study.

Many aspects of early age neutering on health and well-being in cats are not fully understood. To date, this lapse in knowledge evokes controversy and discussion about the implementation of early age neutering. Therefore, the general aim of this work was to gain more information about prepubertal gonadectomy (PPG, i.e. between the estimated age of 8 to 12 weeks) compared to traditional age gonadectomy (TAG, i.e. between 6 to 8 months of age) in a large-scale randomized clinical trial set-up in cats.

The specific aims of this doctoral thesis were three-fold:

The first aim was to design safe and feasible **anaesthetic and surgical protocols** to perform PPG in cats (Chapters 1 and 4). Additionally, anaesthesia- and surgery-related parameters were compared between kittens (PPG) and young adult cats (TAG) with a comparable anaesthetic protocol and surgical technique (Chapters 1 and 4). One of the anaesthetic protocols used in the kittens (PPG) was based on the oral transmucosal (OTM) administration of the premedication (dexmedetomdine-buprenorphine combination) as a painless and practical alternative to intramuscular injection. To better understand the clinical interpretation of the sedative and analgesic effects of the combined drugs (dexmedetomidine and buprenorphine) following OTM and IM administration, a pharmacodynamic study (Chapter 2) and a pharmacokinetic study (Chapter 3) were performed in experimental cats.

The second aim was to investigate the **short- and long-term effects** following early age neutering on **health**. For this purpose, the mortality rate between the arrival of the kittens at the Faculty of Veterinary Medicine and the end of the first week after the kittens returned to the shelter, and miscellaneous health problems during the first month after adoption (short-term), as well as the incidence of feline lower urinary tract disease (FLUTD), urethral obstruction, lameness, fractures and hypersensitivity skin disorders until 24 months of age (long-term) were compared between PPG and TAG cats (Chapter 5). To assess a potential relationship between early age gonadectomy and the development of overweight, body weight, body condition score and plasma leptin concentrations were determined and compared between PPG cats and TAG cats (sexual intact until then) at 6 to 8 months of age (Chapter 6).

The third aim was to evaluate the influence of PPG on the **behavioural development** (post-adoption developmental stages into social maturity) and, particularly, the occurrence of behaviour problems (Chapter 7). In this study, owners were asked to complete several web-

based surveys during 24 months after adoption. The occurrence of (potentially) undesirable behaviour in PPG and TAG cats was compared while taking into account the variation in living conditions (social and environmental factors).

To better understand the feasibility of PPG and to document short- and long-term effects on health and well-being of cats undergoing early-age neutering, the FPS Health, Food Chain Safety and Environment funded a large-scale project in shelter cats.

Between April 2010 and August 2012, 800 shelter cats were recruited from shelters in Flanders, Belgium.

Before adoption, kittens were randomly assigned to two different treatment groups, using unequal group sizes:

- 2/3 PPG: prepubertal gonadectomy group (gonadectomy upon assignment, between the estimated age of 8 to 12 weeks [0.7-1.4 kg]).

- 1/3 TAG: traditional age gonadectomy (gonadectomy was postponed until kitten had the age of 6 to 8 months).

All kittens (PPG and TAG) received a microchip (ID) and were offered for adoption (⌂).

Data of the anaesthesia ( ) and surgery ( ) were recorded for PPG as well as for TAG to investigate the anaeshetic and surgical safety and feasibility of PPG, and to compare the findings with a comparable anaesthetic and surgical protocol for TAG.

All cats (PPG and TAG) were invited to the Faculty of Veterinary Medicine at 6 to 8 months of age for a health check-up (HCU), at which time point TAG cats were also neutered.

Furthermore, cat owners were reached by phone calls (☎) to acquire information about the health of their cat when it was 12, 18 and 24 months of age.

To evaluate the behavioural development of PPG and TAG cats, online short- and long-term follow-up surveys were conducted using a 30-day diary ( ) immediately post adoption (PA) and surveys ( ) at 2, 6, 12, 18 and 24 months post adoption.
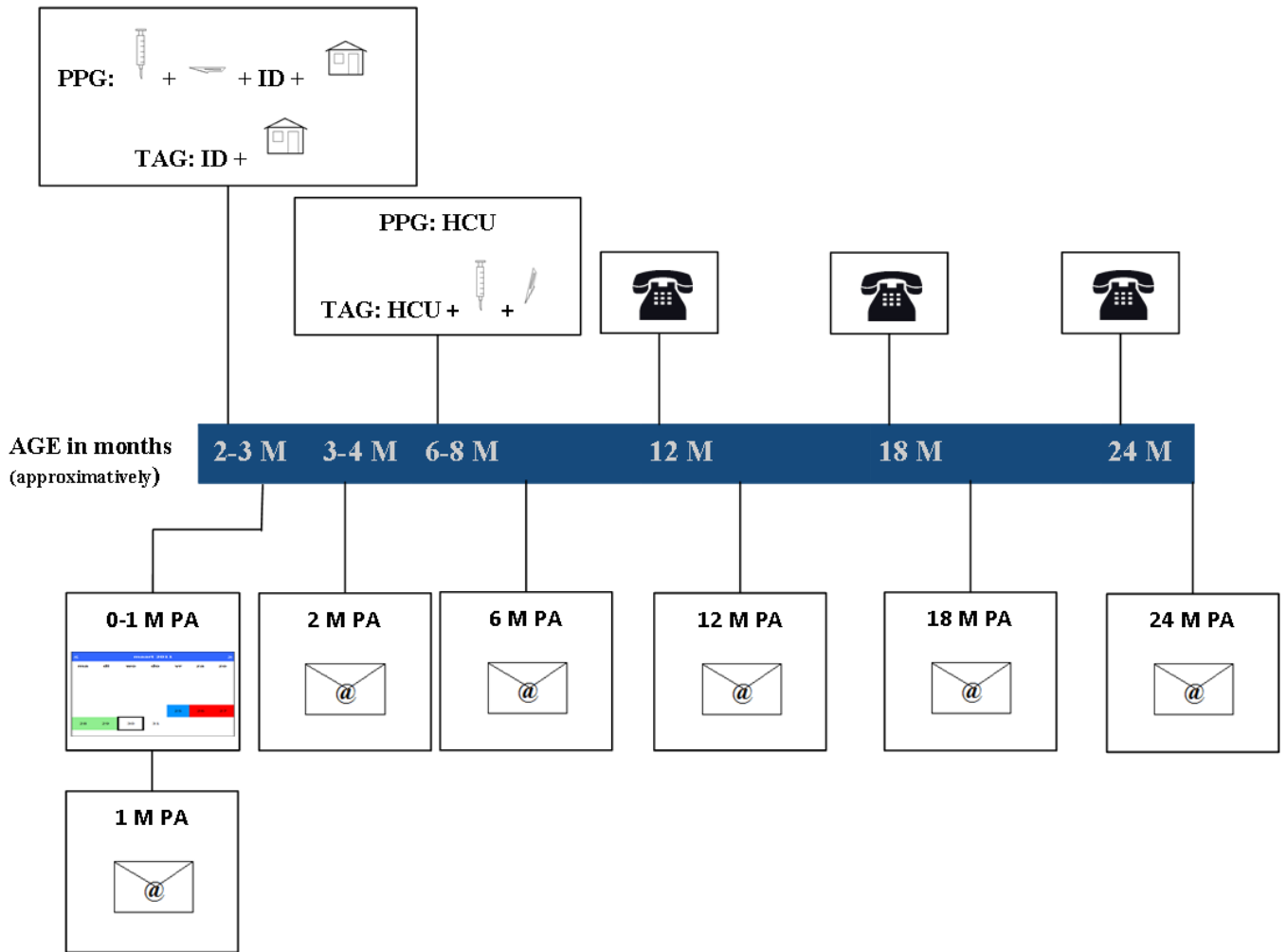
**Prepubertal gonadectomy in cats:
different injectable anaesthetic combinations and
comparison with gonadectomy at traditional age**

1. Abstract

Anaesthetic and analgesic effects of different injectable anaesthetic combinations for prepubertal gonadectomy (PPG) in cats were studied. One anaesthetic protocol was compared to a similar one for gonadectomy at tradional age (TAG).

Kittens were randomly assigned to PPG or TAG. For PPG, three different protocols were compared: (1) an intramuscular (IM) administration of 60 µg/kg dexmedetomidine combined with 20 µg/kg buprenorphine followed by an IM injection of the anaesthetic agent (20 mg/kg ketamine) (protocol DB-IM), (2) an oral transmucosal (OTM) administration of 80 µg/kg dexmedetomidine plus 20 µg/kg buprenorphine followed by an IM injection of 20 mg/kg ketamine combined with a small dosage of dexmedetomidine (20 µg/kg) (protocol DB-OTM), (3) and the last protocol consisting of an IM injection of a 40 µg/kg medetomidine - 20 µg/kg buprenorphine - 20 mg/kg ketamine combination (protocol MBK-IM). For TAG, only the DB-IM protocol was used, with the same products but other dosages for dexmedetomidine (40 µg/kg) and ketamine (5 mg/kg). All cats (PPG and TAG) received an NSAID before surgery. Anaesthetic and analgesic effects were assessed pre- and postoperatively (until 6 hours).

Compared to the DB-OTM protocol, the DB-IM and MBK-IM protocols provided a more stable plane of anaesthesia with fewer adverse effects in PPG cats. Postoperative pain was not significantly different between anaesthetic protocols. PPG and TAG cats with the DB-IM protocol differed significantly only for sedation and/or pain scores, but sedation and pain scores were generally low.

Although there were no anaesthesia-related mortalities in the present study and all anaesthetic protocols for PPG provided a surgical plane of anaesthesia with analgesia up to 6 hours postoperatively, our findings were in favour of the intramuscular (DB-IM and MBK-IM) protocols.

2. Introduction

Prepubertal gonadectomy (PPG) is promoted for population control in cats (Stubbs et al. 1995; Howe 1997; Looney et al. 2008a; Joyce & Yates 2011; Sparkes et al. 2013; Polson et al. 2014) and is gradually becoming an acceptable technique in veterinary practice. Nevertheless, some veterinarians are still hesitant to perform PPG partially because of the lack of experience with pediatric anaesthesia (Root Kustritz et al. 2000; Spain et al. 2002). To better introduce PPG to both the veterinary and lay public, the Belgian government supported a large-scale project on early gonadectomy in cats. The present study, as part of the project, describes the characteristics of anaesthesia and postoperative pain in cats following PPG and gonadectomy at traditional age (TAG).

Previous studies described anaesthetic protocols for PPG in cats, but often with a limited number of animals, without being case-controlled (Faggella & Aronsohn 1993; Robertson et al. 2003a) or with cats and dogs evaluated together (Howe 1997). Moreover, postoperative pain was often not assessed. Lately, injectable anaesthetic drug combinations were used to anaesthetize cats that underwent neutering procedures (Ko & Berman 2010; Joyce & Yates 2011; Ko et al. 2011). Also, pain of kittens following PPG has gained recent attention (Polson et al. 2012; Polson et al. 2014).

The purpose of the present study was two-fold: (1) to compare the characteristics of anaesthesia and postoperative analgesia between different anaesthetic protocols for PPG in cats (while taking into account the used surgical technique), and (2) to compare anaesthetic and analgesic effects in kittens (PPG) and young adult cats (TAG) with a comparable anaesthetic protocol (and comparable surgical) technique based on a large randomized clinical trial.

3. Materials and Methods

The study protocol was approved by the local Ethical Committee (Faculty of Veterinary Medicine, Ghent University, Belgium) (licence number EC 2010/019 and 2011/077) and Deontological Committee (Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium).

3.1. Animals

Healthy, dewormed and vaccinated kittens were recruited from animal shelters in Flanders (Belgium). Female and male kittens were enrolled between the estimated age of 8 to 12 weeks on the basis of their body weight (between 0.7 and 1.4 kg) (Lawler 2008). A complete physical examination was performed before the kittens were transported to the Faculty of Veterinary Medicine, Ghent University. During transport and their stay at the Faculty, kittens from the same litter were housed together to minimize stress and discomfort (Faggella & Aronsohn 1994; Howe 1999).

Between May 2011 and August 2012, standardized anaesthetic protocols as well as standardized surgical techniques were used. Kittens were randomly assigned by a stratified randomization scheme (using different anaesthetic and surgical protocols as stratification factor to ensure treatment assignment balance within each stratum) to one of the two treatment groups, using unequal group sizes (2/3 PPG; 1/3 TAG). Kittens belonging to the PPG group were gonadectomised upon assignment. In the TAG group, gonadectomy was postponed until kittens were 6 to 8 months of age. All kittens (PPG and TAG) were microchipped and remained hospitalized until all neutered kittens (PPG) were monitored for at least 6 hours postoperatively. Then, all kittens were returned to the shelter and were offered for adoption. TAG cats returned to the Faculty around the age of 6 to 8 months for neutering and 6 hours postoperative follow-up. Only cats (PPG and TAG) with macroscopic normal anatomy of the uterus or normal descent of both testicles were included in the present study.

At 3 and 6 hours after surgery (PPG and TAG), vital functions (heart/respiratory rate, capillary refill time, femoral pulse) and body temperature were monitored, sedation and pain were assessed. Environmental conditions were kept as constant as possible throughout all procedures and recovery. Caretakers from the shelters (PPG) and pet owners (TAG) received written instructions for postoperative care beyond 6 hours postoperatively.

3.2. Anaesthesia

Prior to treatment, food, but not water, was withheld for 2 to 4 hours (PPG) or 12 hours (TAG) and the health of the cats was assessed by a general physical examination and body weight measurement (accurate to 0.1 kg). All cats (PPG and TAG) received general anaesthesia according to their assigned protocol (see below). An intramuscular (IM) injection

was given in the quadriceps muscle; a subcutaneous (SC) injection in the back region. Oral transmucosal (OTM) administration was performed by inserting the nozzle of a 1 ml syringe into the buccal cavity of the cat's mouth and gently squirting the content into the buccal area (Robertson et al. 2003b; Slingsby et al. 2009). For IM administration, cats were gently handled and restrained if necessary with a scruffing technique (i.e. holds on the skin of the cat's neck) by a veterinary student or nurse, while for OTM administration cats were gently handled and restrained by the main researcher (NP) and no additional handler was involved.

For PPG three different anaesthetic protocols were compared:

(1) Dexmedetomidine-buprenorphine intramuscular group (DB-IM group): an IM injection of 60 µg/kg (0.12 ml/kg) dexmedetomidine combined with 20 µg/kg (0.07 ml/kg) buprenorphine (Dexdomitor, Orion Corporate and Vetergesic Multidose, Alstoe Animal Health), followed by an IM injection of 20 mg/kg (0.2 ml/kg) ketamine (Anesketin, Eurovet) and a SC injection of 4 mg/kg (0.08 ml/kg) carprofen (Rimadyl, Zoetis).

(2) Dexmedetomidine-buprenorphine oral transmucosal group (DB-OTM group): an OTM administration of 80 µg/kg (0.16 ml/kg) dexmedetomidine combined with 20 µg/kg (0.07 ml/kg) buprenorphine, followed by an IM injection of 20 mg/kg (0.2 ml/kg) ketamine combined with 20µg/kg (0.04 ml/kg) dexmedetomidine and a SC injection of 4 mg/kg (0.08 ml/kg) carprofen.

(3) Medetomidine-buprenorphine-ketamine intramuscular group (MBK-IM group): a single IM injection of 40 µg/kg (0.04 ml/kg) medetomidine (Sedator, Eurovet) plus 20 µg/kg (0.07 ml/kg) buprenorphine plus 20 mg/kg (0.2 ml/kg) ketamine followed by a SC injection of 0.3 mg/kg (0.06 ml/kg) meloxicam (Acticam, Ecuphar). The latter, based on a protocol previously used in kittens (Robertson et al. 2003a) was only implemented in the third study year (2012) on specific demand of shelter vets already involved in PPG but with specific concerns about protocol efficiency and potential safety side effects.

TAG cats were anaesthetized similarly to the PPG DB-IM group, but using different drug dosages: 40 µg/kg (0.08 ml/kg) dexmedetomidine combined with 20 µg/kg (0.07 ml/kg) buprenorphine IM, followed by 5 mg/kg (0.05 ml/kg) ketamine IM and 4 mg/kg (0.08 ml/kg) carprofen SC.

In the DB-IM and the DB-OTM groups, ketamine was only injected when a profound sedation (i.e. sedation score of at least 3 [Table 1]) was reached 15 minutes after

administration the premedication (DB). Otherwise, sedation was reassessed every 5 minutes until profound sedation. If score 3 was still not observed at 30 minutes after premedication, ketamine was administered. Immediately after the ketamine injection, carprofen was administered. Surgical preparation (clipping, scrubbing) was started 3 to 5 minutes later.

In the MBK-IM group, after kittens were profoundly sedated (as soon as a sedation score 3 was reached [Table 1], meloxicam was administered and surgical preparation was started. Kittens were in a surgical plane of anaesthesia when they were not responsive to surgical preparation.

In case of an inadequate plane of anaesthesia (movement and/or vocalisation), systemic or local anaesthetic drugs were supplemented: a second injection of ketamine was administered IM (PPG: 10 mg/kg; TAG: 5 mg/kg) or, during skin closure, 2 mg/kg lidocaïne was gently squirted over the incision site (Xylocaïne 2%, Astrazeneca). Surgery was resumed 5 minutes (systemic) or 1 minute (local) after supplemental drug administration. Atipamezole (Antisedan, Orion Corporate) at half the volume of (dex)medetomidine given, was injected IM at 3 hours postoperatively if the cat still had a sedation score greater than 1. Postoperative rescue analgesia (buprenorphine 20 µg/kg) was administered if a DIVAS score greater than 50 mm or a 4A Vet score greater than 6 was recorded, or when the cat looked uncomfortable.

3.3. Surgical Procedures

Kittens (PPG) were randomly assigned to groups with a different surgical technique, described in detail elsewhere (Porters et al. 2014d). Ovarian pedicle haemostasis for PPG was achieved by ligatures, vascular clips, bipolar electrocoagulation or pedicle tie; for TAG ligatures were used. In male PPG cats, closed castration was performed by spermatic cord knot or by ligature; in TAG an open castration by spermatic cord knot was performed. All surgeries (PPG and TAG) were performed by the same surgeon (NP).

3.4. Data Recording

Sedation – The extent of sedation was assessed subjectively with a Numerical Rating Scale (NRS) (Table 1) modified from scales used previously in cats (Slingsby et al. 1998; Ansah et al. 2000; Dobbins et al. 2002).

Table 1. Numeric Rating Scale (NRS) for sedation scoring based on sedation scoring systems previously used in cats (Slingsby et al. 1998; Ansah et al. 2000; Dobbins et al. 2002).

Score	Definition
0	Completely awake, able to stand and walk, normal posture
1	Stands but staggers when attempting to walk
2	Sternal recumbency, able to lift head up, occasionally makes weak attempts to rise but unable to do so
3	Lateral recumbency, responsive to light stroking and handclapping (can slightly lift up head, tail or limb)
4	Lateral recumbency, unresponsive to light stroking and handclapping

Pain – Pain was assessed using three scoring methods: a Dynamic and Interactive Visual Analogue Scale (DIVAS), a multidimensional pain scoring system (e.g. 4AVet) and by a mechanical nociceptive threshold (MNT) testing device (ProD-Plus – pressure rate onset device; Topcat Metrology Ltd., U.K.). Frightened kittens, hiding from, trying to escape from and showing aggressive behaviour towards the investigator (NP), were excluded for pain assessment.

(1) DIVAS – The 100 mm DIVAS lines were anchored so that 0 mm represented 'no pain' and 100 mm represented 'worst imaginable pain' (Steagall et al. 2009b). DIVAS score of each cat was based on an observation, interaction and wound palpation (Slingsby &

Waterman-Pearson 2002; Steagall et al. 2009b). The wound was four times palpated using the flat part of two or three fingers of the observer's right hand (Grint et al. 2006).

(2) 4AVet (4AVet 2010) (Table 2) – In this multidimensional composite pain scale, the score of each cat was not only based on observation, interaction and wound palpation, but also on monitoring of a physiological parameter (heart rate) and general attitude (activity, appetite, …).

(3) MNT – The antinociceptive effect (MNT) and wound sensitivity (MNT wound; only females) were measured by the cat's response to the handheld MNT testing device. The small probe (PPG: 2.5 mm diameter; TAG: 4 mm diameter) at the angled head of the ProD-Plus was held against the pectoral muscles at the level of the shoulder joint. In female cats, the small probe was also applied 1 cm lateral from the wound edge (MNT wound). The lights of the device guided to alter the force manually at a controlled rate (2 Newton/second). A MNT was recorded when the cat jumped, withdrew or raised a limb, turned the head or vocalised (Steagall et al. 2007; Steagall et al. 2009a; Dixon et al. 2010). Ear withdrawal, tail twitching or any other escape behaviour were also considered as a response to the nociceptive stimulus (Ansah et al. 2000). Whenever a response was observed, the ProD-Plus was withdrawn and the displayed peak reading of force in Newton (N) was subsequently recorded. Since analgesics were used, the cut-off value was set at 20 N to avoid tissue trauma (Dixon et al. 2010). At every time point, the mean of 3 MNT measurements, obtained with an interval of 3 to 5 minutes, was used for statistical analysis.

Table 2. Multidimensional composite pain scale 4AVet used for pain assessment in cats (adapted from 4AVet 2010).

	Identification Cat: Owner:	Baseline	3hrs po	6hrs po
	Date:			
	Time:			
Subjective global evaluation	No pain ↓ Severe pain	0 1 2 3	0 1 2 3	0 1 2 3
General attitude (with the following symtoms):	• Modification of respiration	□	□	□
	• Vaulted back	□	□	□
	• Antalgic posture	□	□	□
	• Agitated or depressed	□	□	□
	• Loss of grooming	□	□	□
	• Look at or lick the operated area	□	□	□
	• Urinate or defecate on itself (or outside the litter box)	□	□	□
	• Loss of appetite	□	□	□
	✓ 0: no symptoms present ✓ 1: 1 symptom present ✓ 2: 2 to 4 symptoms present ✓ 3: 5 to 8 symptoms present	0 1 2 3	0 1 2 3	0 1 2 3
Interactive demeanour	Attentive and reacts to human voice and pettings Timid response Delayed response No or aggressive response	0 1 2 3	0 1 2 3	0 1 2 3
Heart rate Initial value: …. / minute	Increase < 10% Increase of 11-30% Increase of 31-50% Increase > 50%	0 1 2 3	0 1 2 3	0 1 2 3

Reaction to manipulation of surgical area	No visible or audible reactions
	Visible or audible reactions
	  - at the 4th manipulation
	  - at the 2nd and 3rd manipulation
	  - at the 1st manipulation or impossible to manipulate
		0
		
		1
		2
		3
			0
			
			1
			2
			3
				0
				
				1
				2
				3

Intensity of this reaction	No reaction Try to escape Groan, turn its head Try to bite	0 1 2 3	0 1 2 3	0 1 2 3
Global pain score	**1-5: weak pain** **6-10: moderate pain** **11-18: severe pain**			

Adverse and physiologic effects – Adverse effects such as salivation, excitation and vomiting were recorded after administration of the premedication and during recovery. The salivary pH was recorded before drug administration by a pH strip, placed in the cat's mouth until it was moist (Robertson et al. 2003b). The color change was compared with the standard pH colors on the pH indicator paper (Carl Roth GmbH).

Before, during and after anaesthesia, respiration rate (RR) in breaths/minute was counted by observing the thoracic excursions, heart rate (HR) in beats/minute by thoracic auscultation and rectal temperature (T) using a digital thermometer. During surgery, all cats were placed on a circulating warm water blanket and pulse oximetry (Mindray PM-60 Veterinary Pulse Oximeter) through a lingual probe was performed to evaluate peripheral arterial haemoglobin oxygen saturation (SpO$_2$) and HR. During recovery, heat sources were provided and body temperature was monitored every 15 minutes until kittens were awake.

Recovery – In PPG kittens, time from induction of anaesthesia (5 min after injection of ketamine) to sternal recumbency and eating was recorded in minutes.

Data collection – Baseline values for pain scores and physiological parameters (HF, RR and T) were recorded for each cat before drug administration. Cats were observed in their cage, then approached and handled, ventral abdomen (female) and scrotal area (male) were palpated and MNT scores were measured, followed by a clinical examination for physiological parameters.

Sedation in the DB-IM and DB-OTM groups was assessed 15 minutes after IM or OTM premedication (dexmedetomidine combined with buprenorphine), and if sedation was noet yet sufficient, the assessment was repeated every 5 minutes until at least sedation score 3 was reached or until 30 minutes after premedication. In the MBK-IM group, time to sedation score 3 was recorded in seconds.

Physiological parameters (HR, RR, SpO$_2$) were recorded every 10 minutes during surgery. Body temperature was measured after administration of the premedication and immediately after surgery.

At 3 and 6 hours postoperatively, sedation and pain were reassessed and physiological parameters measured in the same way as described above for baseline values. If a cat received atipamezole at 3 hours postoperatively, pain assessment was postponed another 15

minutes. Since all assessments were performed by a single observer (NP), occasionally some details of data could not be recorded.

3.5. Statistical Analysis

To compare the different anaesthetic protocols for PPG in female and in male cats, a cumulative logit model for the ordinal variables (NRS, 4AVet), a linear model for the continuous variables (DIVAS, MNT, HR, RR and SpO_2) and an (exact)[1] logistic regression model for dichotomous variables (adverse effects, need for supplementary anaesthesia and rescue analgesia) were fitted with anaesthetic protocol, surgical technique, period (2011 and 2012) and two-way interactions between anaesthetic protocol, surgical technique and period as fixed effects at the 5% global significance level. Non-significant interactions were removed and will not be discussed. Pairwise multiple comparisons are based on the Tukey-Kramer method and adjusted P-values are reported.

To compare PPG and TAG only data of cats with a similar anaesthetic protocol (DB-IM) and an identical surgical technique (ligatures for females, knot for males) were withheld and the same analyses were repeated (similar models with group, period and the interaction between group and period as fixed effects) using the following data: sedation (NRS) and pain scores (DIVAS, 4AVet, MNT), the occurrence of adverse effects, need for supplementary anaesthesia and rescue analgesia.

Ordinal response variables (NRS, 4AVet) were summarized by the median [range], continuous variables (DIVAS, MNT, HR, RR, SpO_2) by the mean [standard deviation] and dichotomous variables (adverse effects, supplemental anaesthetics and rescue analgesia) by the frequencies for each group. The analysis was performed in SAS version 9.3 (SAS Institute Inc., USA).

4. Results

Four hundred and forty-eight kittens were included. Of those, 380 belonged to the PPG group (female: n = 197; male: n = 183) and 68 to the TAG group (female: n = 34; male: n = 34).

---

[1] if data were sparse

Female PPG –One hundred and ninety-seven kittens were recruited: DB-IM group: n = 93, DB-OTM group: n = 84 and MBK-IM group: n = 20. Mean [sd] body weight was 1.01 [0.20] kg and was not statistically significant among the groups (P = 0.2590). Buccal pH was measured in 135/197 kittens and was between 8 and 9 in 131 kittens and < 8 in 4 kittens.

At baseline, not all pain scores were equal to 0 but did not differ significantly between treatment groups (Table 4). Twenty-three kittens were excluded for pain assessment because they were too frightened to accurately assess pain.

The anaesthetic protocol used was significantly associated with vomiting (P <0.0001) and with salivation (P = 0.0361) within 15 minutes after premedication, with significant differences for vomiting between DB-OTM and DB-IM (P < 0.0001), and between DB-OTM and MBK-IM (P <0.0001). Vomiting was most often observed following OTM administration of the premedication: 54/84 DB-OTM, 14/90 DB-IM and 0/20 MKB-IM. Salivation was observed after OTM and IM administration of the premedication in 5/84 and 1/90 kittens of the DB-OTM and DB-IM group, respectively, and in none of the kittens in the MBK-IM group.

The sedation scores 15 minutes after premedication were significantly higher in the DB-IM group (4 [1-4]) than in the DB-OTM group (4[1-4]) (P = 0.0015). More kittens after IM administration (91/93 DB-IM) than after OTM administration (77/84 DB-OTM) of the premedication were sedated (at least score 3) at 15 minutes after premedication. The mean time to reach sedation score 3 was 1.47 [0.48] minutes after MBK-IM administration.

In every group, at least one kitten needed an additional dose of a systemic or local anaesthetic drug: 5/93 DB-IM, 12/84 DB-OTM, 1/20 MBK-IM (P = 0.0872). In all cases, physiological parameters (HR, RR, SpO$_2$ and T) decreased from baseline values during anaesthesia. No significant differences among groups (Table 3) were detected for HR (P = 0.1278), RR (P = 0.8336) and SpO$_2$ (P = 0.7070).

Table 3. Mean [sd] heart rate (beats per minute), respiratory rate (breaths per minute) and SpO_2 (%) during surgery and rectal temperature (°C) at the end of surgery in female and male kittens randomly assigned to one of the three treatment groups: (1) an intramuscular (IM) administration of 60 µg/kg dexmedetomidine combined plus 20 µg/kg buprenorphine followed by an IM injection of the anaesthetic agent (20 mg/kg ketamine) (DB-IM), (2) an oral transmucosal (OTM) administration of 80 µg/kg dexmedetomidine plus 20 µg/kg buprenorphine followed by an IM injection of 20 mg/kg ketamine combined with a small dosage of dexmedetomidine (20 µg/kg) (DB-OTM), (3) and the last protocol consisting of an IM injection of a 40 µg/kg medetomidine plus 20 µg/kg buprenorphine plus 20 mg/kg ketamine combination (MBK-IM).

Gender	Group	N=	Heart rate (beats per minute)	Respiratory rate (breaths per minute)	SpO$_2$ (%)	Rectal temperature[a] (°C)
Female	DB-IM	93	145 [25]	34 [12]	94 [3]	36.8 [0.8]
	DB-OTM	84	146 [22]	34 [12]	94 [3]	36.9 [0.7]
	MBK-IM	20	138 [23]	33 [9]	95 [3]	36.9 [0.7]
Male	DB-IM	78	150 [20]	28 [12]	94 [4]	36.8 [0.9]
	DB-OTM	75	146 [21]	31 [15]	94 [3]	37.2 [0.8]
	MBK-IM	28	151 [21]	31 [8]	94 [3]	37.3 [0.7]

[a]end of surgery

The anaesthetic protocol used had a significant effect on the incidence of at least one postoperative adverse effect ($P = 0.0065$). Postoperative adverse effects were most often observed in the DB-OTM group (23/84) compared to the DB-IM group (12/93) ($P = 0.0162$) or the MBK-IM group (0/20) ($P = 0.0113$). Salivation (20/84 DB-OTM, 2/93 DB-IM), excitation (8/84 DB-OTM, 5/93 DB-IM) and vomiting (3/84 DB-OTM, 6/93 DB-IM) were observed. Eight kittens (7/84 DB-OTM, 1/93 DB-IM) showed signs of salivation and excitation, one kitten in the DB-OTM group salivation, excitation and vomiting. Sedation scores 3 hours postoperatively were significantly higher in the DB-OTM group (1 [0-4]) compared to the DB-IM group (1 [0-4]) ($P = 0.0010$) or MBK-IM group (1[0-4]) ($P = 0.0432$). More kittens in the DB-OTM group (34/84) than in DB-IM group (19/92) or MBK-IM group (5/20) were still sedated (at least score 2) at 3 hours postoperatively. All kittens recovered uneventfully and recovery times varied significantly between anaesthetic protocols (P =0.0133) (Table 4). Recovery time in the DB-IM group (193 [40] minutes) was significantly shorter than recovery in the DB-OTM group (214 [38] minutes) ($P = 0.0102$). At 6 hours postoperatively, almost all kittens (191/194) were completely awake (score 0), 3

kittens (2/92 DB-IM, 1/83 DB-OTM) were still slightly sedated (score 1) without significant differences among groups ($P = 0.5875$).

Table 4. Mean [sd] recovery time (sternal recumbency and eating) in minutes after prepubertal gonadectomy for female and male kittens randomly assigned to one of the three treatment groups: (1) an intramuscular (IM) administration of 60 µg/kg dexmedetomidine combined plus 20 µg/kg buprenorphine followed by an IM injection of the anaesthetic agent (20 mg/kg ketamine) (DB-IM), (2) an oral transmucosal (OTM) administration of 80 µg/kg dexmedetomidine plus 20 µg/kg buprenorphine followed by an IM injection of 20 mg/kg ketamine combined with a small dosage of dexmedetomidine (20 µg/kg) (DB-OTM), (3) and the last protocol consisting of an IM injection of a 40 µg/kg medetomidine plus 20 µg/kg buprenorphine plus 20 mg/kg ketamine combination (MBK-IM).

Gender	Group	N=	Recovery time (minutes)
Female	DB-IM	69	193 [40][a]
	DB-OTM	53	214 [38]
	MBK-IM	19	176 [37]
Male	DB-IM	63	175 [46]
	DB-OTM	52	191 [40]
	MBK-IM	28	157 [46]

[a] significant difference between IM and OTM group ($P < 0.05$)

Table 4 shows the DIVAS, 4AVet, MNT and MNT wound scores at the various time points. No significant differences were detected between the three groups at 3 hours postoperatively (DIVAS $P = 0.6790$, 4AVet $P = 0.1568$, MNT $P = 0.3953$, MNT wound $P = 0.5869$) and at 6 hours postoperatively (DIVAS $P = 0.3606$, 4AVet $P = 0.9910$, MNT $P = 0.3405$, MNT wound $P = 0.5907$). Rescue analgesia was administered in one kitten of the DB-IM group 3 hours postoperatively and in 5 kittens (3/78 DB-IM, 2/79 DB-OTM) 6 hours postoperatively without significant differences among groups ($P = 0.7995$).

Table 5. Comparison of pain assessment data over time, presented as mean [sd] for DIVAS and MNT (Newton) scores, and presented as median [range] for 4AVet scores following prepubertal gonadectomy in female (F) and male (M) kittens randomly assigned to one of the three treatment groups: (1) an intramuscular (IM) administration of 60 µg/kg dexmedetomidine combined plus 20 µg/kg buprenorphine followed by an IM injection of the anaesthetic agent (20 mg/kg ketamine) (DB-IM), (2) an oral transmucosal (OTM) administration of 80 µg/kg dexmedetomidine plus 20 µg/kg buprenorphine followed by an IM injection of 20 mg/kg ketamine combined with a small dosage of dexmedetomidine (20 µg/kg) (DB-OTM), (3) and the last protocol consisting of an IM injection of a 40 µg/kg medetomidine plus 20 µg/kg buprenorphine plus 20 mg/kg ketamine combination (MBK-IM).

Gender	Group	Baseline				3 hours postoperatively				6 hours postoperatively			
		DIVAS	4AVet	MNT	MNT wound	DIVAS	4AVet	MNT	MNT wound	DIVAS	4AVet	MNT	MNT wound
Female	DB-IM	3 [5]	0 [0-4]	8.5 [4.2]	4.7 [2.2]	16 [12]	4 [0-7]	7.5 [4.1]	2.6 [1.4]	11 [12]	1 [0-6]	6.3 [3.5]	2.4 [1.1]
	DB-OTM	3 [4]	0 [0-4]	7.4 [3.2]	4.6 [2.3]	16 [12]	4 [1-8]	7.0 [3.5]	2.4 [1.2]	11 [11]	2 [0-9]	5.8 [2.8]	2.2 [1.1]
	MBK-IM	2 [5]	0 [0-3]	5.0 [1.2]	2.8 [0.6]	13 [7]	2 [0-5]	4.4 [1.0]	2.0 [0.3]	8 [7]	2 [0-4]	4.2 [1.6]	2.0 [0.6]
Male	DB-IM	1 [2]	1.5 [0-5][a]	7.3 [3.4]		4 [4]	0 [0-3]	6.6 [3.3]		2 [3]	0 [0-2]	5.9 [2.8]	
	DB-OTM	1 [2]	2 [0-5][b]	7.4 [3.5]		4 [3]	1 [0-3]	6.5 [3.3]		2 [2]	0 [0-6]	5.8 [3.0]	
	MBK-IM	1 [2]	1 [0-4]	4.4 [1.3]		3 [3]	0 [0-3]	3.9 [1.4]		1 [2]	0 [0-2]	3.5 [1.1]	

[a] significant differences between OTM and IM (P <0.05); [b] significant differences between OTM and MBK (P < 0.05)

Female PPG and TAG – The female PPG kittens from the DB-IM group with ligatures as surgical technique (n = 23) were compared to 34 female TAG cats. Mean [sd] body weights were 1.00 [0.21] kg and 2.61 [0.34] kg, respectively.

Three PPG cats vomited after premedication (P = 0.2552) whereas one TAG cat salivated after premedication (P = 1.000). In both groups all cats were sedated (at least score 3) at 15 minutes after premedication (P = 0.7284), and all cats reached a surgical plane of anaesthesia after ketamine injection. Only 1 out of the 23 PPG and 2 out of the 34 TAG cats needed an additional systemic anaesthetic drug to allow ovarian isolation (P = 0.5478).

Significant differences in postoperative adverse effects were not detected between PPG (3/23) and TAG (1/34) cats (P = 0.6315). At 3 hours postoperatively, the sedation score was significantly higher in PPG (1 [0-4]) compared to TAG (0 [0-2]) cats (P = 0.0220). More PPG kittens (6/23) than TAG cats (2/31) were still sedated (at least score 2). Also, DIVAS pain scores 3 hours postoperatively were significantly higher in PPG (17 [11]) compared to TAG (6 [9]) cats (P = 0.0011) (Table 6). Only one cat (TAG) received rescue analgesia at 6 hours postoperatively without a significant difference between PPG and TAG (P = 0.4615)

Table 6. Comparison of pain assessment data over time, presented as mean [sd] for DIVAS and MNT (Newton) scores, and presented as median [range] for 4AVet scores in female cats randomly assigned to the prepubertal gonadectomy (PPG) group and the group gonadectomy at traditional age (TAG), both anaesthetized with the DB-IM protocol (PPG: IM injection of 60 µg/kg dexmedetomidine combined with 20 µg/kg buprenorphine, 20 mg/kg ketamine IM, 5 mg/kg carprofen SC; TAG: IM injection of 40 µg/kg dexmedetomidine combined with 20 µg/kg buprenorphine, 5 mg/kg ketamine IM, 5 mg/kg carprofen SC).

	Baseline		3 hours postoperatively		6 hours postoperatively	
	PPG	**TAG**	**PPG**	**TAG**	**PPG**	**TAG**
DIVAS	2 [3]	2 [4]	17 [11][a]	6 [9]	10 [9]	7 [12]
4AVet	1 [0-3]	0 [0-3]	5 [1-7]	3 [0-6]	2 [0-5]	2 [0-7]
MNT[b]	8.2 [4.6]	6.9 [3.2]	8.0 [4.8]	7.4 [3.3]	6.8 [4.1]	6.4 [3.3]
MNT wound[b]	4.6 [2.3]	4.8 [2.1]	2.3 [1.6]	3.9 [1.7]	2.2 [1.1]	3.4 [1.8]

[a] significant difference between PPG and TAG cats ($P < 0.05$); [b] no statistical analysis between PPG and TAG cats due to use of different probe tip for PPG and TAG cats

Male PPG – One hundred and eighty-three kittens were recruited: DB-IM: n = 78, DB-OTM: n = 76 and MBK-IM: n = 29. Mean [sd] body weight was 1.04 [0.21] kg and was not

statistically significant among the groups ($P = 0.1000$). Buccal pH was measured in 100/183 kittens and was between 8 and 9 in 98 kittens, and < 8 in 2 kittens.

At baseline, mean pain scores were not equal to 0 (Table 4). Only 4AVet baseline values in kittens assigned to the DB-OTM group were significantly higher than the values in kittens assigned to the DB-IM group ($P = 0.0296$) or MBK-IM group ($P = 0.0008$). Seventeen kittens were not assessed for pain due to their frightened reaction to the investigator.

The anaesthetic protocol used was significantly associated with vomiting after administration of the premedication ($P < 0.0001$) with significant differences between groups. Of the 76 kittens in the DB-OTM group, 55 kittens vomited in contrast to 15 of the 77 kittens in the DB-IM group (P <0.0001) and no kittens in the MBK-IM group ($P = 0.0010$). Salivation was observed occasionally, irrespective of the anaesthetic protocol used ($P = 0.3258$): 2/76 DB-OTM, 0/78 DB-IM and 1/29 MBK-IM. Sedation scores (15 minutes after premedication) were significantly higher in the DB-IM group (4 [2-4]) than in the DB-OTM group (4 [0-4]) ($P = 0.0461$). More kittens from the DB-IM group (77/78) than from the DB-OTM group (69/76) were sedated (at least score 3) at 15 minutes after premedication. Mean time to reach sedation score 3 was 1.42 [0.63] minutes after MBK-IM administration.

A surgical plane of anaesthesia was reached in all kittens without the need for an additional local or systemic drug. In all cases, physiological parameters (HR, RR, SpO_2 and T) decreased from baseline values during anaesthesia. No significant differences among groups (Table 2) were detected for HR ($P = 0.2348$), RR ($P = 0.0605$) and SpO_2 ($P = 0.9033$).

The anaesthetic protocol used had a significant effect on the incidence of at least one postoperative adverse effect ($P = 0.0100$). Postoperative adverse effects were more often observed in the DB-OTM group (22/73) compared to the DB-IM group (9/76) ($P = 0.0219$): salivation (13/73 DB-OTM, 2/76 DB-IM), excitation (10/73 DB-OTM, 6/76 DB-IM), vomiting (4/73 DB-OTM, 4/76 DB-IM). In the MBK-IM group excitation was observed in one kitten. Six kittens (4/73 DB-OTM, 2/76 DB-IM) were observed with signs of salivation and excitation. Sedation score at 3 hours postoperatively was significantly higher in the DB-OTM group (1 [0-4]) compared to the DB-IM group (1 [0-4]) ($P = 0.0002$) and the MBK-IM group (0 [0-4]) ($P = 0.0010$). More kittens in the DB-OTM group (26/75) were still sedated (at least score 2) compared to kittens in the DB-IM (13/77) and the MBK-IM group (1/29). At 6 hours postoperatively all but 3 kittens were completely awake (score 0) whereas those 3 kittens (2/75 DB-OTM, 1/75 DB-IM) were slightly sedated (score 1) without significant differences among treatment groups ($P = 0.5037$). All kittens recovered uneventfully. There

was no significant difference between different anaesthetic protocols in the recovery time (P = 0.1349) (Table 3) and in pain scores at 3 hours postoperatively (DIVAS P = 0.7101, 4AVet P = 0.5108, MNT P = 0.8635) and at 6 hours postoperatively (DIVAS P = 0.4933, 4AVet P = 0.3409, MNT P = 0.8873) (Table 4). Rescue analgesia was only deemed necessary at 6 hours postoperatively in 4 kittens (2/78 DB-IM, 2/76 DB-OTM).

Male PPG and TAG – The male PPG kittens from the DB-IM group with knot as surgical technique (n = 39) were compared to 34 male TAG cats. Mean [sd] body weight was 1.04 [0.21] kg (PPG) and 3.32 [0.44] kg (TAG).

Vomiting after administration of the premedication was seen in PPG (6/39) as well as TAG (2/34) cats (P = 0.3223). Salivation was observed in 2 TAG cats (P = 0.2265). Adequate sedation was reached 15 minutes after premedication in both groups (P = 0.3331). Only one cat (TAG) needed an additional local anaesthetic drug during surgery (P = 1.0000).

Significant differences in postoperative adverse effects were not detected between PPG (5/38) and TAG (2/34) cats (P = 0.6845). At 3 hours postoperatively the NRS sedation score was significantly higher in PPG (1 [0-4]) than in the TAG (0 [0-1]) group (P = 0.0017): more PPG kittens (5/39) than TAG cats (0/34) were still sedated (at least score 2). Furthermore, 4AVet values were significantly lower in PPG (0 [0-3]) compared to TAG (1 [0-4]) cats (P = 0.0267) at 3 hours postoperatively (Table 7). DIVAS score 6 hours postoperatively was significantly lower in PPG (2 [2]) than in TAG (6 [8]) cats (P = 0.0181) (Table 6). There was no need for rescue analgesia.

Table 7. Comparison of pain assessment data over time, presented as mean [sd] for DIVAS and MNT (Newton) scores, and presented as median [range] for 4AVet scores in male cats randomly assigned to the prepubertal gonadectomy (PPG) group and the group gonadectomy at traditional age (TAG), both anaesthetized with the DB-IM protocol (PPG: IM injection of 60 µg/kg dexmedetomidine combined with 20 µg/kg buprenorphine, 20 mg/kg ketamine IM, 5 mg/kg carprofen SC; TAG: IM injection of 40 µg/kg dexmedetomidine combined with 20 µg/kg buprenorphine, 5 mg/kg ketamine IM, 5 mg/kg carprofen SC).

	Preoperatively		3 hours postoperatively		6 hours postoperatively	
	PPG	**TAG**	**PPG**	**TAG**	**PPG**	**TAG**
DIVAS	1 [2]	2 [5]	4 [4]	4 [6]	2 [2][a]	6 [8]
4AVet	1 [0-4]	2 [0-5]	0 [0-3][a]	1 [0-4]	0 [0-1]	0 [0-0]
MNT[c]	8.1 [3.7]	10.5 [5.2]	7.0 [3.4]	10.2 [5.0]	6.0 [3.1]	9.7 [4.8]

[a] significant difference between PPG and TAG cats (P < 0.05); [c] no statistical analysis between PPG and TAG cats due to use of different probe tip for PPG and TAG cats

5. Discussion

There were no anaesthesia-related mortalities in this study. This finding is in agreement with previous reports using similar drug combinations for neutering kittens (Robertson et al. 2003a; Joyce & Yates 2011; Polson et al. 2012; Polson et al. 2014). Furthermore, there were no clinically relevant differences in any of the anaesthetic/analgesic parameters between PPG and TAG.

Although the criteria (anxiolysis, analgesia, sedation and loss of consciousness) for balanced anaesthesia (Looney et al. 2008a) were fulfilled in all three anaesthetic PPG protocols, the current results are in favour of the intramuscular administered protocols (DB-IM and MBK-IM). Compared to IM premedication (DB-IM), less sedative effects and more pre-anaesthetic adverse effects were observed following OTM administration of the premedication (DB-OTM). To facilitate the clinical interpretation of the sedative and analgesic effects following different routes of administration, a pharmacodynamic (Porters et al. 2014a) and pharmacokinetic study (Porters et al. 2014b) were performed to compare OTM and IM administered dexmedetomidine plus buprenorphine in experimental adult cats. Dexmedetomidine combined with buprenorphine was not as well absorbed from the oral mucosa as from the muscular injection site (Porters et al.2014b). This finding supports the fact that higher dosages of dexmedetomidine were selected for sedation in the DB-OTM group compared to the DB-IM group. Although the alkaline pH of the buccal saliva of the kittens would suggest a high uptake of such drugs (Robertson et al. 2003b; Slingsby et al. 2009), the simultaneous administration of the pharmaceutical drug formulations of dexmedetomidine hydrochloride and buprenorphine hydrochloride and the rather acidic pH of their combined solution (i.e. 4.383) were likely to negatively affect OTM absorption. Furthermore, some kittens seemed to resent OTM administration, possibly due to the bad taste of the solution.

After MBK-IM administration, adverse effects were rarely observed, likely due to the rapid onset of sedation and quick induction of anaesthesia which reduces activation of the vomiting center (Ko & Berman 2010). On the other hand, it should be mentioned that adverse reactions (vocalizing, withdrawal, biting) on the injection were more prominent in the MBK-IM group than in the DB-IM group (data not shown), precluding single handler injections in the former.

This observation has been reported previously with similar combinations with dissociative drug combinations (Ko & Berman 2010).

Recovery times following PPG were considerably long seeing the relative short duration of surgery (Porters et al. 2014d). These observations were also reported in cats aged 1 month to 6 years (Polson et al. 2012). Atipamezole was never administered at the end of (or shortly after) surgery, although routine use of a postoperative alpha-2 antagonist probably would have shortened recovery time without inducing adverse effects (Ko et al. 2011; Polson et al. 2014) and without compromising postoperative pain relief (Polson et al. 2014). A shorter recovery time reduces the risk for hypoglycaemia as well as for hypothermia (Polson et al. 2014), which is why an alpha-2 antagonist may be beneficial, particularly in kittens.

Different methods for pain assessment were combined since a validated method in cats was not available when this study started in 2010. Besides DIVAS and 4AVet pain scores, MNT were measured as being a more objective method to evaluate postoperative pain in cats (Hunt et al. 2013). Due to the use of a probe tip with a different diameter, MNT pain scores between PPG and TAG cats were not compared. To obtain consistent pain scores, a single person assessed the pain (Cambridge et al. 2000; Grint et al. 2006; Polson et al. 2014). Frightened kittens were excluded for pain assessment based on the inability to palpate surgical site and the lack of differentiation between fear and stress behaviours from pain behaviours (Harrison et al. 2011). It should also be noticed that pre-operative baseline values were not equal to zero. Anxiety and stress (following the transportation, novel environment, strange person [NP]) will likely have resulted in an overestimation of these baseline pain scores.

In the postoperative period, on the other hand, the cat's level of sedation and consciousness would affect pain assessment in the opposite direction (Steagall et al. 2009b). For this reason, sedation was assessed and, if needed, atipamezole was administered before pain assessment. For all protocols, pain scores remained low at 3 and 6 hours postoperatively, and very few kittens required rescue analgesia. Although some significant differences in DIVAS values between the PPG and TAG group were observed, pain scores were low in both groups and thus the clinical relevance of the statistical differences is questionable. The low scores in the present study using anaesthetic protocols based on the pre-emptive and multimodal analgesia are likely to support the finding that most kittens and young adult cats experienced only mild pain following gonadectomy (Polson et al. 2012; Polson et al. 2014). For practical considerations, analgesia was only evaluated up to 6 hours postoperatively. Also for animal welfare reasons, it was beneficial that (frightened) kittens only spent a minimal period of time

at the Faculty. Pain in the later postoperative period was further assessed at the shelter (PPG) or at home (TAG). Caretakers (PPG) and owners (TAG) where requested to fill out the 4AVet pain scale up to 48 hours postoperatively since it was the most accessible method for lay persons to assess pain. Pain behavior in all kittens and cats was diminishing over time according to the caretakers (PPG) and owners (TAG) (data not published). Despite the variety in evaluators, this finding is not unimportant because caretakers or owners are well placed to evaluate the level of pain or anxiety experienced by their own cats (Mathews 2000). This clinical study was subject to a number of limitations in terms of data collection. Since a single person (NP) performed all acts concerning anaesthesia and surgery, data collection was not blinded. The data of kittens initially enrolled in the large-scale project on PPG were not included in the present study. Lower dosages of dexmedetomidine (DB-OTM and DB-IM) and ketamine were used at that time, but since an inadequate surgical plane of anaesthesia was reached in many cases, dosages of dexmedetomidine and ketamine were gradually increased. Other research groups also used higher dosages of ketamine (Robertson and others 2003a) or medetomidine-ketamine (Joyce and Yates 2011) in kittens. It is quite likely that slightly lower dosages of dexmedetomidine and ketamine would have been sufficient towards the end of the research project when surgical times were shorter (Porters et al. 2014d). Two different NSAIDs were used for PPG; however, it is advisable to use meloxicam as NSAID for PPG in kittens since, if necessary, it can be administered as a very palatable oral suspension beyond 24 hours postoperatively.

6. Conclusion

There were no anaesthesia-related mortalities in the present study and all anaesthetic protocols for PPG provided a surgical plane of anaesthesia with analgesia up to 6 hours postoperatively. Despite its painless and easy administration, DB-OTM could not be withheld as an ideal route for premedicating kittens. The MBK-IM protocol is the most attractive in shelter medicine.

7. Funding

8. Acknowledgements

Sedative and antinociceptive effects of dexmedetomidine combined with buprenorphine after oral transmucosal or intramuscular administration in cats

1. Abstract

Oral transmucosal (OTM) administration may offer a painless alternative for sedative and analgesic drug administration in cats. The present study compares sedation and antinociception after OTM and intramuscular (IM) administration of a dexmedetomidine-buprenorphine combination in healthy adult cats.

According to a cross-over protocol (1 month wash-out), a combination of dexmedetomidine (40 µg/kg) and buprenorphine (20 µg/kg) was given OTM (buccal cavity) or IM (quadriceps muscle) in 6 female neutered cats. Sedation was measured using a numerical rating scale, at baseline and at various time points until 6 hours after treatment. At the same time points, analgesia was scored using a dynamic and interactive visual analogue scale, based on the response to an ear pinch, and by the cat's response to a mechanical stimulus exerted by a pressure rate onset device. Physiological and adverse effects were recorded, and oral pH measured.

There were no differences in sedation or antinociception scores between OTM and IM dosing at any of the time points. Nociceptive thresholds increased after both treatments but without significant difference between groups. Buccal pH remained between 8 and 8.5. Salivation was noted after OTM administration (n = 2) and vomiting after both OTM (n = 4), and IM (n = 3) dosing.

In healthy adult cats, OTM administration of dexmedetomidine and buprenorphine resulted in comparable levels of sedation and antinociception compared to IM dosing. The OTM administration may offer an alternative route to administer this sedative-analgesic combination in cats.

2. Introduction

The search for painless alternatives for sedative and analgesic drug administration has recently gained interest in both human (Anttila et al. 2003; Zub et al. 2005) and veterinary medicine (Grove & Ramsay 2000; Robertson et al. 2003b). Oral transmucosal (OTM) administration of drugs is simple and painless (Robertson et al. 2003b), which can be helpful when working with an animal species sometimes difficult to restrain. Moreover, drugs are rapidly absorbed by venous drainage in the mouth and first-pass metabolism in the liver is avoided (Robertson et al. 2005). For all these reasons, OTM administration may offer an improved route for sedative-analgesic administration in cats.

Alpha-2 adrenergic receptor agonists, including dexmedetomidine, are commonly used as sedative-analgesics in feline anaesthesia (Ansah et al. 1998; Selmi et al. 2003). with increasing use of the active dextro-rotatory isomer dexmedetomidine (Savola & Virtanen 1991; Ansah et al. 1998; Kuusela et al. 2000; Ansah 2004). When combined with opioid agonists, alpha-2 agonists have shown to exert additive and/or synergistic analgesic and sedative effects (Drasner & Fields 1988; Ossipov et al. 1989). Consequently, a low dose of dexmedetomidine combined with an opioid agonist results in anaesthetic-sparing and analgesic effects superior to those obtained with an opioid alone (Lamont 2008; Grint et al. 2009). Moreover, the combination of dexmedetomidine and buprenorphine, a semi-synthetic partial agonist at mu opioid receptors, has been described as an effective multimodal analgesic regimen in feline surgery (Slingsby et al. 2010).

Previous studies have investigated the analgesic efficacy of OTM administration of buprenorphine (Robertson et al. 2003b; Robertson et al. 2005) and the sedative and thermal antinociceptive effect of dexmedetomidine administered OTM (Slingsby et al. 2009). Only one previous study reported the sedative and cardiorespiratory effects of the combination of dexmedetomidine and buprenorphine given IM or OTM (Santos et al. 2010). The aim of this study was to compare the sedative and mechanical antinociceptive effects of the combination of dexmedetomidine with buprenorphine following either OTM or IM administration in healthy adult cats, and to record adverse and physiological effects observed with each administration route.

3. *Materials and methods*

3.1. Animals

Six purpose-bred female neutered cats with a weight of 6.1 (5.3 − 7.5) kg and an age of 7.2 ± 0.015 years were used in this study. The study protocol was approved by the Animal Research Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (licence number EC 54/2011). All cats were housed together and were well acclimatized to the study environment. Cats were considered healthy based on physical examination. Food, but not water, was withheld for 12 hours before treatment.

3.2. Study protocol

Treatment administration was in a randomized crossover design, with a washout period of one month between treatments. The combination of 40 μg/kg dexmedetomidine (Dexdomitor, Orion Corporation) and 20 μg/kg buprenorphine (Vetergesic Multidose, Alstoe Animal Health) was mixed in a syringe immediately before administration. Cats were manually restrained while the drugs were administered either IM (using a 25 gauge needle into the quadriceps muscle) or OTM (into the buccal cavity). OTM administration was performed by inserting the nozzle of a 1 ml syringe into the buccal cavity of the cat's mouth (Robertson et al. 2003; Slingsby et al. 2009). The content was then gently squirted into the cheek area (Slingsby et al. 2009). Cats were differently restrained for OTM and IM administration. All administrations were performed with the cats on an examination table in the study environment. For IM administration, cats were tightly fixated by a single veterinary nurse in the neck and lower back region, while for OTM administration cats were only gently restrained allowing movement. For the latter, resistance to administration was recorded.

3.3. Sedative and nociceptive testing

The sedative and antinociceptive scoring was performed by three observers, all of whom were unaware of drug administration route. The extent of sedation was assessed at baseline, 15, 30 and 45 minutes, and 1, 2, 4 and 6 hours after drug administration, using a numerical rating scale (NRS) (Table 1), which was modified from scales used previously in cats (Slingsby et al. 1998; Ansah et al. 2000; Dobbins et al. 2002).

Table 1. NRS sedation scoring system based on sedation scoring systems previously used in cats (Slingsby et al. 1998; Ansah et al. 2000; Dobbins et al. 2002).

Score	Definition
0	Completely awake, able to stand and walk, normal posture
1	Stands but staggers when attempting to walk
2	Sternal recumbency, able to lift head up, occasionally makes weak attempts to rise but unable to do so
3	Lateral recumbency, responsive to light stroking and handclapping (can slightly lift up head, tail or limb)
4	Lateral recumbency, unresponsive to light stroking and handclapping

At baseline, 30 minutes and 1, 2, 4 and 6 hours after treatment, antinociceptive effects were measured using a dynamic and interactive visual analogue scale (DIVAS) and by a mechanical threshold (MNT) testing device (ProD-Plus, Topcat Metrology Ltd). The DIVAS was based on the cat's response to an ear pinch with the fingernails to the apex of the external ear (Ansah et al. 1998; Ansah et al. 2000). To obtain a constant intensity, the same observer performed all pinches, however the response of each cat was scored by all three observers. The DIVAS was a 100 mm scale, with 0 mm indicating no response, and 100 mm indicating a response at the highest intensity. The mean of the three observations for the NRS and DIVAS was taken for statistical analysis.

The MNT testing was performed as follows. The small (4 mm diameter) probe of the ProD-Plus device was held perpendicularly to the pectoral muscles at the level of the shoulder joint, and the pressure increased manually at a rate of 2 Newtons (N) per second. A positive response was recorded when the cat jumped, withdrew or raised a limb, turned the head or vocalised (Steagall et al. 2007; Steagall et al. 2009a; Dixon et al. 2010). Ear withdrawal, tail twitching or any other escape behaviour were also considered as a response to the nociceptive stimulus (Ansah et al. 2000). Whenever a response was observed, the ProD-Plus was withdrawn and the displayed force recorded. Since analgesics were used, a maximum force of 20 N was set to avoid tissue trauma (Dixon et al. 2010). At every time point, two MNT measurements were made, three to five minutes apart, and the mean of the two measurements was used for statistical analysis.

3.4.   Adverse and physiologic effects

At least two observers were permanently present in the study environment from 15 minutes to 6 hours after drug administration. Incidence of adverse effects, such as salivation and vomiting, were recorded at this time. At baseline, 30 minutes, and at 1, 2, 4, and 6 hours after treatment, respiratory rate (RR), heart rate (HR), buccal pH and rectal temperature were also recorded; RR (breaths per minute) was counted by observing thoracic excursions and HR (beats per minute) was determined by thoracic auscultation. Bradycardia was defined as a HR of less than 100 beats per minute (Monteiro et al. 2009). Buccal pH was recorded using pH indicator paper, placed in the cat's mouth until it was moist (Robertson et al. 2003). The colour change was compared with the standard pH colours on pH indicator paper (Universal indicator paper pH 1-14, Carl Roth GmbH). The rectal temperature was measured using a digital thermometer.

3.5. Statistical analyses

The two treatments were compared at each time point for each response variable. For the analysis at a particular time point, the mean of the three observers was calculated for each cat at that particular time. As some of the variables were ordinal, i.e., NRS and DIVAS, and too few data were available for the other variables to test for normality, the nonparametric Wilcoxon signed rank test, stratified for cat, was used and results were summarized based on the median and range. Analyses were performed at a significance level of 5%. Bonferroni's multiple comparisons technique was used for the comparisons at the specific time points, leading to a comparison wise significance level of $\alpha/g = 0.05/5 = 0.01$. Data are presented as median and range.

For the nociceptive testing, a MNT reference range for healthy cats not treated with an analgesic was obtained by taking all baseline values for all cats, obtained before drug administration. The 95% reference range was then calculated from these values, as mean ± 1.96 * standard error.

4. Results

Sedative and antinociceptive effects – There was no difference in level of sedation, as measured by the NRS, between cats in the OTM or IM group at any of the time points (Table 2). At 4 hours after drug administration, 3 of the cats in the IM group, and all cats in the OTM group were able to stand (had a sedation score 0 or 1).

There were no significant differences in DIVAS scores between IM and OTM drug administration at any of the individual time points (Table 2). Both treatments caused changes in MNT values over time, with an increase compared to baseline values (Figure 1), but without significant difference between administration routes.

Table 2. Sedation and antinociception (DIVAS) scores in 6 cats, at baseline and after oral transmucosal (OTM) or intramuscular (IM) administration of dexmedetomidine and buprenorphine (40 and 20 µg/kg respectively). For sedation, 0 = no sedation, 4 = maximum sedation. For DIVAS scoring, 0 = no response to nociceptive stimulation, 100 = maximum response. Data are median (range).

Time (minutes)	Sedation score		DIVAS (mm)	
	OTM	IM	OTM	IM
baseline	0 (0-0)	0 (0-0)	17 (1-25)	19 (4-32)
15	3 (1-4)	4 (0-4)		
30	4 (1-4)	4 (0-4)	1 (0-34)	1 (0-1)
45	4 (3-4)	4 (0-4)		
60	4 (3-4)	4 (3-4)	1 (0-5)	0 (0-4)
120	3 (1-4)	4 (3-4)	1 (0-13)	0 (0-2)
240	0 (0-1)	2 (1-3)	5 (2-24)	1 (0-2)
360	0 (0-0)	0 (0-2)	7 (2-12)	4 (0-6)

Table 3. Heart rate (HR), respiratory rate (RR) and rectal temperature (RT) (median and range) at baseline and at different time points after drug administration in 6 healthy cats receiving dexmedetomidine and buprenorphine (40 and 20 µg/kg respectively) either oral transmucosal (OTM) or IM.

Time (minutes)	Heart rate (beats per minute)		Respiratory rate (breaths per minute)		Rectal temperature (°C)	
	OTM	IM	OTM	IM	OTM	IM
baseline	220 (160-248)	190 (160-240)	65 (56-80)	54 (40-72)	38.4 (37.9-38.8)	38.3 (37.4-38.6)
30	134 (88-180)	118 (108-216)	36 (32-60)	30 (20-60)	38.2 (37.9-38.7)	37.8 (37.5-38.0)
60	106 (88-120)	110 (100-132)	30 (24-40)	30 (16-44)	37.3 (36.5-38.0)	36.9 (36.6-38.1)
120	102 (92-116)	96 (88-108)	22 (16-36)	18 (16-40)	36.2 (35.5-36.8)	35.6 (34.8-37.3)
240	176 (140-204)	82 (76-108)	41 (24-44)	24 (20-54)	35.9 (35.1-37.4)	35.0 (34.7-35.7)
360	190 (176-208)	162 (80-240)	50 (32-70)	30 (16-72)	36.7 (35.1-38.0)	35.6 (34.8-38.5)
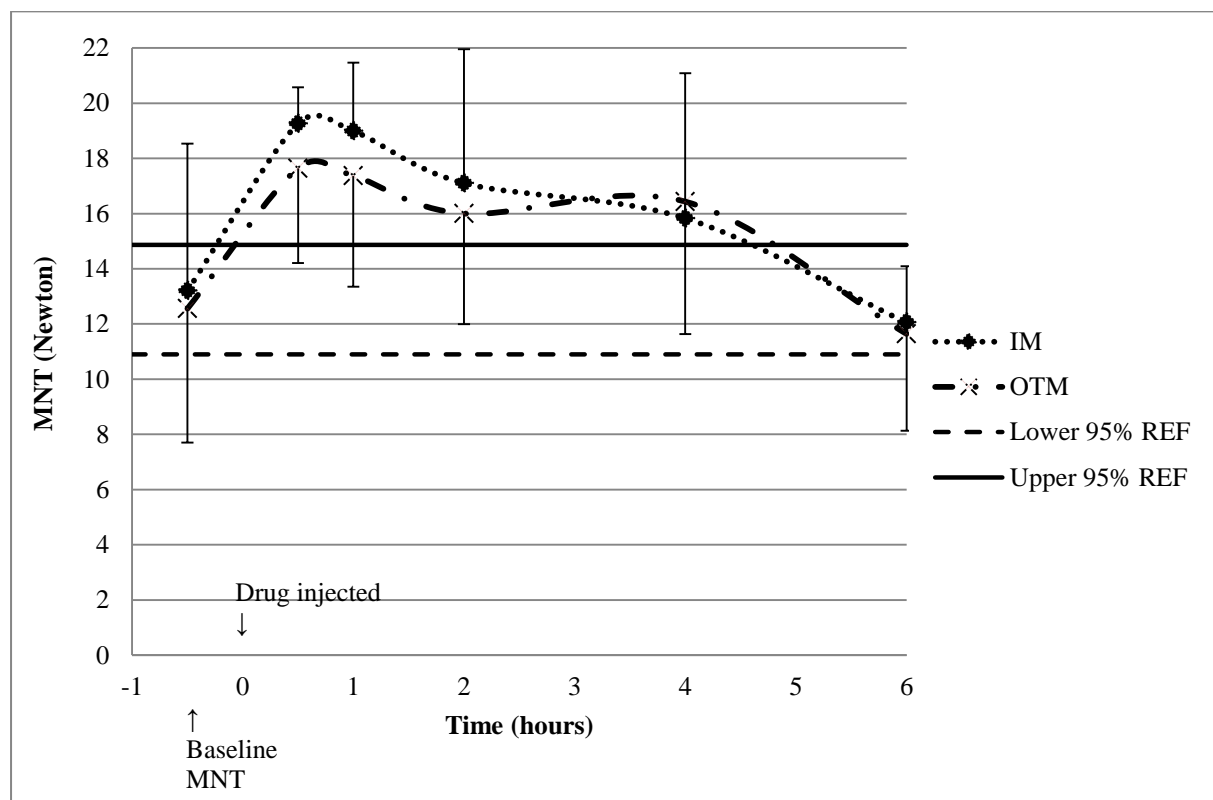
Figure 1. Mean ± standard deviation (SD) mechanical thresholds (MNT) over time in 6 healthy cats after treatment with dexmedetomidine 40 µg/kg and buprenorphine 20 µg/kg administered by the oral transmucosal route (OTM) or IM (time 0). The horizontal lines represent the upper and lower 95% reference ranges (REF) for the pooled baseline MNT values. Values above the reference range were deemed to represent an analgesic effect.

Adverse and physiological effects – Three cats resisted the OTM administration. Four cats vomited within 15 minutes of OTM dexmedetomidine-buprenorphine administration, and a further two showed signs of salivation. Due to the necessary restraint, interpretation of individual resistance to IM injection was not possible. Three cats vomited after IM administration; two within 15 minutes, and one between 15 and 30 minutes, and again at 6 hours.

Compared with baseline values, a decrease in RR, HR and rectal temperature was observed in both groups, but without significant differences between treatments (Table 3). During the two treatments, the buccal pH remained between 8 and 8.5 in all cats at all occasions.

5. *Discussion*

In the present study, dexmedetomidine and buprenorphine (40 and 20 μg/kg respectively) were administered in combination by either the OTM or IM route, and sedation and antinociception were evaluated. Both routes of administration provided measurable sedative and antinociceptive effects in the cats, with no detectable differences in sedation or antinociception at any of the time points during the study period.

Although the alkaline environment of the buccal cavity would theoretically favour the uptake of the unionised dexmedetomidine and buprenorphine molecules (Robertson et al. 2003b; Slingsby et al. 2009), sedation and antinociception were comparable after each method of administration, with no differences detected at any time points. A similar previous study, reporting lower doses of dexmedetomidine and buprenorphine (20 and 20 μg/kg, respectively) demonstrated superior sedation associated with IM compared to OTM dosing (Santos et al. 2010), whereas a comparison of OTM or IM dexmedetomidine alone (40 μg/kg) demonstrated thermal antinociceptive and sedative effects that were similar after OTM and IM administration (Slingsby et al. 2009). Similarly, the effect of buprenorphine (20 μg/kg) on thermal thresholds in the cat, has also been reported to be similar after OTM and IM administration (Robertson et al. 2005).

Stress and anxiety may have an influence on the sedative effect of dexmedetomidine because of the competitive nature of adrenaline at the adrenoreceptors (Grint et al. 2009). This may explain the variability seen in sedation scores between cats in the same treatment group, despite efforts to minimize stress in the cats.

To evaluate antinociception, a DIVAS and a MNT testing system were used. There are a number of limitations of MNT testing, not least that the mechanical stimulus is different to clinical pain. In addition, the positive responses to increasing mechanical force may have been suppressed by dexmedetomidine-induced sedation. Despite these limitations, the highest value on the MNT was measured at 30 minutes, which correlates well with the onset of analgesia by buprenorphine (20 µg/kg) measured previously by thermal threshold testing (Robertson et al. 2005). The response returned to baseline between 4 and 6 hours, suggesting a duration of analgesia of buprenorphine and/or dexmedetomidine of no longer than 6 hours, similar to that reported elsewhere (Pascoe 2000a; Robertson et al. 2005).

The main adverse effects observed after OTM administration were salivation and vomiting. Unexpectedly, resistance to administration was also observed. This contrasts to previous studies, which showed no salivation or resentment with OTM buprenorphine (Robertson et al. 2005) or dexmedetomidine (Slingsby et al. 2009). In the previous buprenorphine study (Robertson et al. 2005), buprenorphine hydrochloride without preservative was used. In contrast, a multidose buprenorphine preparation, which includes the preservative chlorocresol, was used in the present study, which may explain these differences. Emesis was noted following both methods of administration, and was also seen in previous studies examining OTM dexmedetomidine, alone (Slingsby et al. 2009) and in combination with buprenorphine (Santos et al. 2010). No cats vomited after OTM buprenorphine (Robertson et al. 2003b; Robertson et al. 2005), and therefore it is feasible that the emesis may have been triggered by the dexmedetomidine in the present study.

The systemic uptake of buprenorphine after OTM and IV administration has been previously demonstrated to be equivalent (Robertson et al. 2005), however studies examining the comparative bioavailability of dexmedetomidine after OTM and IV administration in cats are lacking. Drug availability may be influenced by emesis, salivation (by diluting the drug in the mouth), or swallowing (via increased gastrointestinal absorption and first-pass metabolism), and therefore different formulations and doses may result in differences in sedation and antinociception.

Using a greater sample size may have increased the reliability of the results in this study, and the study may have been under-powered to detect small differences in sedation and antinociception. Subtle differences in sedation may also not have been detected using the methodology selected for this study. Choice of sample size was based on practical and ethical considerations, as well as the numbers of cats used in previous similar studies (Selmi et al.

2003; Johnson et al. 2007). When executing a two-sided signed rank test using small sample numbers, statistical significance would imply a particularly extreme result (for example all cats having a higher sedation score following one treatment compared with the other), which was not identified in this study. Using the ad hoc method of Lehmann (Lehmann 2006), power calculation can provide a suggestion of the size of the effect that is required before it will lead to a significance test with a power of 80 %. Assuming an underlying normal distribution for continuous variables, power analysis can be performed. For the two-sided paired t-test with 6 cats and $\alpha = 0.05$, a true difference of 6.9 in the DIVAS score will lead to a significant effect with a power of 80 %. As nonparametric tests have typically less power than parametric tests, 15 % additional subjects might be required to achieve the same power. Theoretically, the actual sample size of 6 cats needs to be reduced to 5 cats under the same settings. Consequently, a true difference of 7.5 will lead to a significant effect in the nonparametric test with a power of 80 %. Such difference, although quite large, could be observed for the DIVAS variable, but is unlikely for the MNT testing.

6. Conclusion

Administration of the described clinical dosages of dexmedetomidine and buprenorphine in healthy adult cats provided comparable antinociceptive effects whether administered by the OTM or IM route. The OTM administration of dexmedetomidine and buprenorphine in cats may be considered a useful technique for analgesic and sedative dosing, although further work examining the influence of vomiting and salivation may be beneficial.

Pharmacokinetics of oral transmucosal and intramuscular dexmedetomidine combined with buprenorphine in cats

1. *Abstract*

Plasma concentrations and pharmacokinetics of dexmedetomidine and buprenorphine were compared after oral transmucosal (OTM) and intramuscular (IM) administration of dexmedetomidine combined with buprenorphine in healthy adult cats.

According to a cross-over protocol (1 month wash-out), a combination of dexmedetomidine (40 µg/kg) and buprenorphine (20 µg/kg) was given OTM (buccal cavity) or IM (quadriceps muscle) in 6 female neutered cats. Plasma samples were collected through a jugular catheter during a 24-hour period. Plasma dexmedetomidine and buprenorphine concentrations were determined by liquid chromatography-tandem mass spectrometry. Plasma concentration-time data were fitted to compartmental models (MW/Pharm, Mediware).

For dexmedetomidine, the area under the plasma concentration-time curve (AUC) from 0 to 12 h was significantly different between OTM (9.59 ± 3.88 ng·h/ml) (mean ± SD) and IM administration (38.56 ± 8.66 ng·h/ml). Dexmedetomidine achieved significantly lower maximum plasma concentrations (C_{max}) after OTM than after IM administration (3.59 ± 2.02 ng/ml and 22.07 ± 8.91 ng/ml, respectively). For buprenorphine, the AUC from 0 to 6 h was significantly different between OTM (1.69 ± 0.77 ng·h/ml) versus IM (17.86 ± 7.15 ng·h/ml) administration. Buprenorphine achieved maximum plasma concentrations of 0.52 ± 0.31 ng/ml at 1.18 ± 0.34 h after OTM administration and of 10.43 ± 5.20 ng/ml at 0.15 ± 0.15 h after IM injection with a significant difference between both treatments.

Data suggested that dexmedetomidine (40 µg/kg) combined with buprenorphine (20 µg/kg) are not as well absorbed from the oral mucosa site as from the intramuscular injection site.

2. Introduction

Oral transmucosal (OTM) drug delivery refers to the local and systemic delivery of drugs to or via any oral cavity membrane (Rathbone et al. 1994) and can be subdivided into: (1) sublingual delivery (via the membrane of the ventral surface of the tongue and floor of the mouth) into to the systemic circulation, (2) buccal delivery (via the buccal membrane lining the cheek) into the systemic circulation, and (3) local delivery for the treatment of conditions of the oral cavity (Harris & Robinson 1992). Since it is more suitable in feline medicine to administer drugs into the side of the mouth than sublingual, OTM administration in the current study refers to the administration of drugs via the buccal mucosa.

There are several advantages of OTM administration of drugs compared to other routes of administration. It is a painless and often more practical alternative compared to the conventional gastrointestinal and parenteral routes of administration (Rathbone et al. 1994). Only minimal restraint is needed for drug delivery and the patient will barely experience discomfort.

The combination of an opioid with an alpha-2 agonist has been described as part of an effective multimodal analgesic regimen in veterinary medicine (Corletto 2007; Slingsby et al. 2010). Buprenorphine, a partial µ-opioid agonist, combined with a low dose of dexmedetomidine, results in anaesthetic-sparing and analgesic effects superior to that obtained with an opioid alone (Lamont 2008; Grint et al. 2009). Furthermore, dexmedetomidine is widely used for feline sedation and premedication (Slingsby & Taylor 2008) and, combined with an opioid, it provides a good sedative and analgesic effect (Slingsby et al. 2010).

Recently, there has been growing interest in the clinical usefulness of the OTM administration of dexmedetomidine (Slingsby et al. 2006; Slingsby et al. 2009), buprenorphine (Robertson et al. 2003b; Robertson et al. 2005; Giordano et al. 2010; Catbagan et al. 2011; Bortolami et al. 2012) and the combination of dexmedetomidine and buprenorphine (Santos et al. 2010) in cats. Therefore, our group investigated the sedative, analgesic and adverse effects of the IM versus OTM administration of a dexmedetomidine/buprenorphine combination as premedication for early age neutering in cats (Porters et al. manuscript 2014c). The small size of the 8- to 12-week old kittens and the surgical intervention made it impossible to combine that study with a pharmacokinetic study. To facilitate the clinical interpretation of the differences in the sedative and analgesic effects of the combined drugs following OTM and IM administration, a pharmacodynamic study

(Porters et al. 2014a) and the present pharmacokinetic study were performed in adult experimental cats.

To our knowledge, only one study reports the plasma concentration of medetomidine after spraying it into the cat's mouth (Ansah 2004) and three studies have investigated the pharmacokinetic data of different dosages of buprenorphine after OTM administration in cats (Robertson et al. 2003b; Robertson et al. 2005; Hedges et al. 2013a), but pharmacokinetic data of the sedative-analgesic combination of dexmedetomidine/buprenorphine are missing.

In the present study, we tested the null hypothesis that this sedative-analgesic combination would be equally absorbed systemically after OTM administration as after IM injection in healthy adult cats. Therefore, the pharmacokinetics of the combination of dexmedetomidine (40 µg/kg) and buprenorphine (20 µg/kg) after a single OTM or IM administration in healthy adult cats were determined and compared.

3. *Materials and Methods*

3.1. Animals

Six purpose-bred neutered female cats with a weight of 5.7 ± 0.4 kg (mean ± SD) and age of 7.3 ± 0.015 years were used in this study. The study protocol was approved by the Animal Research Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (licence number EC 54/2011). All cats were housed together and were well acclimatized to the study environment. Cats were considered healthy based on a general physical examination and their body weight was measured the day before each treatment. Food, but not water, was withheld for 12 h before treatment.

3.2. Study Protocol

Seventeen hours prior to treatment, the cats were anaesthetized with propofol (4-6 mg/kg Propoflo Plus, Abbott Laboratories Ltd.) for placement of a jugular catheter. A 20 G 8 cm polyurethane catheter (arterial leader-catheter PE, Vygon, Ltd.) was aseptically inserted into a jugular vein and secured with nylon suture material (Ethilon, Ethicon, Inc., Johnson & Johnson Medical S.A.) and an elastic bandage to facilitate subsequent blood sampling.

Treatments were administered in a randomized crossover design with a wash-out period of 1 month between treatments. The combination of dexmedetomidine hydrochloride

(Dexdomitor, Orion Corporation) at 40 µg/kg and buprenorphine hydrochloride (Vetergesic Multidose, Alstoe Animal Health, Ltd.) at 20 µg/kg was mixed in a syringe immediately before administration. To allow easy comparison between administration routes, the same drug dosages for OTM and IM treatment were used. Cats were manually restrained while the drugs were administered. Oral transmucosal administration was performed by inserting the nozzle of a 1 ml syringe into the buccal cavity of the cat's mouth. Afterwards, the content (volume range from 0.77 to 0.91 ml) was gently squirted into the cheek area. For the IM route, a 25 gauge needle was used to inject the drugs (volume range from 0.75 to 0.88 ml) into the quadriceps muscle. Blood samples were collected from the jugular catheter before and at 1, 5, 10, 20, 30 and 60 min, and at 2, 4, 6, 12 and 24 h after drug administration. The volume of blood collected was 2 ml per sample so that in total less than 10% of the cat's estimated blood volume was removed. An equal volume of 0.9% saline was injected through the jugular catheter after each sample (Robertson et al. 2005). The catheter was flushed with 1 ml of 10 IU/ml heparin saline solution (Heparine LEO 5000 IU/ml, LEO pharmaceutical products) to ensure catheter patency (Lainesse et al. 2006). Blood was transferred into heparinized tubes (Vacuette 2 ml Lithium Heparine), enrolled in aluminium foil and centrifuged (2000 g) for 10 min within 2 h after sampling (Robertson et al. 2005). Plasma was separated and stored at -80°C until analysed.

3.3. Drug analysis

Plasma concentrations of dexmedetomidine and buprenorphine were determined using a high-performance liquid chromatography (HPLC) method. Analytical standards of dexmedetomidine and buprenorphine were purchased from Tocris Bioscience (Bristol, UK) and Sigma (Bornem, Belgium), respectively. Norbuprenorphine, the primary active N-dealkylated metabolite of buprenorphine, was provided by DoCoLab (Doping Control Laboratory, Zwijnaarde, Belgium). Tolazoline was used as internal standard for the analysis of dexmedetomidine while nalorphine served as internal standard for the analysis of buprenorphine and norbuprenorphine. Both internal standards were purchased from Sigma. Stock solutions of 1 mg/ml of all components were prepared in methanol and stored at -20 °C. Working solutions were obtained by further dilution of these stock solutions in methanol, and were stored at 4 °C.

The following extraction and clean-up procedure was followed for the analysis of dexmedetomidine, buprenorphine and norbuprenorphine in the feline plasma samples. To a

250 µl plasma sample, 800 µl water of HPLC grade were added, followed by vortex mixing during 15 s. To blank samples used for calibration or quality control samples, 25 µl of a working solution of the analytes dexmedetomidine, buprenorphine and norbuprenorphine were added, with concentrations ranging from 0.25 to 10 ng/ml for dexmedetomidine and from 0.5 to 25 ng/ml for buprenorphine and norbuprenorphine, followed by vortex mixing during 15 s. Also 25 µl of a working solution of the internal standards tolazoline (25 ng/ml) and nalorphine (50 ng/ml) were added, again followed by vortex mixing during 15 s. After an ultracentrifugation step (13000 rpm, 10 min), the supernatans was applied on an Oasis® MCX (Mixed-mode Cation eXchange, Waters, Milford, MA, USA) solid-phase clean-up cartridge, conditioned previously with respectively 3 ml methanol of HPLC grade, 3 ml water of HPLC grade, and 3 ml of a 0.1 N HCl solution in water of HPLC grade. When the sample was passed entirely through the cartridge, it was rinsed with successively 3 ml of a 0.1 N HCl solution in water of HPLC grade, 3 ml water of HPLC grade, and 3 ml methanol of HPLC grade. The cartridge was vacuum dried, and elution was achieved by applying 3 ml of a 25 % ammonia solution in methanol of HPLC grade (10/90, v/v). The extract was dried at 40 °C under a gentle stream of nitrogen, and subsequently redissolved into 125 µl of a 0.1 % solution of formic acid in water of an Ultra Performance Liquid Chromatography (UPLC) grade. A 5 µl portion of the redissolved extract was injected into an ACQUITY UPLC^{TM} BEH C_{18} reversed-phase column (1.7 µm, 50 mm x 2.1 mm I.D.) on an ACQUITY UPLC^{TM} Binary Solvent Manager and Sample Manager combination (all from Waters, Milford, MA, USA). The column was maintained at a temperature of 30 °C. Mobile phase A was acetonitrile, while mobile phase B was a solution of 20 mM ammonium formate in water. The following gradient program was used to separate all components: 0 min: 0.5 % A, 99.5 % B, 0.00 – 4.50 min: linear to 100 % A, 4.50 – 7.00 min: 100 % A, 7.00 – 7.10 min: linear to 0.5 % A, 99.5 % B, 7.10 – 10.00 min: 0.5 % A, 99.5 % B. Detection was achieved on a Micromass® Quattro Premier^{TM} XE mass spectrometer instrument (Waters), equipped with an ESI ion source used in the positive ion mode. A divert valve was used to send the UPLC effluent only to the detector from 1 to 5 min, which is the time window when all analytes elute from the column. The following MRM (multiple reaction monitoring) transitions – using the most prominent product ions – 201.27 > 95.02, 160.85 > 90.87, 468.37 > 55.01, 414.3 > 101.04, and 312.31 > 201.06 were retained for the quantification of dexmedetomidine, tolazoline, buprenorphine, norbuprenorphine and nalorphine, respectively, using the MassLynx® software (Waters).

The analytical method was sufficiently validated according to EC guidelines[2]. In particular, the following validation parameters were evaluated: linearity, accuracy, precision, limit of quantification (LOQ), limit of detection (LOD), specificity, and carry-over. For dexmedetomidine, the LOQ was 0.025 ng/ml with a linear range up to at least 1 ng/ml. For buprenorphine and norbuprenorphine, the LOQ was 0.05 ng/ml with a linear range up to at least 2.5 ng/ml. A LOD of 0.012, 0.016 and 0.023 ng/ml was determined for dexmedetomidine, buprenorphine and norbuprenorphine, respectively.

3.4.   Pharmacokinetic analysis

Pharmacokinetic parameters were calculated on an individual basis for each cat following OTM and IM treatment. Pharmacokinetic analysis was performed by use of a computer program (MW/PHARM 3.15) (Proost & Meijer 1992). The individual plasma drug concentration-time data were fitted to a one- or two- compartmental open model. The appropriate model was selected by evaluating correlation of the observed and predicted plasma concentrations and by visual examination of the line fits and the residual plots. Values below the LOQ of the method were not taken into account in the pharmacokinetic analysis. The following pharmacokinetic parameters were calculated: the area under the concentration-time curve (AUC_{0-t}), time to maximal plasma concentration (T_{max}), maximal plasma concentration (C_{max}), absorption rate constant (K_a), elimination rate constant (K_{10}), elimination rate constant from central to peripheral compartment and peripheral to central compartment (K_{12} and K_{21}, respectively), absorption half-life ($t_{1/2a}$), elimination half-life ($t_{1/2e}$ for one-compartmental model, $t_{1/2\alpha}$ and $t_{1/2\beta}$ for two-compartmental model) and mean residence time (MRT).

3.5.   Statistical analysis

Descriptive statistical parameters were calculated as mean $\pm$ standard deviation (SD). Since there were only six values in each treatment group and a Gaussian distribution of the data could not be assumed, a non-parametric test was used (Powers 1990). A Wilcoxon signed rank test was performed to examine significant differences between the 2 routes of

---

[2] Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performances of analytical methods and the interpretation of results. Official Journal of the European Communities, No L221, 17.8.2002, Decision of 12 August 2002, European Commission, Directorate General for Public Health and Consumers Protection, 2002/657/EC, 17-08-2002 (SANCO).

administration. The null hypothesis implied that pharmacokinetic parameters are unaffected by the route of administration. Values of $P < 0.05$ were considered significant. Statistical software SPSS (SPSS Statistics, Version 20, IBM Corp., USA) was used.

4. Results

For dexmedetomidine, the mean estimated plasma profiles over time obtained from OTM and IM administration are depicted in Figure 1. After both treatments, plasma dexmedetomidine concentrations were quantified until 12 h in all cats. Depending on the individual cat, a one- or two-compartmental model best described the changes in plasma dexmedetomidine concentration following OTM or IM administration. Pharmacokinetic parameters for dexmedetomidine in cats are summarized in Table 1. For dexmedetomidine, the AUC_{0-12} was significantly smaller ($P = 0.028$) after OTM administration (9.59 ± 3.88 ng·h/ml) than after IM injection (38.56 ± 8.66 ng·h/ml). The C_{max} of dexmedetomidine was significantly lower ($P = 0.028$) after OTM (3.59 ± 2.02 ng/ml) than after IM administration (22.07 ± 8.91 ng/ml). No other significant differences between pharmacokinetic parameters among the OTM and IM route were detected for dexmedetomidine.

Figure 1. Plasma dexmedetomidine concentration over time (mean ± SD) following oral transmucosal (OTM) and intramuscular (IM) administration of dexmedetomidine (40 μg/kg) plus buprenorphine (20 μg/kg) in 6 healthy adult cats.

Table 1. Pharmacokinetic parameters of dexmedetomidine after OTM (oral transmucosal) and IM (intramuscular) administration of dexmedetomidine (40 µg/kg) plus buprenorphine (20 µg/kg) in 6 healthy adult cats (individual cats 1 - 6 and mean ± SD).

Cat	1		2		3		4		5		6		Mean ± SD	
Treatment	OTM	IM	OTM	IM	OTM	IM	OTM	IM	OTM	IM	OTM	IM	OTM	IM
Weight (kg)	6.2	6.0	5.5	5.5	5.3	5.1	5.3	5.3	6.1	6.0	5.9	5.8	5.7 ± 0.4	5.6 ± 0.4
Dose (mg)	0.25	0.24	0.22	0.22	0.21	0.20	0.21	0.21	0.24	0.24	0.24	0.23	0.23 ± 0.02	0.23 ± 0.02
CA	1	1	2	2	2	2	1	1	1	2	2	2		
AUC_{0-12} (ng·h/ml)	11.95	41.78	11.41	45.56	14.92	42.85	8.73	21.53	5.11	41.24	5.42	38.41	9.59 ± 3.88[a]	38.56 ± 8.66
T_{max} (h)	0.72	0.01	0.44	0.24	0.59	0.74	0.27	0.27	1.12	0.17	0.80	0.38	0.66 ± 0.30	0.30 ± 0.25
C_{max} (ng/ml)	3.07	26.30	5.61	28.68	6.51	15.88	2.86	8.67	1.64	32.73	1.83	20.14	3.59 ± 2.02[a]	22.07 ± 8.91
K_a (1/h)	3.70	909.60	2.65	10.36	1.91	1.72	14.0	12.8	0.89	7.09	1.40	3.35	4.09 ± 4.95	157.49 ± 368.5
K_{10} (1/h)	0.32	0.63	1.20	0.83	1.08	0.82	0.36	0.46	0.89	1.84	0.82	1.18	0.78 ± 0.36	0.96 ± 0.49
K_{12} (1/h)			1.04	0.31	0.58	0.43				3.80	0.40	1.32	0.67 ± 0.33	1.47 ± 1.62
K_{21} (1/h)			0.74	0.82	0.55	0.88				1.93	0.44	1.31	0.58 ± 0.15	1.24 ± 0.51
$t_{1/2a}$ (h)	0.19	0.76×10^{-3}	0.26	0.07	0.36	0.40	0.05	0.05		0.10	0.49	0.21	0.36 ± 0.26	0.14 ± 0.15
$t_{1/2e}$ (h)	2.15	1.10					1.95	1.51	0.78				1.33[b]	1.27[b]
$t_{1/2\alpha}$ (h)			0.26	0.46	0.36	0.40				0.10	0.49	0.21	0.30[b]	0.21[b]
$t_{1/2\beta}$ (h)			2.06	1.53	2.23	1.63				1.38	2.68	1.51	2.29[b]	1.51[b]
MRT (h)	3.10	1.58	2.00	1.65	1.91	1.81	2.81	2.18	1.12	1.61	2.32	1.72	2.21 ± 0.75	1.76 ± 0.22

CA: one- or two-compartmental analysis; AUC_{0-12}: area under the plasma concentration-time curve from 0 to 12h (time of last quantified point); T_{max}: time to maximum plasma concentration; C_{max}: maximum plasma concentration; K_a: absorption rate constant; K_{10}, K_{12}, K_{21}: elimination rate constant, rate constant from central to peripheral compartment, and from peripheral to central compartment; $t_{1/2a}$: absorption half-life; $t_{1/2e}$: elimination half-life for one-compartmental model; $t_{1/2\alpha}$, $t_{1/2\beta}$: elimination half-life for first and second slope; MRT: mean residence time; [a] significantly different from IM ($P < 0.05$); [b] harmonic mean

For buprenorphine, the mean plasma concentration versus time curves following OTM and IM administration are shown in Figure 2.

Following OTM administration, plasma buprenorphine concentration was first detected at 5 min and remained detectable until 6 h in 5, and until 24 h in 3 out of 6 cats. In one cat, however, buprenorphine was first measured at 20 min only and the concentration fell below the detection threshold from 6 h after OTM administration. The median concentration of buprenorphine in this cat was 0.11 ng/ml (range of 0.05 − 0.32 ng/ml). On the other hand, plasma buprenorphine concentrations following IM administration were detected in all cats throughout the whole study. Compartmental analysis of buprenorphine yielded a one-compartmental model after OTM administration. After IM injection, a two-compartmental model best described the changes in plasma buprenorphine concentration. The pharmacokinetic parameters obtained for buprenorphine are shown in Table 2. The AUC_{0-6} was significantly smaller ($P = 0.028$) following OTM (1.69 ± 0.77 ng·h/ml) compared to IM administration (17.86 ± 7.15 ng·h/ml). Buprenorphine reached a C_{max} of 0.52 ± 0.31 ng/ml at 1.18 ± 0.34 h following OTM administration and 10.43 ± 5.20 ng/ml at 0.15 ± 0.15 h after IM injection, with a significant difference between both treatments ($P = 0.028$). A significant difference ($P = 0.028$) was also found for $t_{1/2a}$, $t_{1/2e}$ and MRT after OTM administration compared to the IM route. The K_a was significantly lower after OTM than after IM administration. Norbuprenorphine was not detected in any cat at concentrations above the LOD at any time point. No other significant differences between pharmacokinetic parameters among the different routes of administration were detected for buprenorphine.
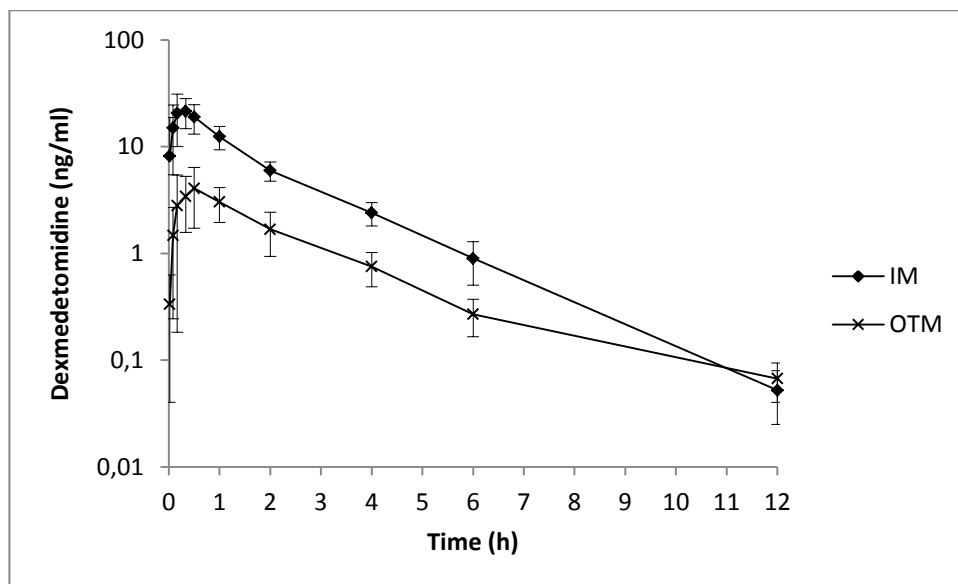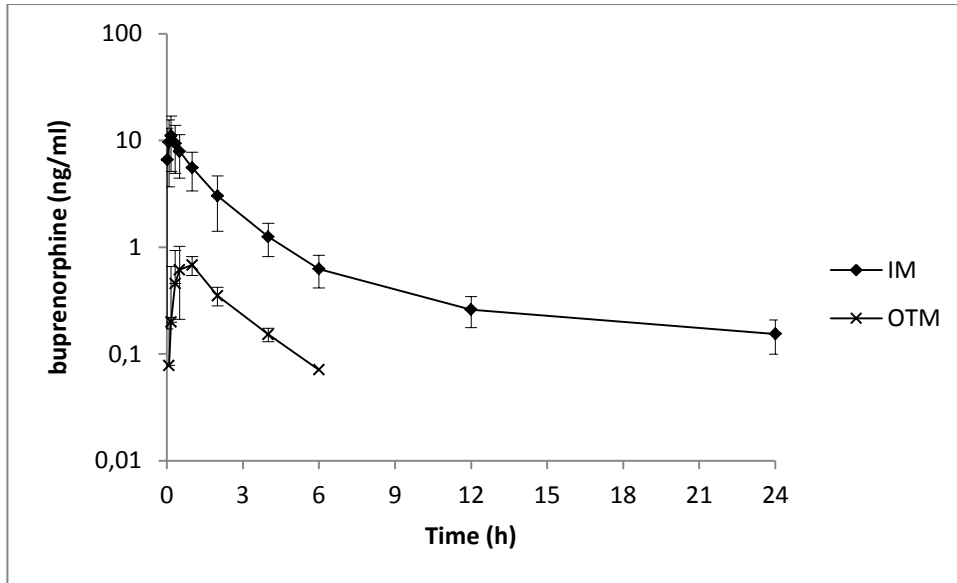
Figure 2. Plasma buprenorphine concentration over time (mean ± SD) following oral transmucosal (OTM) and intramuscular (IM) administration of dexmedetomidine (40 µg/kg) plus buprenorphine (20 µg/kg) in 6 healthy adult cats.

Table 2. Pharmacokinetic parameters of buprenorphine after OTM (oral transmucosal) and (IM) intramuscular administration of dexmedetomidine (40 µg/kg) plus buprenorphine (20 µg/kg) in 6 healthy adult cats (individual cats 1-6 and mean ± SD).

Cat	1		2		3		4		5		6		Mean ± SD	
Treatment	OTM	IM	OTM	IM	OTM	IM	OTM	IM	OTM	IM	OTM	IM	OTM	IM
Weight (kg)	6.2	6.0	5.5	5.5	5.3	5.1	5.3	5.3	6.1	6.0	5.9	5.8	5.7 ± 0.4	5.6 ± 0.4
Dose (mg)	0.12	0.12	0.11	0.11	0.11	0.10	0.11	0.11	0.12	0.12	0.12	0.12	0.11 ± 0.01	0.11 ± 0.01
CA	1	2	1	2	1	2	1	2	1	2	1	2		2
AUC_{0-6} (ng·h/ml)	1.61	27.05	2.97	23.73	1.91	11.25	1.83	9.41	0.74	21.15	1.10	14.59	1.69 ± 0.77[a]	17.86 ± 7.15
AUC_{0-24} (ng·h/ml)		30.34		27.7		17.54		15.04		27.78		18.24		22.77 ± 6.55
T_{max} (h)	1.83	0.04^{-1}	0.85	0.13	1.01	0.44	1.06	0.09	1.21	0.09	1.09	0.16	1.18 ± 0.34[a]	0.15 ± 0.15
C_{max} (ng/ml)	0.33	16.20	1.05	15.76	0.63	4.07	0.59	5.15	0.18	12.28	0.31	9.13	0.52 ± 0.31[a]	10.43 ± 5.20
K_a (1/h)	0.55	21.83	2.16	31.57	1.40	6.54	1.50	45.69	1.75	48.14	1.69	21.43	1.51 ± 0.54[a]	29.2 ± 15.90
K_{10} (1/h)	0.55	0.38	0.55	0.57	0.68	0.23	0.54	0.31	0.31	0.38	0.42	0.55	0.51 ± 0.13	0.40 ± 0.13
K_{12} (1/h)		0.23		0.17		0.22		0.45		0.20		0.26		0.26 ± 0.10
K_{21} (1/h)		0.01		0.07		0.11		0.16		0.07		0.15		0.10 ± 0.05
$t_{1/2a}$ (h)	1.27	0.32^{-3}	0.32	0.02	0.50	0.11	0.46	0.02	0.40	0.01	0.41	0.03	0.56 ± 0.35[a]	0.03 ± 0.04
$t_{1/2e}$ (h)	1.27		1.26		1.02		1.27		2.27		1.63		1.36[a,b]	
$t_{1/2u}$ (h)		1.12		0.91		1.36		0.81		1.14		0.80		0.99[b]
$t_{1/2\beta}$ (h)		77.13		13.87		14.60		12.34		15.19		7.16		13.63[b]
MRT (h)	1.83	44.51	1.83	6.29	1.48	13.52	1.84	12.56	3.28	9.78	2.36	4.94	2.10 ± 0.64[a]	15.27 ± 14.72

CA: one- or two-compartmental analysis; $AUC_{0-6/24}$: area under the plasma concentration-time curve from 0 to 6h and 24 h(time of last quantified point after OTM and IM administration respectively); T_{max}: time to maximum plasma concentration; C_{max}: maximum plasma concentration; K_a: absorption rate constant; K_{10}, K_{12}, K_{21}: elimination rate constant, rate constant from central to peripheral compartment, and from peripheral to central compartment; $t_{1/2a}$: absorption half-life; $t_{1/2e}$: elimination half-life for one-compartmental model; $t_{1/2a}$, $t_{1/2\beta}$: elimination half-life for first and second slope; MRT: mean residence time; [a] significantly different from $t_{1/2\beta}$ IM ($P < 0.05$); [b] harmonic mean

83

5. *Discussion*

The present study showed that the pharmacokinetic profile of OTM administration of dexmedetomidine (40 µg/kg)/buprenorphine (20 µg/kg) in cats is different from the one obtained after IM administration of the identical drug combination. The lower AUC, the lower C_{max} and the later T_{max} after OTM administration compared to IM injection suggest a smaller therapeutic efficacy of the combined drugs following OTM compared to IM administration. The longer time to C_{max} after OTM administration probably reflects a slower absorption of these drugs from the site of administration due to the vasoconstriction caused by dexmedetomidine, a finding reported previously for medetomidine (Ansah 2004). In the pharmacodynamic study (Porters et al. 2014a), using the same cats as for the current pharmacokinetic study, statistical analysis failed to pick up a difference in sedative and analgesic effects between OTM and IM administration of dexmedetomidine combined with buprenorphine. Nevertheless, the sedative effect was more distinct after IM injection than OTM administration of the premedication in our large-scale clinical study in kittens (manuscript submitted). That finding relates the pharmacodynamic results to the pharmacokinetic data of the drug combination for the 2 routes of administration described in the present study. Our clinical impression is in accordance with the findings published by Santos et al. (2010) who described less sedative effect following OTM administration compared to IM injection of dexmedetomidine (20 µg/kg) plus buprenorphine (20 µg/kg), and by Giordano et al. (2010), who observed a lower quality postoperative analgesia following OTM administration of buprenorphine (10 µg/kg) compared to IM administration.

The results of this pharmacokinetic study should be interpreted in view of strengths and limitations. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was used to determine plasma concentrations as it selectively distinguishes the parent buprenorphine compound from its metabolites in contrast to Radio Immuno Assays (RIA) with cross-reactivity between buprenorphine and its phase I metabolite norbuprenorphine and phase II glucuronide metabolites (Kuhlman et al. 1996). This might partially explain why in the present study different pharmacokinetics were observed after OTM and IM administration of buprenorphine, whereas previous studies (in which data were obtained with RIA) (Robertson et al. 2003b; Robertson et al. 2005) reported similar pharmacokinetics after OTM, IM and IV administration of buprenorphine. In agreement with our results, a comparable low bioavailability of buprenorphine following OTM administration was observed in a previous study in cats that also used LC-MS/MS to analyse buprenorphine (Hedges et al. 2013a). It

should be noted that modeling of data of buprenorphine following OTM administration of dexmedetomidine combined with buprenorphine resulted in a one compartmental model probably due to the observed low concentrations of the drug in relation to the LOQ and LOD of the analytical method. Modeling of data of buprenorphine after IM injection of this drug combination indicated a second compartment from which buprenorphine is slowly released, as expected for a lipophilic drug (Bullingham et al. 1981). This difference in compartmental model caused the apparent discrepancy in the elimination half-life parameters of buprenorphine following OTM and IM administration of dexmedetomidine combined with buprenorphine.

Besides the use of different analytical methods, the concomitant use of dexmedetomidine may also explain why the pharmacokinetic profile of buprenorphine following OTM administration is not in accordance to the findings of Robertson and colleagues (Robertson et al. 2003b; Robertson et al. 2005). The mixture of drugs may interfere with absorption or disposition of buprenorphine after OTM administration due to possible alteration of the pH of the mixture. The pH in the cats mouth was not measured before drug administration in the present study, but was measured at the time of the pharmacodynamic study (Porters et al. 2014a). In the latter, the oral pH was between 8 and 8.5 which was less than 9 reported by Robertson et al. (2005). Not only small alterations in buccal pH (Hedges et al. 2013b), but also the low pH of the combination of the pharmaceutical drug formulations of dexmedetomidine hydrochloride with buprenorphine hydrochloride (pH = 4.383) may have enhanced drug ionization and therefore decreased drug absorption by passive diffusion through lipid membranes. The taste of OTM administered drugs will also influence the degree of absorption. Previous studies reported no resistance or salivation when either preservative-free buprenorphine hydrochloride (Robertson et al. 2003b; Robertson et al. 2005; Giordano et al. 2010) or preservative-containing dexmedetomidine hydrochloride (Slingsby et al. 2009) was used for OTM administration in cats. In contrast, adverse effects (resentment to administration and salivation) observed in the pharmacodynamic study (Porters et al. 2014a) and the present study are likely to be caused by the preservative 0.135% chlorocresol in Vetergesic Multidose. Chlorocresol is reported to be responsible for the aversive behaviour of the cats to OTM administration of the drug formulation (Bortolami et al. 2012). Some amount of drugs was probably swallowed or lost through salivation and thus, the dosage after OTM administration was likely to be less precise than after IM injection.

Hence, preservative-free buprenorphine is preferable for OTM administration to prevent adverse reactions (Bortolami et al. 2012).

Furthermore, only six experimental cats were involved, but the study design was set up as a crossover trial to filter out cat variation and increase the power of the study. It should also be noticed that venous blood samples were used as well after IM as after OTM drug administration. Recently, it has been claimed that arterial blood samples should be used for time-concentration drug profiles after OTM drug administration since blood perfusing the buccal mucosa drains in the jugular vein (Hedges et al. 2013b). Based on this knowledge, our results of the pharmacokinetic profile of dexmedetomidine and buprenorphine following OTM administration may be overestimated, suggesting the real difference between the pharmacokinetic profiles following OTM and IM administration is even more distinct.

The results of the present study indicate that absorption of a combination of injectable dexmedetomidine and buprenorphine at the clinical doses studied was less after OTM administration than after IM injection. Usefulness of OTM administration of the sedative-analgesic combination in a clinical setting may therefore be questionable.

6. Conclusion

The lower AUC and C_{max}, the later T_{max} following OTM administration compared to IM injection of dexmedetomidine (40 µg/kg) combined with buprenorphine (20 µg/kg) suggest that this sedative-analgesic combination is less absorbed from the oral mucosa site than from the intramuscular injection site.

**Prepubertal gonadectomy in cats:
different surgical techniques and comparison with
gonadectomy at traditional age**

1. Abstract

Feasibility, surgical time and complications of different surgical techniques for prepubertal gonadectomy (PPG; 8 to 12 weeks of age) in cats were studied and compared to gonadectomy at traditional age (TAG; 6 to 8 months of age). Kittens were randomly assigned to PPG or TAG. Ovarian pedicle haemostasis for PPG was achieved by ligatures (n = 47), vascular clips (n = 50), bipolar electrocoagulation (n = 50), or pedicle tie (n = 50); for TAG (n = 34) ligatures were used. In male cats, PPG consisted of closed castration by spermatic cord knot (n = 92) or ligature (n = 91) while TAG (n = 34) was an open castration by spermatic cord knot.

For female PPG, clips and coagulation were the fastest procedures; placement of ligatures was most time-consuming. In male PPG, knot placement was significantly faster than ligation. In both sexes, very few intraoperative or wound complications were observed, irrespective of the surgical technique used. Surgical times in females (ligatures) as well as in males (knot) were significantly shorter for PPG than for TAG.

PPG was as safe as TAG, yet took less time to perform and did not result in a greater rate of postoperative complications.

2. Introduction

Surgical gonadectomy of cats remains the most useful tool to prevent unwanted reproduction and to reduce subsequent overpopulation (Howe 1997; Looney et al. 2008b; Reichler 2009). Traditionally, gonadectomy is often delayed until the animals are at least 6 months of age. Many cats will be adopted when they are still sexually intact. They may remain unneutered or may have one or more unplanned litters before being neutered. Therefore, prepubertal gonadectomy (PPG) is being promoted for population control in cats (Stubbs et al. 1995; Howe 1997; Joyce & Yates 2011). The number of animal shelters that have initiated programs in which juvenile and adult cats are castrated prior to adoption, has increased. However, some veterinarians are concerned about anaesthetic and surgical complications in PPG. To change the traditional attitude of the veterinary and public community in Belgium, the government funded a large-scale project to investigate the concept of early age neutering in shelter cats, in particular the short- and long-term effects of PPG on health and welfare of cats. The present study on surgical techniques for PPG was part of this project.

Previous studies have investigated anaesthetic protocols (Faggella & Aronsohn 1993; Theran 1993; Howe 1997; Joyce & Yates 2011) and surgical techniques (Aronsohn & Faggella 1993; Theran 1993; Howe 1997; Joyce & Yates 2011) for PPG in cats. Some studies included only a limited number of cases and were not case-controlled (Aronsohn & Faggella 1993; Faggella & Aronsohn 1993). In other studies, data on dogs and cats were evaluated together whereas surgery was performed by students (Howe 1997) or different surgeons (Theran 1993). To the author's knowledge, clinical studies comparing different surgical techniques for PPG and comparing PPG with gonadectomy at traditional age (TAG) under standardized conditions are not yet available.

The aim of this study was two-fold: (1) to compare feasibility, surgical time and complications between different surgical techniques for PPG in cats, and (2) to compare surgical time and complications between PPG and TAG in cats.

3. Materials and Methods

The study protocol was approved by local Ethical Committee (Faculty of Veterinary Medicine, Ghent University, Belgium) (licence number EC 2010/019 and 2011/077) and Deontological Committee (Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium).

3.1. Animals

Healthy kittens were recruited from animal shelters in Flanders (Belgium). Kittens had been dewormed and vaccinated against feline panleukopenia virus, feline herpesvirus and feline calicivirus at least once (Aronsohn & Faggella 1993; Looney et al. 2008b). Kittens were enrolled between the estimated age of 8 to 12 weeks on basis of their body weight (between 0.7 and 1.4 kg) (Lawler 2008). A complete physical examination was performed before the kittens were transported to the Faculty of Veterinary Medicine, Ghent University. During transportation and during their stay at the Faculty, kittens from the same litter were housed together to minimize stress and discomfort (Theran 1993; Faggella & Aronsohn 1994; Howe 1999). A "pilot study" was conducted prior to the analyses of the study period. The data from the first year of kitten recruitment (May 2010 – April 2011) are not part of the presented herein analysis. During the pilot study, the originally designed anaesthetic and surgical protocols were improved based on the experience gained while working with kittens. Moreover, the kittens (PPG and TAG) treated from May 2010 to April 2011 were hospitalized for five days following the procedure, while hospitalization was shortened to only one day for those treated from April 2011 until August 2012. The reduction in time spent at the Faculty resulted from the fact that, given the observed limited surgical and anaesthetic complications, there was no obvious benefit for the long postoperative follow-up period at the Faculty whereas the stress caused by the environmental changes was deemed to increase the observed incidence of anorexia and upper respiratory tract diseases.

Between May 2011 and August 2012, standardized anaesthetic protocols as well as standardized surgical techniques were used. Kittens were randomly assigned by a stratified randomization scheme (using different anaesthetic and surgical protocols as stratification factors to ensure treatment assignment balance within each stratum) to one of the two treatment groups, using unequal group sizes (2/3 PPG; 1/3 TAG). Kittens belonging to the PPG group were gonadectomised after being assigned to the group. In the TAG group, gonadectomy was postponed until the kittens were 6 to 8 months of age. Only cats with macroscopic normal anatomy of the uterus or normal descent of both testicles were included in the present study (PPG and TAG). All kittens were hospitalised at the Faculty of Veterinary Medicine and every PPG kitten was supervised by the surgeon (NP) until at least 6 hours postoperatively. Then, kittens were returned to the shelter and offered for adoption. All TAG cats returned to the Faculty around the age of 6 to 8 months to be neutered. They were supervised by the surgeon (NP) until 6 hours postoperatively. At 3 and 6 hours after

surgery (PPG and TAG), vital functions and body temperature were monitored and wound incisions were palpated. Caretakers from the shelters (PPG) and pet owners (TAG) were given written instructions for postoperative care beyond 6 hours.

3.2. Anaesthetic Protocols

All cats received general anaesthesia according to their assigned protocol. For PPG, three different anaesthetic protocols were compared and described in detail elsewhere (Porters et al. 2014c): (1) an intramuscular (IM) administration of 60 µg/kg dexmedetomidine combined with 20 µg/kg buprenorphine followed by an IM injection of the anaesthetic agent (20 mg/kg ketamine) (DB-IM), (2) an oral transmucosal (OTM) administration of 80 µg/kg dexmedetomidine plus 20 µg/kg buprenorphine followed by an IM injection of 20 mg/kg ketamine combined with a small dosage of dexmedetomidine (20 µg/kg) (DB-OTM), and (3) an IM injection of a 40 µg/kg medetomidine - 20 µg/kg buprenorphine - 20 mg/kg ketamine combination (MBK-IM). All cats received a subcutaneous injection of an NSAID before surgery. For TAG, only the DB-IM protocol was used, with the same products but other dosages for dexmedetomidine (40 µg/kg) and ketamine (5 mg/kg). It should be noted that TAG cats that were assigned to a different analgesic protocol than the PPG cats were excluded.

An intramuscular injection was given in the quadriceps muscle; oral transmucosal administration was performed by inserting the nozzle of a 1 ml syringe into the buccal cavity of the cat's mouth and gently squirting the content into the buccal area (Robertson et al. 2003b, Slingsby et al. 2009).

3.3. Surgical Procedures

All surgeries (PPG and TAG) were performed by the same surgeon (NP). Environmental conditions were kept as constant as possible throughout all surgeries. Before surgery, the bladder of female cats was emptied by gentle manual pressure for optimal visualization of the abdominal organs during surgery. All cats were placed in dorsal recumbency on a warm water blanket.

Female PPG – The fur of the ventral abdomen was shaved as little as possible and non-alcoholic products were used to scrub and disinfect the skin to minimise heat loss. A small skin incision (1 to 1.5 cm) was made midway between the umbilicus and pubis. The subcutaneous tissue was bluntly dissected to free the linea alba and the abdomen was opened

by a stab incision through the linea alba. To avoid inadvertent damage to the spleen, an ovariectomy hook was used only to identify the right uterine horn; the left ovary was approached by following the left uterine horn starting at the bifurcation. Ovarian pedicle haemostasis was performed by one of the following techniques:

(1) Ligature group: a double ligature using 4-0 polyglecaprone 25 (Monocryl®; Ethicon) was placed around the ovarian pedicle and a single ligature around the tip of the uterine horn.

(2) Clip group: small titanium clips (Premium Surgiclips S-9.0 of S-9.75®) were applied, one clip on the ovarian pedicle and one clip on the tip of the uterine horn.

(3) Coagulation group: the ovarian pedicle and the tip of the uterine horn were progressively coagulated until sectioning using a bipolar forceps (ERBE C100®, 20 Watt).

(4) Pedicle tie group: the ovarian pedicle was tied similar to the spermatic cord (Griffin & Brestle 2010): Holes were created by blunt dissection cranially and caudally to the ovarian vessels. A first haemostat was placed on the suspensory ligament and a second on the proper ligament; both were left to the side of the surgery field. While the ovary was lifted, a closed curved haemostat was passed through the hole in the broad ligament and turned around the pedicle with the tip down. The haemostat was opened to clamp the pedicle (Figure 1). The pedicle was sectioned between the haemostat and the ovary. The loop of the ovarian pedicle was pushed off the tip of the haemostat to finalize the knot that was subsequently pulled tight. The tip of the uterine horn was ligated using 4-0 polyglecaprone 25.

Figure 1. Intraoperative image of the pedicle tie. Haemostats are placed on the suspensory ligament (S) and on the proper ligament (cranial to the uterus [U]). While the ovary is lifted, a closed curved haemostat is passed through the hole in the broad ligament and turned around the pedicle (P) with the tip down. The haemostat (with the tip up) is opened to clamp the pedicle.

In every animal following gonadectomy, a 7.5 x 7.5 cm gauze surgical swab was applied to the ovarian pedicle area, on both sides, to remove any blood and to check for on-going haemorrhage. The abdomen was closed with simple interrupted sutures or a continuous

mattress suture, the subcutis with a continuous mattress suture and the skin with an intradermal suture using 4-0 polyglecaprone 25.

Female TAG – A traditional approach was used and ovarian pedicle sectioning was performed after placement of ligatures: a double ligature around the ovarian pedicle and a single ligature around the tip of the uterine horn, using 3-0 polyglecaprone 25. After controlling haemostasis, the peritoneal cavity, the subcutis and skin were closed in a similar way as for PPG, using 3-0 polyglecaprone 25.

Male PPG – The fur of the scrotum was shaved atraumatically and as little as possible and non-alcoholic products were used to scrub and disinfect the skin to minimise heat loss. After stabilization of one testicle in the scrotum between two fingers, a longitudinal incision in the scrotal skin was made over the individual testicle. Haemostasis in the spermatic cord was achieved by one of the following techniques:

(1) Knot group: the spermatic cord was tied on itself in a single throw without opening the vaginal tunic (Tobias 2010). The spermatic cord was sectioned between the knot and the testicle, and the latter was removed. The knot was pulled tight and the stump placed in the scrotum before repeating the same procedure for the second testicle. The skin incisions were left open.

(2) Ligature group: a single ligature using 4-0 polyglecaprone 25 was placed around the spermatic cord without opening the vaginal tunic. The spermatic cord was sectioned between the ligature and the testicle, and the latter was removed. The stump placed in the scrotum before repeating the same procedure for the second testicle. The skin wounds were also left open.

Male TAG – A traditional approach was used and haemostasis of the spermatic cord was performed by creating a knot similar to the technique in PPG, but in TAG the tunica vaginalis was first incised.

3.4.   Data Recording

Surgical time was recorded as time (minutes) from first skin incision to completion of the skin closure in female cats and to replacement of the second stump in the scrotum in male cats (PPG and TAG).

The occurrence of complications during and after surgery were recorded. Aside from complications related to the feasibility of the surgical techniques, intra-operative complications included bleeding, originating from the ovarian pedicle or other tissues, and fragility of tissues. Postoperative complications such as wound complications (signs of wound dehiscence, swelling, redness, discharge) that required conservative or surgical treatment were recorded.

3.5. Statistical Analysis

To compare different surgical techniques for PPG in female and male kittens, a linear model was fitted with surgical time as response variable and anaesthetic protocol, surgical technique, period (first or second year of the study), and the interaction between anaesthetic protocol, surgical technique and period as fixed effects. A Tukey-Kramer correction was used for multiple comparisons between surgical techniques in females. An (exact[3]) logistic regression was performed for complication rate.

To compare PPG and TAG only data of cats with a similar anaesthetic protocol (IM) and an identical surgical technique (ligatures for females, knot for males) were analysed. To compare surgical time in PPG and TAG for female and male cats, a linear model was used with surgical time as response variable and treatment group, period (first of second year of the study) and the interaction between treatment group and period as fixed effects. An (exact[1]) logistic regression was performed for complication rate.

Surgical time was expressed as mean (sd) value. Categorical data (complications) were expressed as frequencies for each group.

Descriptive statistics were performed using Excel 2010 (Microsoft Corporation, USA) and SPSS version 21 (IBM corp., USA). The analysis was performed in SAS version 9.3 (SAS Institute Inc., USA). Values of $P < 0.05$ were considered significant for all analyses.

4. Results

Four hundred forty eight kittens were included for analysis of which 380 belonged to the PPG group (female: n = 197, male: n = 183) and 68 to the TAG group (female: n = 34, male: n = 34).

---

[3] If data were sparse.

No female kittens were excluded because of uterine abnormalities. In six kittens cysts were observed on the prepubertal ovaries, but this finding did not lead to exclusion because it was without surgical relevance. One female TAG cat was excluded as she was pregnant and required ovariohysterectomy. Four male PPG kittens were excluded because of unilateral cryptorchidism (abdominal: n = 1, inguinal: n = 3). Cryptorchidism was not observed in any of the TAG cats. Fifty-nine TAG assigned cats did not return to the Faculty at 6 to 8 months of age because they were dead or missing, neutered by a local practitioner, or the owner could not be reached or was not willing to participate.

Female PPG – One hundred ninety seven kittens were recruited and were randomly assigned to the ligature group (n = 47), clip group (n = 50), coagulation group (n = 50), or pedicle tie group (n = 50). Mean (sd) body weight was 1.01 (0.20) kg and was not statistically different among the groups ($P = 0.2011$).

Surgical technique and period were significantly associated with the length of surgery (both $P < 0.0001$). Mean (sd) surgical time (minutes) in each group was: coagulation 15.55 (6.23), clip 16.77 (6.60), pedicle tie 20.37 (8.38), ligatures 22.02 (5.70). Surgical time was significantly shorter when coagulation ($P < 0.0001$), clips ($P < 0.0001$) or pedicle tie ($P = 0.0270$) were used for ovarian pedicle haemostasis in kittens compared to ligatures. In addition, use of coagulation ($P = 0.0012$) and placement of clips ($P = 0.0349$) resulted in significantly shorter surgical times compared to the creation of a pedicle tie. Surgical time was significantly shorter in 2012 (14.15 [0.53]) compared to 2011 (20.04 [0.61]) ($P < 0.0001$).

During the early phase of the pilot study period preceding the current study, one kitten died shortly after surgery because of intraoperative blood loss due to splenic laceration created by the ovariectomy hook. Although the haemorrhage was successfully addressed prior to abdominal closure and supportive fluid therapy was immediately installed, this kitten could not compensate the intraoperative blood loss and died within 3 hours after surgery. This fatal event led to the immediate adaptation of the surgical approach used.

Intraoperative complications were encountered in 7 kittens during the studied period, without significance between the different groups ($P = 0.0711$). Also, a logistic regression failed to identify the surgical group approaching the closest to significance ($P = 0.5233$). Minor bleeding at the uterus was observed in 3 kittens (1/50 clip, 1/50 coagulation and 1/50 pedicle tie) whereas ovarian pedicle haemorrhage occurred in one (1/50 pedicle tie). Friable tissue was seen once at the uterus (1/50 pedicle tie) and once at the ovarian site (1/50 pedicle tie).

At one occasion it was not feasible to create a pedicle tie as the ovarian pedicle was relatively too short (too much traction), although it was possible to perform a pedicle tie on the contralateral ovarian pedicle. The surgeon decided to ligate the short ovarian pedicle. All kittens recovered uneventfully. Seven kittens were re-presented with a wound complication, without significance between the different groups ($P = 0.5233$). One kitten from the coagulation group was re-presented with a seroma, 2 kittens (1/50 clip, 1/50 pedicle tie) with wound redness, wound discharge and wound dehiscence that required surgical treatment, whereas the remaining 4 kittens (2/50 clip, 1/50 coagulation, 1/50 pedicle tie) with wound redness and/or wound discharge were treated conservatively (topical antiseptic and/or antibiotics).

Female PPG and TAG – The female PPG kittens from the IM group with ligatures as surgical technique (n = 23) were compared to 34 female TAG cats. Mean (sd) body weights were 1.00 (0.21) kg and 2.61 (0.34) kg, respectively.

Surgical time was significantly shorter for PPG (22.22 [3.88]) than for TAG (23.58 [7.32]) ($P = 0.0071$). Intraoperative complications were not observed in this subset of cats. Three TAG cats were re-presented because of wound complications. One cat with a seroma received NSAIDs. Another cat was re-presented with wound redness and limited dehiscence that resolved after topical application of an antiseptic. In the remaining cat, redness was observed and a surgical site infection was suspected. This cat responded well to antibiotics and NSAIDs.

Male PPG – One hundred eighty three kittens were recruited and were assigned to the knot group (n = 92) or the ligature group (n = 91). Mean (sd) body weight was 1.04 (0.21) kg and was not significantly different between the two groups ($P = 0.0979$).

Surgical technique and period were significantly associated with the length of surgery; surgical time was significantly shorter in the knot group (1.75 [0.83]) than in the ligature group (3.87 [1.55]) ($P = 0.0005$), and in 2012 (2.42 [0.15]) compared to 2011 (3.10 [0.02]) ($P < 0.0001$).

Only two complications were encountered. In one kitten from the ligature group one testicle could not be adequately fixed within the scrotal skin, resulting in a parascrotal rather than a scrotal skin incision, which was closed with a single interrupted skin suture. One kitten from the ligature group was re-presented 2 days following castration with purulent discharge from the scrotal incisions which resolved with antibiotics.

Male PPG and TAG – The male PPG kittens from the IM group with knot as surgical technique (n = 39) were compared to 34 male TAG cats. Mean (sd) body weight was 1.04 (0.21) kg in PPG cats and 3.32 (0.44) kg in TAG cats.

In male cats, period was significantly associated with group for the length of surgery (P = 0.0458). Surgical time was shorter for PPG than for TAG, but significance was only reached in 2012 (P = 0.0408): PPG 1.33 (0.65) and TAG 2.58 (1.93). Intraoperative and wound complications were not observed.

5. *Discussion*

The topic of early age neutering in cats has evoked extensive discussions among veterinarians (Spain et al. 2002; Murray et al. 2008; Farnsworth et al. 2013), cat owners (Welsh et al. 2013), breeders and cat rescue organisations (Pernestal & Axner 2012). Apart from reluctance regarding technical challenges when performing anaesthesia and surgery in young and small animals, the assumed risk of short- and long-term complications raised animal welfare concerns. To better inform both the veterinary and lay public, the Belgian government supported a large-scale project on early age gonadectomy in shelter cats. The current study on surgical techniques for PPG was a part of this initiative and the results show that there is no surgical counter indication to perform PPG instead of TAG.

In female kittens, all four surgical techniques for PPG proved to be technically feasible although they can be differentiated based on the observed differences in surgical time. Because of the randomization and standardization in the present study, there is little doubt that the variability in surgical time is solely attributable to the surgical technique. For PPG in female kittens, the use of clips and coagulation were the fastest surgical techniques, but obviously also the most expensive. Especially in shelter medicine, the use of clips will be limited by the costs of the disposable device. Notwithstanding the initial cost of the cautery device, coagulation is attractive for the high volume spay conditions in rescue or feral cats. The pedicle tie might also be an effective technique in shelter medicine since this technique requires no additional material and is faster than the conventional technique using ligatures. The pedicle tie on the ovarian pedicle (Griffin & Brestle 2010; Bushby & Griffin 2011; Bushby 2013) is not yet well established in the veterinary world, but the manoeuvre is identical to the creation of a knot in the spermatic cord. Although the latter is commonly performed in male cats suggesting that any veterinarian should also easily acquire the pedicle

tie technique, the relative limited length of the ovarian pedicle and subsequent greater stretching of the tissue can result in technical complications. Hence, this technique might be less appropriate for inexperienced surgeons. To allow comparison between PPG and TAG, the ovarian pedicle was double ligated in both age groups. Because of the small vessel size, there is probably no need to double ligate the ovarian pedicle in kittens (Appel & Scarlett 2013). Consequently, the difference in surgical time amongst the different surgical techniques for PPG observed in the current study population will be less pronounced in clinical conditions. However, quite likely, ligature placement will even then remain the slowest procedure.

In male kittens, the possible fragility of immature tissues (Aronsohn & Faggella 1993) can be bypassed by performing a closed castration (Howe 2006; Bushby & Griffin 2011). The spermatic cord within its tunic could be sufficiently exteriorized in the kittens to allow easy knot placement. Furthermore, a knot was the fastest and the cheapest technique for male PPG, since no suturing and suture material were needed.

As expected and previously reported (Howe 1997), PPG was faster than TAG for both sexes. This fact is probably due to the physiological differences between kittens and adult cats. In kittens the gonadal vessels are elastic and small (Aronsohn & Faggella 1993; Howe 1999). Furthermore, in female kittens there is little subcutaneous fat to hinder entrance to the abdominal cavity (Bushby & Griffin 2011) and fat lacks in the ovarian pedicles (Howe 1999). Free peritoneal fluid was present in the abdomen of many female kittens and the amount of the fluid varied greatly. This finding is considered physiologic (Aronsohn & Faggella 1993; Root Kustritz 2002; Howe 2006) and does not influence the surgical approach. The prepubertal ovaries were easily identifiable because they were relatively large compared to the small size of the kitten. Uchikura and others (2010) demonstrated that the ovarian weight in cats rapidly increased until 3 to 4 months of age. Their histological findings indicated that this weight gain was associated with an increase in the number and size of secondary follicles. In the current study, follicular cysts were also observed in six kittens. The testicles in male kittens, on the contrary, are smaller and more mobile than in young adult cats. It was therefore sometimes harder to stabilize them in the scrotal region in preparation for the skin incision, a finding that has been described previously (Howe 2006; Bushby & Griffin 2011).

In the present study, few intra- and postoperative complications were encountered and all cats (PPG and TAG) recovered uneventfully. The only fatal event occurred in a female PPG kitten in the early stage of the pilot study. During the initial surgical protocol, an ovariectomy hook

was used for identification of both ovaries. In the affected kitten, inadvertent damage of the spleen occurred while lifting the left uterine horn. This experience led to the immediate adaptation of the surgical approach: an ovariectomy hook was used only to identify the right uterine horn whereas the left ovary was approached by following the left uterine horn starting at the bifurcation. Respecting this approach was successful in preventing further cases of spleen laceration.

Our data support the scientific evidence for the safety for PPG (Aronsohn & Faggella 1993; Theran 1993; Howe 1997; Joyce & Yates 2011). Complications were more often observed in female cats (TAG and PPG) compared to male cats (TAG and PPG). This finding is plausible because the surgery in female cats is more invasive and takes longer. A higher wound complication rate was observed in female cats following TAG compared to PPG, as reported previously (Howe 1997). Factors contributing to an increased incidence of postoperative complications might be the prolonged surgical time (Brown et al. 1997; Nicholson et al. 2002), and the larger skin incision in adult cats compared to kittens. Also, it is likely that the wounds in pet-owned cats were more often and more closely monitored than those in kittens that were still housed at a shelter (majority of PPG group) and were thus more often reported. Furthermore, minor (self-limiting) complications may not always have been reported.

All the observed intraoperative as well as wound complications were amendable to treatment. Death within the first week after surgery occurred only in the PPG group and necropsy, whenever performed, could attribute these fatalities to infectious diseases. It emphasizes the need to select kittens that are in the best possible physical condition and to minimize stress associated with transport, a novel environment and the actual surgery/anaesthesia (Theran 1993; Faggella & Aronsohn 1994; Howe 1999; Joyce & Yates 2011). The outbreak of postoperative infections is not considered to be directly related to the surgical technique and, therefore, will be described in a future manuscript on the long-term follow-up of PPG and TAG kittens (manuscript in preparation). Some of the alleged long-term complications following early age neutering in cats are the higher risk for an urethral obstruction in male cats, obesity and fractures at the physes due to a delayed closure time (Spain et al. 2002; Murray et al. 2008). Briefly, own observations did not support those findings.

Some findings of the present study may not be completely valid for novice surgeons without any experience with PPG. It should be noted that the early age neutering project funded by the Belgian government started already earlier than the current subset and the surgeon (NP) gained experience with the technique prior to the current study. As mentioned previously, the

data on the kittens that were enrolled in the pilot study were not included in the present study because of the lack of standardization of the anaesthetic and surgical protocols and the environmental conditions.

This study is not the first to describe PPG in cats, and recently published guidelines for spay-neuter programs in shelters (Looney et al. 2008a; Looney et al. 2008b), review articles (Bushby & Griffin 2011; Joyce & Yates 2011; Bushby 2013) and book sections (Appel & Scarlett 2013; Little 2013) are describing and promoting PPG in cats for population control. With this comparative study and further related investigations, the authors would like to further challenge the traditional attitudes and the worldwide perception of PPG in cats to improve feline health and welfare.

In summary, the surgical approach for PPG differs slightly from TAG: it is advised to create the skin incision relatively more caudally (Griffin & Brestle 2010; Bushby & Griffin 2011; Joyce & Yates 2011), an ovariectomy hook should be used exclusively at the right hand side (N. Porters, personal recommendation), tissues should be handled more gently (Aronsohn & Faggella 1993; Theran 1993; Joyce & Yates 2011) and even minimal blood loss can become clinically significant (Aronsohn & Faggella 1993; Theran 1993; Howe 1997). All surgical techniques proved to be safe for prepubertal gonadectomy in cats. Furthermore, prepubertal gonadectomy is faster compared to gonadectomy in young adult cats. In female kittens, the placement of clips or the use of coagulation significantly shortens surgical time while providing excellent haemostasis. In male kittens, creation of a knot in the spermatic cord significantly shortens surgical time.

6. Conclusion

PPG was not only as safe as TAG, but PPG also took less time to perform than TAG and no more postoperative complications were encountered.

7. Funding

8. Acknowledgements

Relationship between age at time of gonadectomy and health issues in adopted shelter kittens

1. Abstract

Prepubertal gonadectomy (PPG) is promoted to manage cat population, but concerns about potential health issues still exist. The objective of the present study was to compare short- and long-term health issues in cats following PPG or gonadectomy at traditional age (TAG).

In a prospective randomized clinical trial, 800 shelter kittens between the estimated age of 8 to 12 weeks were recruited before adoption and assigned randomly to the PPG group (gonadectomy upon assignment) or the TAG group (gonadectomy at 6 to 8 months of age). Short-term health issues included the mortality rate between the arrival of the kittens at the Faculty of Veterinary Medicine and the end of the first week after the kittens returned to the shelter, and the occurrence of miscellaneous health issues during the first month after adoption. Health issues of interest until 24 months of age (long-term) included: feline lower urinary tract disease (FLUTD), urethral obstruction (tomcats), lameness, fractures and hypersensitivity skin disorders.

The short-term results were not significantly different between neutered (PPG) and unneutered (TAG) kittens. Similarly, significant differences among treatment groups were not observed for the long-term medical outcomes.

The present study did not detect differences in type or number of health issues between PPG and TAG shelter cats until 24 months of age.

2. Introduction

Prepubertal gonadectomy (PPG), defined as gonadectomy between 6 to 16 weeks of age (Root Kustritz 2013), is being used in shelters in an effort to control cat population (Joyce & Yates 2011; Clark 2012). Practical and safe anaesthetic and surgical protocols are available (Porters et al. 2014c; Porters et al. 2014d; Aronsohn & Faggella 1993; Faggella & Aronsohn 1993; Theran 1993; Robertson et al. 2003a; Joyce & Yates 2011; Bushby 2013). Nevertheless, there are still concerns about PPG regarding behaviour and health issues (Spain et al. 2002; Murray et al. 2008).

Gonadectomy has been linked to a potentially increased risk for feline lower urinary tract disease (FLUTD) (Fennell 1975; Willeberg & Priester 1976; Lekcharoensuk et al. 2001; Gunn-Moore 2003). Furthermore, lack of gonadal hormones negatively affects closure time of selected physes in male (May et al. 1991; Houlton & McGlennon 1992; Stubbs et al. 1996; Root et al. 1997; McNicholas et al. 2002; Perry et al. 2014) and female cats (Stubbs et al. 1996; Root et al. 1997). This delay might result in lengthening of the associated bones (Root et al. 1997) and might make growth plates more susceptible to injury (Salmeri et al. 1991; Stubbs et al. 1996) and, in particular, to fracture (Root Kustritz 1999). However, the clinical relevance of the slower physeal maturation remains unclear (Stubbs et al. 1996; Root Kustritz 1999; Root Kustritz 2013; Perry et al. 2014).

To date, little research has been performed to study the clinical effects following PPG compared to gonadectomy at traditional age (TAG), resulting in only one short-term survey (Howe 1997) and long-term studies (Stubbs et al. 1996; Howe et al. 2000; Spain et al. 2004). Short-term complications (7 days after surgery) were encountered no more frequently following PPG (< 12 weeks of age) than following TAG; however, dogs and cats were evaluated together (Howe 1997). Although the risk of developing a medical condition such as FLUTD, urethral obstruction, lameness, fractures or allergic skin diseases was not higher in PPG compared to TAG cats in the published long-term surveys, correct interpretation of the reported data is hampered by methodological aspects. Twice, the study design relied on an extensive recall ability by owners of over one year (Howe et al. 2000, Spain et al. 2004) whereas in the third study, the sample size was small (Stubbs et al. 1996). Furthermore, PPG was defined as gonadectomy in cats aged less than 22-24 weeks (Howe et al. 2000, Spain et al. 2004) rather than less than 16 weeks (Root Kustritz 1999, Root Kustritz 2013). Hence, the potential relation between age at the time of gonadectomy and health problems still remains

to be elucidated. This is key to support PPG as a tool to manage the cat population (Stubbs et al. 1995; Howe 1997; Joyce & Yates 2011)

The present prospective study was part of a large-scale project on early gonadectomy in cats supported by the Belgian government to better introduce PPG to both the veterinary and lay public. The aims were to study short- and long-term health effects following PPG, compared to TAG in shelter cats.

3. Materials and Methods

The study protocol was approved by the local Ethical Committee (Faculty of Veterinary Medicine, Ghent University, Belgium) (licence number EC 2010/019 and 2011/077) and the Deontological Committee (Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium).

3.1. Animals

Between May 2010 and August 2012, healthy kittens were recruited from animal shelters in Flanders (Belgium). Kittens were dewormed and vaccinated at least once against feline parvovirus, feline calicivirus and feline herpesvirus (Aronsohn & Faggella 1993; Looney et al. 2008b). Kittens were enrolled between the estimated age of 8 to 12 weeks on the basis of their body weight (between 0.7 and 1.4 kg) (Lawler 2008). A thorough physical examination was performed prior to transportation to the Faculty of Veterinary Medicine at Ghent University. Littermates were housed together as much as possible to minimize stress and discomfort (Faggella & Aronsohn 1994; Howe 1999).

Kittens were randomly assigned by a stratified randomization scheme to one of the two treatment groups, using unequal group sizes (2/3 PPG; 1/3 TAG). All kittens received a microchip for identification. Kittens belonging to the PPG group were gonadectomised upon assignment. In the TAG group, gonadectomy was postponed until the kittens were 6 to 8 months of age. The applied anaesthetic and surgical protocols were described in detail elsewhere (Porters et al. 2014c; Porters et al. 2014d). After their stay at the Faculty, the kittens (PPG and TAG) were subsequently returned to the original shelter and were offered for adoption. The length of time that the kittens (PPG and TAG) stayed at the Faculty was five days from May 2010 to April 2011, and one day from April 2011 until August 2012. The reduction in time spent at the Faculty resulted from the fact that, given the observed limited

surgical and anaesthetic complications, there was no obvious clinical benefit of a prolonged postoperative follow-up period at the Faculty. The stress caused by the environmental changes during transport and temporary housing at the Faculty, however, was deemed to be responsible for the observed increase in the incidence of anorexia and upper respiratory tract diseases. After a stay of one day at the Faculty (April until August 2012), the kittens were observed another 2 days by the shelter staff, before becoming available for adoption.

Individuals interested in adopting a kitten included in the project received a brochure from the shelter staff containing information about the study, its goals and the effort required from adopters. Adopters were asked to sign an informed consent form and to provide contact information. Health condition of PPG and TAG cats was evaluated until 24 months of age by means of web-based surveys, a health check-up and regular phone interviews. Shelter caretakers, shelter veterinarians or adopters were asked to inform the investigator (NP) when a kitten became sick or died shortly after its stay at the Faculty. Owners were also encouraged to immediately report if their cat died or went missing during follow-up until 24 months of age.

At the end of the first month after adoption, the owners received an e-mail invitation (and, if necessary, an automatic reminder) to complete a web-based survey created on a dedicated Microsoft Dynamics CRM platform (ESC, De Pinte, Belgium). Paper copies were available to owners without internet access. When the cat reached the age of 6 to 8 months, owners received an e-mail invitation to present their cat to the Faculty for a health check-up. Non-responders were sent an e-mail reminder or were contacted by phone to make this appointment. During that particular visit, gonadectomy was performed in the TAG cats.

To reach owners by telephone to acquire information about the health of their cat at 12, 18 and 24 months of age, at least 3 attempts were made at various times during the day and in the evening, both on weekdays and weekends. If the owner could still not be reached, an additional e-mail was sent, requesting to contact us in due course.

3.2.   Short-term follow-up

The investigated medical outcomes in the short-term follow-up were limited to kitten mortality between the arrival of the kittens at the Faculty of Veterinary Medicine and the end of the first week after the kittens returned to the shelter, and to the occurrence of health issues as reported by the owners during the first month after adoption.

If a kitten deceased, its corps was submitted to the Department of Pathology, Faculty of Veterinary Medicine, Ghent University for full necropsy, whenever possible.

Data about the health of the kittens during the first month after adoption were gathered via the web-based survey at the end of the first month after adoption. The inclusion criteria in the survey data analysis were: (1) the kittens were adopted within 30 days after their stay at the Faculty (thus limiting the variation in age of kittens to 8 weeks), (2) the kittens homed in foster families (rather than traditional shelters) were not adopted by those families, and (3) the survey was completed by the adopter within 14 days of the initial request. Owners were asked whether (1) the cat was still in the household and, if not, what had happened to the cat and (2) whether the cat had been examined by a veterinarian and if so, for which reasons (only health-related problems were included, not preventive medical care such as registration of the microchip, vaccinations, …).

3.3. Long-term follow-up

During the health check-up at 6 to 8 months of age and during phone-calls at 12, 18 and 24 months of age, cat owners were asked whether the cat was still in the household, and whether the cat had been ill during the period of interest (i.e. time interval between two questionnaires). Medical history data of interest were: FLUTD (including signs of hematuria, stranguria and pollakisuria as well as urinary obstruction), lameness (fracture-related or other) and hypersensitivity skin disorders (including food hypersensitivity with cutaneous manifestations). The consulted veterinarian was contacted for FLUTD and for other complex problems that could not be adequately described by the owner. If cats died in any of the later stages of the follow-up, routine necropsies were not requested.

3.4. Statistical Analysis

For the short-term follow-up, kitten mortality rate between the arrival at the Faculty of Veterinary Medicine and the end of the first week after the kitten returned to the shelter, and the prevalence of health problems reported in the survey at 1 month after adoption were analyzed using logistic regression with treatment group, gender, period, and their interactions as fixed effects. Non-significant interactions were removed. In the case of sparse data, exact inferential techniques were used.

For the long-term follow-up, data about the incidence of FLUTD, urethral obstruction (tomcats only), lameness, fractures and hypersensitivity skin disorders were analyzed by

logistic regression for each medical outcome separately with treatment group, gender, period, and their interactions as fixed effects. Non-significant interactions were removed. In the case of sparse data, exact inferential techniques were used. A response of 1 was noted if at least one episode of a medical outcome was reported for the cat during the follow-up period (minimum 6 months – maximum 24 months). For this analysis, cats not adopted from the shelter, or relinquished or rehomed due to private reasons or owner's allergies prior to the start of the long-term follow-up (6 to 8 months of age) were excluded.

The analysis was performed in SAS version 9.3 (SAS Institute Inc., USA), using a significance of 5%. Binomial variables were presented as the proportion of kittens (number or percentage) belonging to a specific category.

4. Results

Eight hundred kittens (PPG: n = 547, TAG: n = 253) from eighteen animal shelters were recruited between May 2010 and August 2012.

4.1. Short-term follow-up

Forty-two kittens died between the arrival at the Faculty of Veterinary Medicine and the end of the first week after the kittens returned to the shelter (8 to 13 weeks of age), without significant differences between PPG (31/547; 5.7%) and TAG kittens (11/253; 4.3%) ($P = 0.3720$). One female kitten died shortly after surgery because of an intra-operative complication (controlled but excessive blood loss due to inadvertent laceration of the spleen). Infectious diseases were deemed to be the cause of most fatalities in the kittens. Panleukopenia was diagnosed in 11 PPG and 2 TAG kittens based on histology and immunohistochemistry. Furthermore, panleukopenia was highly suspected in another 9 PPG and 2 TAG kittens based on clinical signs and the fact that a littermate died of confirmed panleulopenia. In one additional kitten (PPG), necropsy and histology indicated enteritis with signs suggestive for panleukopenia; however, immunohistochemistry and virus isolation failed to confirm the diagnosis. One kitten (PPG) died because of sepsis (histological examination). Two kittens (PPG) died of interstitial pneumonia (histological examination) which is suggestive for a viral etiology, but this could not be confirmed. In both kittens, feline parvovirus and feline herpesvirus were negative based on immunohistochemistry and in one kitten, feline calicivirus isolation was performed and also negative. The cause of death

of the remaining 13 kittens was not investigated (corpses not available for necropsy). In 6 PPG and 7 TAG kittens symptoms of gastro-intestinal pathology or vague symptoms quickly resulting in death were reported by the owners or caretakers, suggesting an underlying viral cause.

For 539 kittens, the survey at the end of the first month after adoption was completed by the adopter. Taking into account the inclusion criteria, 396 surveys of 275 PPG and 121 TAG kittens (12 to 20 weeks of age) were used for analysis. One hundred and thirty-eight kittens (46.2%) were presented to a veterinarian by their owner for one (n=125) or multiple (n=13) medical issues, without significant differences between PPG (92/275; 33.5%) and TAG (46/121; 38.0%) (P=0.5854). Upper respiratory tract diseases (sneezing, coughing, fever, eye inflammation or infection, tongue ulcers) were the reason to seek veterinary assistance in nearly half of the kittens (46 PPG and 23 TAG). In another 46 kittens (28 PPG and 18 TAG), gastro-intestinal diseases (vomiting, anorexia, diarrhea, endoparasitic infestation) were seen. Dermatological conditions (skin infections, fungal diseases, ear mites) were also commonly reported (24 PPG and 6 TAG). Less frequent health complaints were: broken toe (2 PPG), skinniness (2 PPG) and stiffness (probably due to viral disease) (1 PPG and 1 TAG).

4.2. Long-term follow-up

Long-term follow-up was available for 614 cats (PPG: n = 417, TAG: n = 197). One hundred and eighty-seven cats were lost at the beginning of the long-term follow-up (Table 1).

Table 1. Reasons for dropout of 187 kittens at the beginning of the long-term follow-up at 6 to 8 months of age and the number (%) of cats in the prepubertal gonadectomy group (PPG: n = 547) and traditional age gonadectomy group (TAG: n = 253).

Reason	PPG n (%)	TAG n (%)
Death	54 (9.9)	23 (9.1)
Arrival – 1st week after return to shelter[a]	31 (5.7)	11 (4.3)
Due to trauma	10 (1.8)	6 (2.4)
Due to infectious diseases[b]	11[c] (2.0)	5[d] (2.0)
Other cause	2[e] (0.37)	1[f] (0.40)
No participation owner	35 (6.4)	18 (7.7)
Not adopted at 6 to 8 months of age	19 (3.5)	7 (2.8)
Not returned home	18 (3.3)	6 (2.4)
Returned to shelter or rehomed	6[g] (1.1)	0 (0.0)

[a] between the arrival of the kittens at the Faculty of Veterinary Medicine and the end of the first week after the kittens returned to the shelter, main cause of death in necropsies of kittens were infectious diseases; [b] suspicion, no necropsy performed; [c] clinical signs of feline infectious peritonitis (n = 5), upper respiratory diseases (n = 2), feline leukemia virus (n = 2), feline panleukopenia virus (n = 2); [d] clinical signs of feline infectious peritonitis (n = 4) and feline panleukopenia virus (n : 1); [e] unknown (n = 1), liver pathology (n = 1); [f] intoxication (n = 1); [g] reasons: owner's allergy (n = 2), movement (n = 1), behavioural reasons (n = 2), unknown reasons (n = 1).

Information about the medical condition of the cat at 6 to 8, 12, 18 and 24 months of age could be obtained in the majority of cats for each time-point (Table 2). The number of cats available per time-point decreased towards the end of the study, at which point 78 additional cats (52 PPG, 26 TAG) were lost. Death was the reason for dropout in 40 cats (11, 8 and 21 cats between 8 and 12, 12 and 18, 18 and 24 months of age, respectively). The most common cause of death was a road traffic accident (19 PPG, 12 TAG). Rarely, cats died of clinical signs compatible with feline infectious peritonitis (2 PPG, 1 TAG), feline leukemia virus (1 TAG), liver pathology (1 PPG), unwitnessed trauma (1 PPG) or suspected intoxication (1 PPG, 1 TAG). One kitten (PPG) displayed weakness before it deceased around 12 months of

age. Thirty-eight kittens were lost throughout the long-term follow-up (7, 13 and 18 cats between 8 and 12, 12 and 18, 18 and 24 months of age, respectively) because they never returned home (17 PPG, 9 TAG) or because they were removed from the household by the owner for allergies or other private reasons (9 PPG, 2 TAG), or because the cat displayed undesirable behaviour (1 PPG).

The number of cats for which the owner reported a health-related problem is depicted in Table 2. Throughout the entire long-term follow-up, no significant differences were observed for any medical outcome of interest among treatment groups (Table 3).

Table 2. The number of cats in the prepubertal gonadectomy (PPG) group and the traditional age gonadectomy (TAG) group for which data were obtained about their health status at 6 to 8, 12, 18 and 24 months of age and the number of cats with feline lower urinary tract disease (FLUTD), urethral obstruction (tomcats), lameness, fractures and hypersensitivity skin disorders at each time-point and overall incidence of these medical outcomes.

Age (months)	Group	Cats (n)	FLUTD	Urethral obstruction[a]	Lameness	Fractures	Hypersensitivity disorder
6 to 8	PPG	366/416	0	0	5	1	0
	TAG	185/197	0	0	1	0	0
12	PPG	382/413	3	1	19	3	5
	TAG	175/191	1	0	8	4	1
18	PPG	257/291	5	3	11	0	4
	TAG	85/105	1	1	5	1	1
24	PPG	349/372	4	1	8	0	4
	TAG	160/176	1	0	5	0	3

[a] tomcats only

Table 3. The overall number (%) of cats in the prepubertal gonadectomy (PPG) group and the traditional age gonadectomy (TAG) group with feline lower urinary tract disease (FLUTD), urethral obstruction (tomcats), lameness, fractures and/or hypersensitivity skin disorders during the long-term follow-up (until 24 months of age) without significant differences in type and/or number of health issues between both treatment groups.

	FLUTD	Urethral obstruction[a]	Lameness	Fractures	Hypersensitivity skin disorder
PPG (n=417)	8 (1.9)	4 (1.6)	39 (9.4)	3 (0.72)	9 (2.2)
TAG (n=197)	2 (1.0)	1(0.8)	19 (9.6)	5 (2.5)	4 (2.0)
Overall (n=614)	10 (1.6)	5 (0.81)	58 (9.4)	8 (1.3)	13 (2.1)
P-value[b]	0.4391	1.000	0.8093	0.1101	0.8718

[a] tomcats only (PPG: n = 258, TAG: n = 128); [b] logistic regression model (in the case of sparse data, exact inferential techniques were used) with treatment group, gender and period, and interactions among these as fixed effects. Non-significant interactions were removed.

Ten cats (8 PPG, 2 TAG) were diagnosed with at least one episode of FLUTD, among which 5 male cats (4 PPG, 1 TAG) with an urethral obstruction. Two female PPG cats were diagnosed with cystitis before 12 months of age. In both animals, recurrence of cystitis was observed and bladder stones (struvite) were surgically removed before 18 months of age. One male TAG cat had an episode of cystitis before 12 months of age and 2 male PPG cats were diagnosed with one episode of FLUTD before 24 months of age (one with crystalluria, one with cystitis). One male PPG cat had its first episode of FLUTD with obstruction before 12 months and its second episode before 18 months of age. Two other male PPG cats had an urethral obstruction around 18 months of age and one male PPG cat around 24 months of age. Except for the latter animal, crystals were identified (2 PPG: struvite, 1 PPG: unidentified). One of the PPG cats with an urethral obstruction before 18 months of age, had an episode of FLUTD without obstruction between 18 and 24 months of age. One male TAG cat had an urethral obstruction around 18 months of age (plug or crystals not investigated) and an episode of FLUTD (crystalluria without obstruction) around 24 months of age.

Fifty-eight cats (39 PPG, 19 TAG) had at least one episode of lameness throughout the follow-up. Amongst them, 3 PPG and 5 TAG cats were diagnosed with fractures, all before 18 months of age. One PPG cat experienced 2 fracture events, one before 6 to 8 months of age and one before 12 months of age. All fractures were caused by traumatic insults (road traffic accident, heavy object on foot, kick by a horse), which were witnessed by people nearby in all but one case. Lameness without fractures was reported 56 times in 50 cats (36 PPG, 14 TAG); 2 probably unrelated episodes of lameness were observed in 5 of the PPG cats and in one of the TAG cats. Trauma or fighting was reported in 14 PPG and 5 TAG cats. Lameness in one additional PPG cat was likely due to a viral polyarthritis. However, in the majority of the cases, the cause of lameness was unclear (22 PPG, 8 TAG). Most problems resolved spontaneously (24 PPG, 8 TAG).

In 13 cats (9 PPG, 4 TAG), a hypersensitivity skin disorder was reported. In 8 PPG cats a food hypersensitivity was suspected, based on a history of dermatologic complaints and/or positive response to a hypo-allergenic diet with(out) immune-modulating therapy (e.g. glucocorticoids). The first signs of food hypersensitivity were encountered before the age of one year in 5 cases. Around 12 and 18 months of age, 1 TAG cat was diagnosed with indolent ulcers and around 24 months of age, 1 PPG cat and 1 TAG cat were diagnosed with indolent ulcers. Moreover, around 24 months of age, 2 TAG cats were diagnosed with eosinophilic skin diseases.

5. *Discussion*

Concerns about effects of PPG on physical development and health in cats are a common reason for veterinarians not to support PPG. The present study was designed to provide short- and long-term information about health issues in shelter cats following PPG (8 to 12 weeks of age) compared to TAG (6 to 8 months of age).

The mortality rate between the arrival of the kittens at the Faculty of Veterinary Medicine and the end of the first week after the kittens returned to the shelter was not significantly higher in the neutered kittens (PPG) than in unneutered kittens (TAG). The cause of death in PPG kittens was not directly related to the anaesthesia and/or surgery, and was, similar to non-operated kittens, mainly caused by infectious agents such as feline panleukopenia virus or by respiratory diseases. Also, in previously reported studies, infectious diseases accounted for the greatest number of fatalities during the 7-day period post PPG (Howe 1997). Similarly, fatalities encountered in kittens in animal care and private homes were mainly due to infectious diseases (Cave et al. 2002). Nevertheless, stress associated with transportation from the shelter to the Faculty and the change in environment may have facilitated the outbreak of viral diseases in both PPG and TAG kittens. To obtain the highest level of standardization in the present study, transport of kittens was inevitable, but it should obviously be avoided by performing the surgery at a neuter facility in the shelter itself (Howe 1997). Ideally, neutered kittens should be observed in their familiar shelter environment during 48 hours postoperatively to detect and control pain or discomfort, and to treat miscellaneous diseases before their adoption. Observations such as anorexia, vomiting, diarrhea and upper respiratory tract diseases in these first days were recorded at the Faculty and/or at the shelter as being present or absent. Due to the lack of detailed information about these miscellaneous diseases acquired from some shelters and the self-limiting nature of most of them, it was decided to not report those data in the current study.

Throughout the first month after adoption, nearly one third of each group experienced at least one health problem, although it was often mild and temporary. These findings are in accordance with previous studies about health problems in cats and dogs during the first month after adoption from a shelter (Neidhart & Boyd 2002; Lord et al. 2008). Not unexpectedly, upper respiratory tract diseases were the most commonly reported indication to visit a veterinarian. The reported prevalence of upper respiratory tract diseases in a study involving 8 shelters in California (USA) varied from 8 to 38 % and being a kitten (0 to 3 months of age) was one of the risk factors to develop upper respiratory tract diseases

(Bannasch & Foley 2005). Moreover, cats can be carriers of 1 or more infectious agent related to upper respiratory tract diseases and latent infections can be reactivated in response to stress resulting in clinical disease (Gourkow et al. 2013). Stress induces immunodeficiency and results in vulnerability to various infectious diseases as suggested already by Pesavento & Murphy (2014). In addition, kittens are more vulnerable to infectious diseases than immunized adult animals, because their response to vaccination will be influenced by the level of maternal antibodies (Day et al. 2010). Infectious disease control (such as vaccination protocol, cleaning/disinfecting protocols, hand hygiene,…) is thus essential in shelters (Hurley 2005, Newbury et al. 2010) as well as in neuter facilities (Howe 1997, Looney et al. 2008a). Also, adopters should be notified about the vulnerability of kittens, the high-risk environment in the shelter, and the stress associated with the novel (non-)social environment post adoption. Veterinarians have an important role in reassuring adopters about the health of their shelter kitten by explaining the nature of mostly mild and/or self-limiting infections and by treating those infections (Scarlett et al. 2002). Therefore, adopters should be encouraged to present their new cat to a veterinarian shortly after adoption, in particular since it is established that disease in the immediate post adoption period is correlated with animal relinquishment (Wells & Hepper 1999).

Notwithstanding the methodological improvements such as prospective design and data collection on several time-points to obtain accurate information, the current findings did not differ from those of previous long-term studies (Stubbs et al. 1996; Howe et al. 2000; Spain et al. 2004). In the current long-term follow-up (6 to 24 months of age), no association could be identified between age at time of gonadectomy and the incidence of FLUTD, urethral obstruction, fractures, lameness and hypersensitivity skin disorders.

The finding that PPG was not associated with a higher risk for urethral obstruction in tomcats is in accordance with the observation that the urethral diameter at 22 months of age was not significantly different among male cats left intact, neutered at 7 weeks or at 7 months (Root et al. 1996a). Also, at 10 months of age male cats left intact or neutered at 5 months of age had a similar urethral diameter, irrespective of testosterone supplementation in neutered cats (Herron 1972). In an epidemiological survey of FLUTD, it was shown that neutered cats were at higher risk, but the time of this operation (i.e. < 6 months or late i.e. > 11 months) had no effect on development of FLUTD (Walker et al. 1977).

The incidence of lameness or fractures was not significantly different in PPG compared to TAG cats in the current study. It is well documented that time of closure of specific growth

plates is significantly different between neutered and sexually intact cats (May et al. 1991; Perry et al. 2014). However, there was no significant difference in growth plate closure between cats neutered at 7 weeks or 7 months (Stubbs et al. 1996; Root et al. 1997). In the present study, the reported episodes of lameness in PPG and TAG cats were generally self-limiting and had a traumatic or unknown cause. These findings suggest that, even if physeal closure time would be delayed in PPG compared to TAG cats, it is very unlikely to be clinically relevant, let alone different. Only in one report on 26 cats, PPG (before the age of 6 months) was reported as risk factor for spontaneous femoral capital physeal fractures in adult cats (McNicholas et al. 2002); however, these cats were also overweight. Obesitas in cats is a known risk factor for lameness (Scarlett & Donoghue 1998) as well as for traumatic fractures at the capital femoral epiphysis (Craig 2001). Unlike the plethora of studies comparing obesitas in gonadectomised versus intact animals without regard for time of gonadectomy (Fettman et al. 1997, Robertson 1999, Allan et al. 2000, Martin et al. 2001, Lund et al. 2005, Colliard et al. 2009, Courcier et al. 2010), few studies have specifically addressed the relationship between age at time of gonadectomy and the development of overweight in cats (Root et al. 1996b, Stubbs et al. 1996, Alexander et al. 2011). It is well established that gonadectomy is one of the important predisposing factors for the development of overweight and obesity in cats (Robertson 1999, Lund et al. 2005, Colliard et al. 2009, Courcier et al. 2010, Joyce & Yates 2011); however, the influence of the age at time of gonadectomy remains unclear. Yet, studying overweight in cats is hampered by its multifactorial nature to which numerous uncontrolled variables such as diet, exercise and genetics contribute (Joyce & Yates 2011).

No significant differences in hypersensitivity skin disorders between PPG and TAG cats were observed. It should be noted that, although the incidence of that in the present study was low, it might still be overestimated since diagnoses were mostly suspected rather than based on exclusion of other pruritic diseases (viral, fungal, bacterial and parasitic diseases) (Hobi et al. 2011, Favrot et al. 2012). Based on the current findings, it seems unlikely that age at time of gonadectomy is associated with feline hypersensitivity skin disorders. Yet Spain and colleagues (2004) suggested such association for allergic skin diseases and Howe and colleagues (2000) for problems of the integumentary system (including minor skin allergies). Finally, some additional comments should be made. A follow-up period of 24 months does certainly not reflect the lifespan of cats. Nevertheless it does permit evaluation about the health of kittens and junior cats into adulthood (Hoyumpa Vogt and others 2010). Especially

for conditions like FLUTD, with an increased risk between 2 and 6 to 7 years of age (Willeberg and Priester 1976, Lekcharoensuk and others 2001), a much longer follow-up period would be more ideal. It should also be noted that in the present study the occurrence of any disease of interest in the long-term follow-up was relatively rare ($\leq$ 13/614 cats [$\leq$ 2.1%] for each condition). Therefore, the statistical power for finding significant differences between PPG and TAG cats was rather low. Numbers as high as 1,605 PPG and 827 TAG tomcats should be studied to be able to document a significant difference of 2 % between both treatment groups for urethral obstruction with a power of 80 %.

6. Conclusion

The results of the present study do not indicate that PPG increases the risk for health problems until 24 months of age compared to TAG. Ideally, gonadectomy should be performed at the shelter facilities to avoid stress associated with environmental changes.
 Successful PPG strategies require optimal infectious disease control at the shelter and neuter facility and postoperative clinical observation of the kittens in their familiar shelter environment before adoption.

7. Funding

8. Acknowledgements

118

Prepubertal gonadectomy affects body weight, body condition score and plasma leptin concentrations in young adult cats

1. *Abstract*

Gonadectomy is an important predisposing factor for the development of overweight and/or obesity in cats. However, the influence of the age at time of gonadectomy remains unclear. To better understand a potential development of overweight in kittens following PPG, body weight, body condition score and plasma leptin concentrations were determined in gonadectomised (prepubertal; 8 to 12 weeks of age) and sexually intact young adult cats, both aged 6 to 8 months.

In female cats, mean body weight and plasma leptin concentrations were significantly higher in the PPG (n = 47) than in the TAG (n = 36) group, and a significantly higher proportion of PPG queens was already overweight. In male cats, only leptin concentrations were significantly higher in the PPG group (n = 55) compared to the TAG (n = 47) group, without simultaneous significant differences in body weight.

The risk to become overweight is possibly influenced by age at gonadectomy, especially in female cats. Further long-term investigation comparing PPG with TAG is needed to clarify whether the risk to become overweight at a later stage is indeed influenced by the age at gonadectomy or solely by the gonadectomy itself.

2. Introduction

Overweight and obesity are common nutritional disorders in the domestic feline population in developed countries, with a prevalence ranging between 27 % and 52% (Russell et al. 2000; Lund et al. 2005; Colliard et al. 2009; Courcier et al. 2010; Cave et al. 2012). Animals gain weight when energy acquired from dietary intake exceeds the energy expended by the body through metabolism and physical activity (Fettman et al. 1997). A drop in the concentrations of gonadal hormones following gonadectomy has been linked to a 30% decreased energy requirement in adult cats (Root et al. 1996b) and a change in body composition and development of obesity (Fettman et al. 1997; Robertson 1999; Allan et al. 2000; Russell et al. 2000; Martin et al. 2001; Courcier et al. 2010; Cave et al. 2012; Courcier et al. 2012). As excess energy is stored, body fat mass increases and adipose cells enlarge producing many peptides and nutrient signals such as leptin (Martin et al. 2001), a lipostatic hormone regulating appetite and energy balance of the body (Hoenig & Ferguson 2002). Leptin concentrations are higher in obese than in normal-weight cats (Appleton et al. 2000) and highly correlated with body fat in cats (Appleton et al. 2000; Backus et al. 2000; Hoenig & Ferguson 2002; Martin et al. 2006).

In an effort to control the reproduction rate in cat population, prepubertal gonadectomy (PPG) has been promoted (Stubbs et al. 1995; Howe 1997; Joyce & Yates 2011). Few studies have investigated the effect of age at time of gonadectomy on the likelihood to develop overweight and/or obesity. However, the different studies used different ways to assess and define overweight, hampering direct comparisons. In an experimental study, male and female cats gonadectomised at 7 weeks (n = 12) or at 7 months of age (n = 12) had a decreased metabolic rate compared to sexually intact cats (n = 12) at 12, 18 and 24 months of age (Root et al. 1996b). In another experimental study based on 31 cats of both sexes, falciform fat thickness via radiographic assessment differed significantly between neutered cats (at 7 weeks or at 7 months) and sexually intact cats (Stubbs et al. 1996). However, only cats gonadectomised at 7 months had a significantly higher body weight than the sexually intact cats at the age of 12 months (Stubbs et al. 1996). A retrospective study in 1660 cats with a median follow-up time of 3.9 years claimed that early age gonadectomy (< 22 weeks of age) in male and female cats was not associated with an increased risk of obesity, but these results were only based on body condition score (BCS) assessment by the owners (Spain et al. 2004). More recently, it was demonstrated in an experimental study that female cats (n = 12)

neutered prepubertally (19 weeks of age) had a 24% higher body weight and a 16% higher BCS at 12 months of age compared to their sexually intact counterparts (Alexander et al. 2011). Surprisingly, the expected differences in body and lean mass between gonadectomised and sexually intact cats were not confirmed by means of dual-energy X-ray absorptiometry at 12 months of age (Alexander et al. 2011). The abovementioned apparent discrepancies motivate to further explore the potential relationship between overweight and (age at) gonadectomy by combining several parameters of assessment.

The present study was part of a large-scale project on PPG in cats supported by the Belgian government to better introduce PPG to both the veterinary and lay public. The objective of this study was to investigate whether body weight, being overweight and plasma leptin concentrations in young adult male and female cats at 6 to 8 months of age were affected by the timing of gonadectomy.

3. Material and Methods

The study protocol was approved by the local Ethical Committee (Faculty of Veterinary Medicine, Ghent University, Belgium) (licence number EC 2010/019 and 2011/077) and the Deontological Committee (Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium).

Between May 2010 and August 2012, healthy kittens were recruited from animal shelters in Flanders (Belgium). Kittens were enrolled between the estimated age of 8 to 12 weeks on basis of their body weight (between 0.7 and 1.4 kg) (Lawler 2008). A thorough physical examination was performed prior to transportation to the Small Animal Clinic at the Faculty of Veterinary Medicine at Ghent University. Kittens were randomly assigned to one of the two treatment groups by a stratified randomization scheme, using unequal group sizes (2/3 PPG; 1/3 traditional age gonadectomy [TAG]). All kittens received a microchip for identification. Kittens belonging to the PPG group were gonadectomised upon assignment. In the TAG group, gonadectomy was postponed until the animals were 6 to 8 months of age. The anaesthetic and surgical protocols used were described in detail elsewhere (Porters et al. 2014c; Porters et al. 2014d). After their stay at the Faculty, the litter was subsequently returned to the original shelter and offered for adoption. Individuals interested in adopting a kitten included in the project received a brochure from the shelter staff containing

information about the study, its goals and the effort required from adopters. Adopters were asked to sign an informed consent form and to provide their contact information.

When the cat reached the age of 6 to 8 months, the owners received an e-mail invitation to present their cat to the clinic at the Faculty for a health check-up. Non-responders were sent an e-mail reminder or were contacted by phone to make this appointment.

During the health check-up at 6 to 8 months of age, a physical examination was performed. In addition, body weight and BCS were determined. All examinations and all data collection were performed by a single investigator (NP). For the BCS, a 5-point scale was used in the first period (May 2010 until March 2011) (Thatcher et al. 2000) and a 9-point scale in the second period (April 2011 until March 2013) (Laflamme 1997). Overweight was defined as a score of at least 4 (5-point scale) or at least 6 (9-point scale), while non-overweight animals were classed as all cats with a BCS of less than 4 (5-point scale) or less than 6 (9-point scale). For the determination of plasma leptin concentration, preprandial blood (food withheld for at least 8 hours) was sampled by jugular venipuncture into heparine tubes. After centrifugation of the collected blood samples, plasma was collected and stored at − 20 °C until analysis. Plasma leptin concentrations were determined with a commercially available radioimmunoassay kit (Multispecies Leptin RIA Kit, Catalogue number XL-85K, Merck Millipore) and automatic gamma counter (1277 Gammamaster, Wallac OY). This RIA test kit was developed to quantify leptin in plasma of several species and has been validated for use in cats (Backus et al. 2000).

Analyses of plasma samples for leptin concentrations were performed in duplicate. Only duplicate results with a variance coefficient less than 25% were included. The mean concentration of the duplicate samples were used for statistical analysis.

Data of female and male cats were analyzed separately to examine differences between PPG and TAG cats. An independent t-test (normal distribution) and non-parametric tests (Mann-Whitney; non-normal distribution) were used for the continuous variables BW and plasma leptin concentrations, respectively. A chi-square test was used for the ordinal variable (proportion overweight cats in population). Continuous variables were summarized by the mean (standard deviation) and ordinal variables by the frequencies for each group. The analysis was performed in SPSS version 21 (IBM corp., USA). Significance was set at 0.05.

4. Results

One hundred and eighty-five cats (PPG: n = 102, TAG: n = 83) were included in the current study. Illustrated in Table 1, gonadectomised cats (PPG) were heavier than sexually intact cats (TAG) and more PPG cats than TAG cats were considered as being overweight; however, significance was only reached in female cats (body weight: $P < 0.001$, BCS: $P = 0.009$). Plasma leptin concentration was significantly higher for PPG compared to TAG cats, both for males and females ($P < 0.001$ and $P = 0.003$, respectively).

Table 1. Comparison of body weight and plasma leptin concentrations, presented as mean [sd] and comparison of the proportion of cats with overweight between prepubertal gonadectomised (PPG) cats and sexual intact cats (offered for traditional age gonadectomy [TAG]) at 6 to 8 months of age.

	Male			Female		
	PPG	TAG	P-value[a]	PPG	TAG	P-value[a]
Number of cats	55	47		47	36	
Body weight (kg)	3.55 [0.61]	3.40 [0.44]	0.164	3.22 [0.47]	2.67 [0.44]	< 0.001[a]
Plasma leptin concentration (ng/ml HE)[b]	4.72 [3.36]	2.85 [1.24]	< 0.001[a]	5.25 [4.93]	3.25 [1.61]	0.003[a]
Number of overweight cats (%)[c]	12 (21.8)	9 (19.1)	0.781	17 (36.2)	4 (11.1)	0.009[a]

[a] significance set at P < 0.05; [b] HE = human equivalent (multispecies RIA kit); [c] overweight based upon body condition score: at least 4 (5-point scale) or at least 6 (9-point scale).

5. Discussion

This study revealed that early age gonadectomy was associated with an increase in circulating leptin when the cats were 6 to 8 months of age, which is most likely a result of the increased amount of body fat as described in the literature following gonadectomy in adult cats (Martin et al. 2001; Hoenig & Ferguson 2002; Martin et al. 2006; Belsito et al. 2009). In female PPG cats, this increase in leptin concentration was accompanied by an increase in body weight and in the proportion of cats being overweight. In male cats, body fat potentially increased after PPG, yet without resulting in significant differences in body weight or in the proportion of overweight cats among male PPG and TAG cats. Since an increase in body fat mass and subsequently decrease in lean body mass results in lower energy requirements (Bermingham

et al. 2010), male PPG cats might also be more likely to become overweight at a later stage if energy intake is not restricted.

It should be mentioned that some outliers (high values above 10.00 ng/ml HE) were observed in the data, possibly because not all cats were fasted for at least 8 hours at the time of blood sampling (Backus et al. 2000; Radin et al. 2009; Zoran 2010). Unfortunately, it was impossible to measure body fat mass by means of dual-energy x-ray absorptiometry in the current study. It would also be interesting to re-evaluate body weight and BCS again in adult PPG and TAG cats (around 24 months of age).

The current study represents the influence of PPG on the body weight, BCS as well as plasma leptin concentrations as a marker for body fat mass, in young adult male and female cats. It is established that gonadectomised cats are at greater risk to become overweight (Robertson 1999; Allan et al. 2000; Courcier et al. 2010; Cave et al. 2012; Courcier et al. 2012). The risk is possibly influenced by age at gonadectomy, which has previously been suggested for female kittens (Alexander et al. 2011). Further long-term research in cats should investigate whether the risk to become obese at a later stage is indeed influenced by the age at gonadectomy or solely by the gonadectomy itself.

It should be remembered that prevention and early recognition of overweight in cats is essential to increase both quality and quantity of life (Zoran 2010). Therefore, the role of the veterinarian and owner to avoid excessive weight gain following (early age) gonadectomy by means of energy restriction is crucial (Martin et al. 2001; Hoenig et al. 2003; Reichler 2009; Alexander et al. 2011). A helpful tool for the owner to maintain optimal condition of the cat can be a BCS (Laflamme 2006; Courcier et al. 2010).

6. Conclusion

Plasma leptin concentrations in cats at 6 to 8 months of age were higher in neutered (PPG) cats compared to sexually intact cats. This might be the result of an increased amount of body fat. Further long-term research, comparing PPG and TAG, should investigate whether the risk to become overweight at a later stage is indeed influenced by the age at gonadectomy or solely by the gonadectomy itself.

7. *Funding*

8. *Acknowledgements*

**Development of behaviour in adopted shelter kittens
following gonadectomy performed at an early age
or at a traditional age**

1. Abstract

Prepubertal gonadectomy (PPG) is promoted for population control in cats, but concerns related to health and behavior still exist. From a behavioral point of view, in order for PPG to be an acceptable alternative for traditional age gonadectomy (TAG), the occurrence of undesirable behavior should be unaffected by age at gonadectomy. The aims were to investigate (1) whether the average number of (potentially) undesirable behaviour in shelter kittens during 24 months after adoption was associated with the age at gonadectomy, and (2) whether social or environmental factors were related to the occurrence of commonly reported undesirable behaviours (inappropriate elimination, fearfulness, aggression and destruction).

Eight hundred healthy kittens between the estimated age of 8 to 12 weeks (0.7 and 1.4 kg) were recruited from animal shelters in Flanders, Belgium. Before adoption, kittens were randomly assigned to a PPG (gonadectomy upon assignment) and TAG group (gonadectomy between 6 to 8 months of age). Online short- and long-term follow-up were conducted using a 30-day diary immediately after adoption and surveys at 2, 6, 12, 18 and 24 months after adoption.

Mean number ($\pm$ SD) of potentially undesirable behaviour per day during the first month after adoption was not significantly different between PPG (1.48 $\pm$ 0.957) and TAG (1.39 $\pm$ 0.899) kittens ($P = 0.32$), and neither was the evolution of the mean number of potentially undesirable behaviour and undesirable behaviour during the long-term follow-up ($P = 0.0946$ and $P = 0.10$ respectively). The occurrence of inappropriate elimination, fearful behaviour, non-play related aggression and destruction was associated with other social and environmental variables (e.g. the use of punishment by the owner and friendliness towards a stranger).

In conclusion, this study in shelter cats did not demonstrate an effect of age at time of gonadectomy on the mean number of (potentially) undesirable behaviour during 24 months after adoption. Other factors seem to play a more dominating role in the development of such behaviours.

2. *Introduction*

To date, surgical gonadectomy in cats is the most reliable and permanent method of contraception (Looney et al. 2008b; Reichler 2009; ACC&D 2013). Traditionally, gonadectomy is performed around the age of 6 months or later (Stubbs et al. 1995; Olson et al. 2001; Root Kustritz 2007), but at that age, some cats will have already entered puberty and are able to produce litters (Jemmett & Evans 1977; Jackson 1984). Consequently, to ensure that adopted cats from shelters will not reproduce, prepubertal gonadectomy (PPG) has been promoted (Stubbs et al. 1995; Howe 1997; Bushby & Griffin 2011; Joyce & Yates 2011; Root Kustritz 2012; Root Kustritz 2013; Polson et al. 2014).

Concerns about PPG, defined as gonadectomy between 6 to 16 weeks of age (Root Kustritz 2013), have been voiced in the past, particularly by practicing veterinarians (Spain et al. 2002; Murray et al. 2008). Especially the risks related to anaesthesia and surgery and the possible development of certain medical conditions (e.g. obesity, feline urinary tract diseases, delayed physeal closure time, …) were debated. To date, however, several studies have established safe anaesthetic and surgical protocols for PPG (Porters et al. 2014c; Porters et al. 2014d; Aronsohn & Faggella 1993; Faggella & Aronsohn 1993; Howe 1997; Robertson et al. 2003a; Robertson 2007; Joyce & Yates 2011; Bushby 2013). Furthermore, a number of studies have investigated physical development (e.g. diameter of the urethra, body weight, physeal closure time) following early age neutering in cats without significant differences between cats neutered at 7 weeks or 7 months (Root et al. 1996a; Stubbs et al. 1996; Root et al. 1997). Finally, there have also been concerns about the behavioural development after PPG in cats. Since PPG is performed during the sensitive socialization period, which lasts until 9 to 10 weeks of age (McCune 1995; Adamelli et al. 2005; Overall et al. 2005), this intervention might affect the behavioural development, resulting in behaviour problems.

Commonly reported behaviour problems in cats are inappropriate elimination, fearful behaviour, aggression to other cats or people and destructive behaviour (Heidenberger 1997; Fatjo et al. 2006; Shore et al. 2008; Amat et al. 2009). Behaviour problems may be caused by or result in fear and anxiety, thereby impacting animal welfare (Landsberg 1996; Levine 2008). Furthermore, many feline behaviour problems can be a burden for the owner, affecting the owner-cat bond (Overall et al. 2005). Consequently, the risk for relinquishment or even euthanasia increases (Patronek et al. 1996; Salman et al. 1998; Shore et al. 2003; Casey et al. 2009). In order for PPG to be considered an acceptable alternative for TAG, it should have at least the same advantages without any additional disadvantages compared to TAG.

The link between age at time of gonadectomy and behavioural development in cats has been scarcely investigated (Stubbs et al. 1996; Howe et al. 2000; Spain et al. 2004; Wright & Amoss 2004). In the experimental study of Stubbs (1996) investigating the physical manifestations following (prepubertal) gonadectomy, behavioral manifestations were also assessed at 12 months of age: sexually intact cats displayed greater intraspecies aggression and less affection towards humans than did cats neutered at 7 weeks or 7 months. In one long-term follow-up study no differences were detected in age groups at gonadectomy (< 24 and ≥ 24 weeks of age) for overall behavior problems, destructive behavior, inappropriate elimination and other miscellaneous behavior problems (Howe et al. 2000). Spain and collaegues (2004), on the other hand, reported that early age neutering was associated with a decreased occurrence of hyperactivity and increased shyness in the presence of strangers in both sexes and increased hiding in male cats, but significance disappeared when analysis was restricted to cats whose owners the behavior considered 'serious'. Unfortunately the reported studies have one or more methodological limitations. In some cases, a study design relying on an extensive recall ability by the owners of over one year was used (Howe et al. 2000; Spain et al. 2004). Furthermore, the sample size was quite small (Stubbs et al., 1996). Finally, early age gonadectomy was sometimes defined as neutering cats up until 22 to 24 weeks of age (Howe et al. 2000; Spain et al. 2004) and therefore there is a good chance some cats would not have been prepubertal. In a more recent study, investigating the prevalence of house soiling and aggression in shelter kittens during the first year after adoption, no significant association between age at time of gonadectomy (between 6 and 13 weeks or between 5 to 7 months of age) and the behaviors of interest was found (Wright & Amoss, 2004). Data collection occurred prospectively and at several time points, but, unfortunately, ended at 52 weeks after adoption. Therefore, a prospective long-term study in cats, with randomized PPG and TAG groups, spanning the post-adoption developmental stages into social maturity is currently lacking.

The current study is part of a larger project on early age gonadectomy in cats, supported by the Belgian government. The main aim of the present study was to investigate the effect of age at gonadectomy in both sexes on the mean number of potentially undesirable and undesirable behaviors in shelter cats during the first month after adoption (short-term; potentially undesirable behavior only) and during 24 months after adoption (long-term; both potentially undesirable behavior and undesirable behavior). Furthermore, the present study also aims to investigate the association of age at gonadectomy and other selected social and environmental factors on the most common (potentially) undesirable behaviors in cats as

reported in literature: inappropriate elimination, fearfulness, (non) play-related aggression toward people or animals, and destruction (Heidenberger 1997; Fatjo et al. 2006; Shore et al. 2008; Amat et al. 2009).

3. Materials and Methods

The study protocol was approved by the local Ethical Committee (Faculty of Veterinary Medicine, Ghent University, Belgium) (licence number EC 2010/019 and 2011/077) and the Deontological Committee (Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium).

3.1. Animals

Between May 2010 and August 2012, healthy, dewormed and vaccinated kittens were recruited from animal shelters in Flanders (Belgium). Based on their body weight (between 0.7 and 1.4 kg), kittens were enrolled between the estimated age of 8 to 12 weeks (Lawler 2008). A thorough physical examination was performed to establish the good health of the kittens before they were transported with their littermates to the Faculty of Veterinary Medicine at Ghent University.

The selected kittens were randomly assigned to one of the two treatment groups, by a stratified randomization scheme using unequal group sizes (2/3 PPG; 1/3 TAG). Kittens belonging to the PPG group were gonadectomised upon assignment and received a microchip for identification at the end of the surgery. In the TAG group, kittens also received a microchip for identification, but gonadectomy was postponed until the age of 6 to 8 months.

Before pre-anaesthetic examination (PPG) or placing the microchip (TAG), the reaction of the kittens towards a stranger (NP) at the Faculty was noted. For this purpose, kittens were firstly observed and approached while NP talked to the kittens. Afterwards, kittens were handled and petted. Friendly kittens did not hide, were playful and easy to manipulate. Shy kittens hid, but could be handled and petted without signs of aggression whereas frightened kittens also hid, but when handled tried to escape or showed aggressive behaviour. Following the stay of the kittens (PPG and TAG) at the Faculty, the litter was returned to the shelter and the kittens were offered for adoption.

The length of time that the kittens (PPG and TAG) stayed at the Faculty was five days from May 2010 to April 2011 and one day only from April 2011 until August 2012. The reduction

in time spent at the Faculty resulted from the fact that, given the observed limited surgical and anaesthetic complications, there was no obvious clinical benefit for the long postoperative follow-up period at the Faculty. The stress caused by the environmental changes during transport and temporary housing at the Faculty, however, was deemed to be responsible for the observed increase in the incidence of anorexia and upper respiratory tract diseases compared to the pre-Faculty entry.

The anaesthetic and surgical protocols as well as complication rates in PPG and TAG cats were described in detail elsewhere (Porters et al. 2014c; Porters et al. 2014d).

3.2. Post adoption survey instrument

Individuals interested in adopting a kitten included in the project received a brochure from the shelter staff containing information about the study, its goals and the effort required by the new owners. Adopters were asked to sign an informed consent form and to provide their contact information. Next, over the course of the following 24 months, the owners received e-mail invitations (and, if necessary, automatic reminders) to complete several web-based surveys created on a dedicated Microsoft Dynamics CRM platform (ESC, De Pinte, Belgium). Paper copies were available to owners without internet access.

The surveys were partly based on existing surveys (BSAVA 2009; Corridan 2010) and consisted of open-ended or closed questions, the latter mostly being multiple choice items. The original surveys (in Dutch) are available from the first author.

General information – Immediately after adoption, participants were asked to complete a 17-item survey to obtain general information about the adopter's household (e.g. contact details, family composition, presence of other pets, and the availability to the kitten of food, toys, litter boxes, scratching posts, …). Individuals who adopted more than one kitten from the project, needed to complete this survey only once.

Short-term follow-up – Evidence in literature suggests that kittens are at the highest risk for developing certain behavioural issues (house soiling and aggression towards people or animals) during the first month after adoption (Wright & Amoss 2004). Consequently, participants were requested to keep a diary for 30 days, starting the day after adoption. Each day, the same 34-item questionnaire was presented, requesting information about the kitten's general activity (feeding and drinking, (solitary) play, litter box use, outdoor access), reciprocal interaction with other animals (if applicable) and with the owner (affiliative and

agonistic interactions, including use of punishment by the owner, proximity to owner or other family members and the time spent home alone by the kitten), and the occurrence of potentially undesirable behaviour (inappropriate urination, defecation, and urine marking, (non) social fearful behaviour, (non) play-related aggression towards people or animals, destruction, excessive vocalization, pica and sucking on fabric). In addition, owners were asked to rate on a 10-point Likert scale how satisfied they were with their kitten (Corridan 2010).

Long-term follow up – At 2, 6, 12, 18 and 24 months after adoption, owners were asked to fill out an identical survey containing 75 questions to obtain information about the behaviour and medical condition of the kitten, the attitude of the owner and living conditions. Except for pica, sucking on fabric and play-related aggression to animals, the occurrence of the same list of potentially undesirable behaviours as mentioned in the diary were investigated. In addition, the following behaviours were inquired: negative interaction with other animals, excessive activity, the tendency to keep to itself, attention seeking, disobedience, stealing food, excessive or inadequate coat care, hunting and sexual behaviour (mounting on people/animals/objects, flehmen, urine spraying, genital licking, …). When owners indicated one of these potentially undesirable behaviours, they were asked to state whether the behaviour bothered them, in which case it was also counted as an undesirable behaviour. Owners were asked whether their cat had shown the previous behaviours during the past month, or during the past 4 or 6 months for the survey at 2, 6 and 12/18/24 months after adoption, respectively. Other aspects that were covered in the survey were as mentioned above for the diary, with the addition of certain cat-related factors (medical condition, adapted to the household, solely entertainment), owner-related factors (using advice about cat's behaviour) and environmental-related factors (number, type and cleaning frequency of litter box, distance between litter box and feeding bowl, (un)limited feeding regimen, feeding enrichment, use of scratching posts, changes in daily routine).

3.3. (Potentially) undesirable behaviour

In this study, the behaviours as reported by the owner were examined, including the "undesirability" thereof, as it is the personal perceptual element which defines the concept of an "undesirable" or "problematic" behaviour (Blackwell et al. 2008; Ramos & Mills 2009). The use of the term "behaviour problem" was avoided, as this is commonly used to refer to a clinical problem, which is difficult to establish without a detailed behavioural history.

Consequently, the term potentially undesirable behaviour will be used to describe behaviour that is reported by the owner and that might potentially become troublesome to the owner (Blackwell et al. 2008). The term undesirable behaviour, on the other hand, refers to behaviour that has been indicated by the owner to be truly troublesome or problematic, and thus, considered to be unacceptable to the owner (Blackwell et al. 2008; Amat et al. 2009).

3.4. Statistical Analysis

The inclusion criteria for the kittens in the data analysis were: kittens adopted within 30 days after their stay at the Faculty (variation in age of kittens limited to 8 weeks), kittens in foster families were not adopted by those families, at least 3 days of the diary had been filled out by the owners (short-term follow-up), surveys (long-term follow up) were filled out within a specific time period following the invitation (60 days for the survey at two months and 90 days for the survey at 6, 12, 18 and 24 months after adoption), cats had not been rehomed after adoption and had not been seen by a behaviour consultant.

1) Effect of group and gender on the mean number of (potentially) undesirable behaviour:
Before analysis, data were tested for normality and for equal distribution of male and female cats in the PPG and TAG group using a chi-square test. If data were not normally distributed, a log10-transformation of the data was performed for analysis and results were reported as back transformed means ± standard deviation.
Short-term follow-up – The mean number of potentially undesirable behaviour was calculated as the sum of the reported behaviours per kitten divided by the number of days the diary was completed by the owner. To examine the effects of age at gonadectomy and gender, a linear model was fitted with group, gender and the interaction between group* gender as fixed effects. If gender and/or group*gender were not significant, they were removed from the model.
Long-term follow-up – A similar model as for the short-term follow-up was developed to assess the evolution of the mean number of potentially undesirable and undesirable behaviour over time with group, gender and time (2, 6, 12, 18 and 24 months after adoption) and interactions among these as fixed effects.

2) Identification of social and environmental factors associated with common (potentially) undesirable behaviours:

Short-term follow-up – Responses of the diary were transformed to binary responses for each of the selected potentially undesirable behaviours reported: a score of 0 was assigned if the behaviour was reported less than 3 times, to exclude accidentally performed behaviours in the analysis. A logistic regression model was fitted for each behavioural outcome and investigated the effect of the above-mentioned social and environmental factors on the response. Retention of those factors was verified by means of backward elimination technique.

Long-term follow-up – A longitudinal logistic regression model was fitted for each undesirable behaviour of interest, with as fixed effects group, time, group*time and 10 social/environmental covariates, selected based on their expected biological relevance for the particular behavioural outcome (Table 1). During the model-building process, insignificant variables were removed one at a time, starting with the least biologically relevant one.

For the short-term as well as for the long-term follow-up, group and the interaction group*time respectively, were always retained in the model, irrespective of their significance. Cut-off for retention of social and environmental factors in the models was set at P = 0.10, given the large number of covariates. Thereby, statistical trends ($0.05 \leq$ P-value < 0.10) were also reported. For the variables retained in the models, odds ratios and 95 % confidence intervals were calculated.

Continuous variables were expressed as mean ($\pm$ standard deviation), categorical variables as the proportion kittens (number or percentage) belonging to a specific category.

All data were exported form the CRM platform to excel 2010. The analysis was performed in SAS version 9.2 (SAS Institute Inc., USA). Significance was set at 0.05.

Table 1. Selection and ranking of 10 social and non-social environmental factors according to expected biological relevance for five undesirable behaviours.

	Inappropriate elimination	Fearful behavior	Non play-related aggression (people)	Play-related aggression (people)	Destruction
1.	Maintenance litter boxes	Reaction as kitten to stranger	Reaction as kitten to stranger	Outdoor access	Outdoor access
2.	Number of litter boxes compared to the number of cats in household	Punishment	Punishment	Positive interaction	Positive interaction
3.	Type of litter box (open, closed)	Outdoor	Adaptation to household	Punishment	Feeding enrichment
4.	Distance between litter box and food bowl	Adaptation to household	Changes in daily routine	Play	Solely entertainment
5.	Outdoor access	Changes in daily routine	Medical condition	Proximity	Feeding regimen
6.	Medical condition	Proximity	Outdoor access	Feeding regimen	Play
7.	Adaptation to household	Play	Feeding regimen	Reaction as kitten to stranger	Use of scratch post as a resting place
8.	Changes in daily routine	Positive interaction	Play	Adaptation to household	Proximity
9.	Reaction as kitten to stranger	Solely entertainment	Positive interaction	Changes in daily routine	Reaction as kitten to stranger
10.	Punishment	Other cat in household	Proximity	Other cat in household	Other cat in household

4. Results

Out of a total of 800 recruited kittens, the general information survey was completed for 678 kittens (PPG: n = 465 and TAG: n = 213). For the majority of the kittens, the responding owner was a female (PPG: 65%, TAG: 62%), between 18 and 38 years of age (PPG: 60%, TAG: 65%) and without children (PPG: 56%, TAG: 62%).Most kittens lived in a house (PPG: 81%, TAG: 80%) and/or had access to a garden, a terrace and/or a court (PPG: 91%, TAG: 91%). More than half of all PPG and TAG kittens (59%) lived together with at least one other cat in the household (mean 1.5 ± 1.5, range: 1 to 15 cats). Ninety-five respondents had adopted more than one kitten: 45 owners one PPG and one TAG kitten, 39 owners two PPG kittens, seven owners two TAG kittens, 2 owners three PPG kittens, one owner two PPG and one TAG kitten and one owner four PPG and one TAG kitten.

During the short-term follow-up, the diary was completed for 612 kittens. Following application of inclusion criteria, data on 480 kittens (PPG: n = 334, TAG: n = 146) were retained for analysis. On average, compliant owners completed 22 ± 9.5 days of the diary. The gender distribution for kittens in the PPG (164 male and 170 female) and TAG (68 male and 78 female) group was not significantly different ($X^2 = 0.2597$, df = 1, $P = 0.61$).

During the long-term follow-up, 1456 out of 2072 potential surveys were completed. According to the inclusion criteria, data of 1250 surveys of 495 kittens (PPG: n = 345, TAG: n = 150) were retained for the longitudinal analysis (Table 2). Long-term data until 24 months post adoption could not be collected for every recruited kitten due to the end date of the project. There was also some drop-out of kittens (91/465 PPG; 35/213 TAG) throughout the study, but the response rate per survey remained sufficiently high. The gender distribution for kittens in the PPG (165 male and 180 female) and TAG (77 male and 73 female) group was also not significantly different in the long-term follow-up ($X^2 = 0.5147$, df = 1, $P = 0.47$).

Table 2. Distribution of completed and analyzed surveys during the long-term follow-up and the number (%) of cats with at least one (potentially) undesirable behaviour at 2, 6, 12, 18 and 24 months after adoption from a shelter.

Time points of long-term follow-up surveys (months after adoption)	2	6	12	18	24
Cats available per survey[a]	649	616	402	275	130
Number of cats survey completed	497	402	284	173	79
Response Rate (%)	76.6	65.3	70.6	62.9	60.8
Number of cats survey analysed[b]	430	357	242	151	70
Number of cats with at least one potentially undesirable behaviour (%)	385 (89.5)	333 (93.3)	234 (96.7)	144 (95.4)	69 (98.6)
Number of cats with at least one undesirable behaviour (%)	195 (45.3)	172 (48.2)	96 (39.6)	65 (43.0)	33 (47.1)

[a] number of cats available per survey decreases towards end of project, ending date of the project did not permit a follow-up of every kitten until 24 months after adoption; [b] taking into account the inclusion criteria: kittens were adopted within 30 days after their stay at the Faculty, surveys were filled out within a specific time period following the invitation (60 days for the survey at two months and 90 days for the survey at 6, 12, 18 and 24 months after adoption), cats had not been rehomed after adoption and had not been seen by a behaviour consultant

1) Effect of group and gender on the mean number of (potentially) undesirable behaviour:

Short-term follow-up – Of the 480 kittens included for analysis, only 10 kittens were reported by their owner not to show any of the potentially undesirable behaviours during the first month after adoption. The most frequently reported potentially undesirable behaviours in PPG and TAG kittens were play-related aggression towards people, destruction, sucking on fabric and fearful behaviour (towards noise/movement) (Table 3). The mean number of potentially undesirable behaviour per day did not differ significantly between PPG kittens (1.48 ± 0. 957) and TAG kittens (1.39 ± 0.899) ($P = 0.32$). There was a tendency ($P = 0.055$) that male kittens, irrespective of the age at gonadectomy, exhibited on average more potentially undesirable behaviour per day during the first month after adoption compared to female kittens (1.50 ± 0.980 and 1.37 ± 0. 893, respectively).

Table 3. Potentially undesirable behaviours included in the short-term follow-up and the number (%) of kittens in the early gonadectomy (PPG: n = 334) and traditional age gonadectomy (TAG: n = 146) group that displayed them. The daily averages of each potentially undesirable behaviour, calculated across the kittens within the PPG and TAG groups, is also shown.

Potentially undesirable behaviour	Group	Kittens[a] n (%)	Mean ± SD per day
Inappropriate elimination	PPG	49 (14.7)	0.058 ± 0.13
	TAG	20 (13.7)	0.064 ± 0.14
Fearful behaviour (people, animals)	PPG	83 (24.9)	0.12 ± 0.21
	TAG	32 (21.9)	0.10 ± 0.18
Fearful behaviour (movement, noise)	PPG	160 (47.9)	0.21 ± 0.26
	TAG	61 (41.8)	0.17 ± 0.21
Non play-related aggression (people)	PPG	45 (13.5)	0.056 ± 0.13
	TAG	14 (9.6)	0.047 ± 0.11
Non play-related aggression (animals)	PPG	33 (9.9)	0.045 ± 0.13
	TAG	13 (8.9)	0.049 ± 0.14
Play-related aggression (people)	PPG	221 (66.2)	0.36 ± 0.33
	TAG	93 (63.7)	0.37 ± 0.33
Play-related aggression (animals)	PPG	29 (8.7)	0.044 ± 0.16
	TAG	9 (6.2)	0.032 ± 0.14
Destruction	PPG	193 (57.8)	0.30 ± 0.30
	TAG	88 (60.3)	0.30 ± 0.29
Excessive vocalization	PPG	25 (7.5)	0.028 ± 0.094
	TAG	9 (6.2)	0.026 ± 0.088
Pica	PPG	13 (3.9)	0.019 ± 0.066
	TAG	5 (3.4)	0.024 ± 0.080
Sucking on fabric	PPG	122 (36.5)	0.23 ± 0.32
	TAG	56 (38.4)	0.20 ± 0.27

[a] kittens displayed a specific potentially undesirable behaviour if this behaviour was at least 3 days reported in the diary to exclude accidentally performed behaviours in the analysis.

Long-term follow-up – At 2, 6, 12, 18 and 24 months after adoption, the percentage of kittens displaying at least one potentially undesirable behaviour ranged between 89.5 % (2 months) and 98.6 % (24 months). In almost half of the cases, the behaviours were disturbing to the owner, hence undesirable behaviours (Table 2). In PPG as well as in TAG cats, the most frequently reported potentially undesirable behaviours at each time point were hunting, destruction, sexual behaviour, fearful behaviour and attention seeking. Common undesirable behaviours were destruction, stealing food and (non) play-related aggression towards people (Table 4). As can be seen in Figures 1 and 2, evolution over time of the mean number of potentially undesirable behaviour and undesirable behaviour respectively was comparable for PPG and TAG cats. The mean number of potentially undesirable behaviours rose slightly, without statistical significance between PPG and TAG cats ($P = 0.095$). In PPG cats, the mean number of undesirable behaviour also slightly increased over time, whereas it decreased slightly for TAG cats, but this difference was not statistically significant ($P = 0.10$). There was also a numerical, but not statistically significant, decrease in mean

number of (potentially) undesirable behaviour in PPG and TAG cats for the survey at 12 months after adoption.

Figure 1. Evolution over time of the mean number (± SEM) of potentially undesirable behaviours of the prepubertal gonadectomy (PPG) and the traditional age gonadectomy (TAG) group as reported by the owner in the long-term survey at 2, 6, 12, 18 and 24 months after adoption from a shelter.
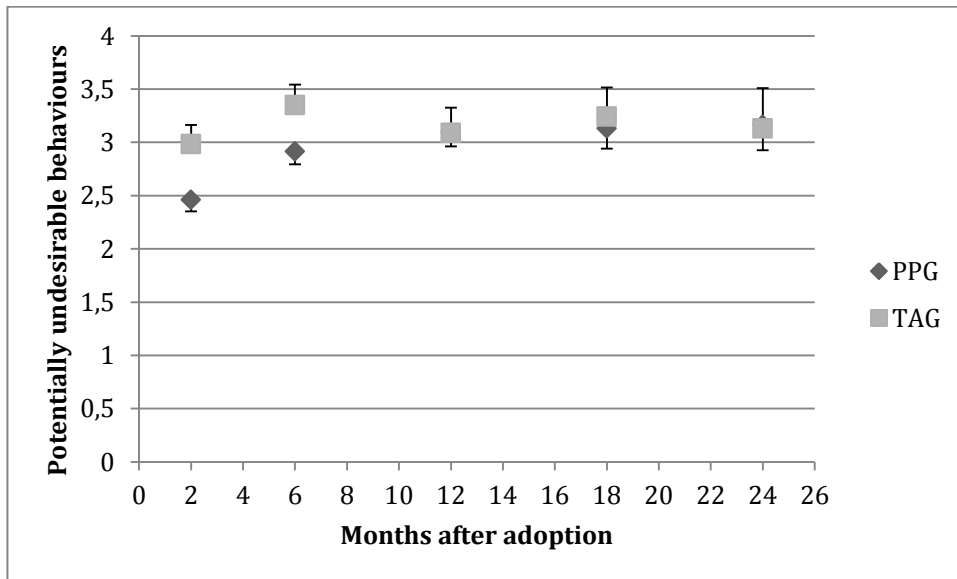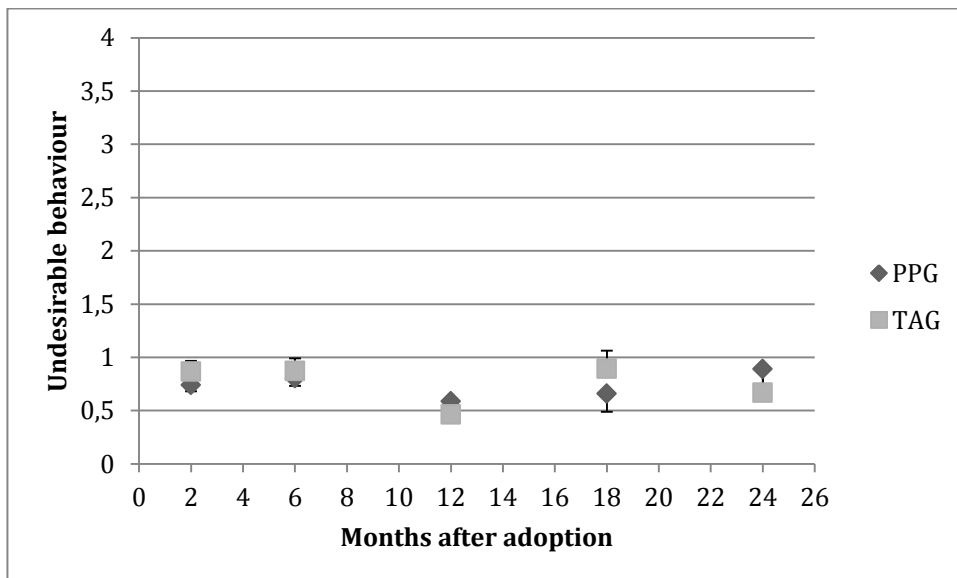

Figure 2. Evolution over time of the mean number (± SEM) of undesirable behaviours of the prepubertal gonadectomy (PPG) and the traditional age gonadectomy (TAG) group as reported by the cat owner in the long-term survey at 2, 6, 12, 18 and 24 months after adoption from a shelter.

Table 4. Potentially undesirable and undesirable behaviours included in the long-term follow-up and the number (%) of cats in the prepubertal gonadectomy group (PPG: n = 295, 240, 186, 114 and 55 at 2, 6, 12, 18 and 24 months after adoption resp.) and traditional age gonadectomy group (TAG: n = 135, 117, 56, 37 and 15 at 2, 6, 12, 18 and 24 months after adoption) that displayed them.

| Behaviours | Group | Time points of long-term surveys (months after adoption) | | | | | | | | | |
		2 Potentially undesirable	2 Undesirable	6 Potentially undesirable	6 Undesirable	12 Potentially undesirable	12 Undesirable	18 Potentially undesirable	18 Undesirable	24 Potentially undesirable	24 Undesirable
Inappropriate elimination	PPG	12 (4.1)	12 (4.1)	8 (3.3)	8 (3.3)	6 (3.2)	6 (3.2)	4 (3.5)	4 (3.5)	1 (1.8)	1 (1.8)
	TAG	1 (0.7)	1 (0.7)	2 (1.7)	2 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fearful behaviour	PPG	39 (13.2)	3 (1.0)	76 (31.7)	6 (2.5)	60 (32.2)	1 (0.5)	49 (43.0)	2 (1.8)	21 (38.2)	1 (1.8)
	TAG	17 (12.6)	0 (0.0)	29 (24.8)	1 (0.9)	14 (25.0)	0 (0.0)	9 (24.3)	0 (0.0)	2 (13.3)	0 (0.0)
Non play-related aggression (people)	PPG	19 (6.4)	11 (3.7)	28 (11.7)	11 (4.6)	9 (4.8)	2 (1.1)	10 (8.8)	6 (5.3)	6 (10.9)	5 (9.1)
	TAG	12 (8.9)	7 (5.2)	10 (8.5)	6 (5.2)	6 (10.7)	3 (5.4)	4 (10.8)	4 (10.8)	1 (6.7)	1 (6.7)
Non play-related aggression (animals)	PPG	1 (0.3)	0 (0.0)	3 (1.3)	1 (0.4)	6 (3.2)	2 (1.1)	3 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)
	TAG	1 (0.7)	1 (0.7)	2 (1.7)	0 (0.0)	1 (1.8)	0 (0.0)	1 (2.7)	1 (2.7)	1 (6.7)	0 (0.0)
Play-related aggression (people)	PPG	23 (7.8)	13 (4.4)	21 (8.8)	12 (5.0)	12 (6.5)	5 (2.7)	10 (8.8)	7 (6.1)	1 (1.8)	1 (1.8)
	TAG	15 (11.1)	12 (8.9)	15 (12.8)	9 (7.7)	5 (8.9)	2 (3.6)	5 (13.5)	3 (8.1)	5 (33.3)	2 (13.3)
Destruction	PPG	117 (39.7)	79 (26.8)	102 (42.5)	67 (27.9)	75 (40.3)	46 (24.7)	39 (34.2)	24 (21.1)	22 (40.0)	16 (29.1)
	TAG	64 (47.7)	45 (33.1)	55 (47.0)	33 (28.2)	23 (41.1)	12 (21.4)	14 (37.8)	8 (21.6)	5 (33.3)	1 (6.7)
Negative interaction with animals	PPG	3 (1.0)	2 (0.7)	6 (2.5)	2 (0.8)	3 (1.6)	1 (0.5)	7 (6.1)	2 (1.8)	3 (5.5)	3 (5.5)
	TAG	3 (2.2)	3 (2.2)	4 (3.4)	3 (2.6)	0 (0.0)	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)
Excessive vocalisation	PPG	38 (12.9)	13 (4.4)	34 (14.2)	12 (5.0)	29 (15.6)	5 (2.7)	16 (14.0)	2 (1.8)	9 (16.4)	1 (1.8)
	TAG	19 (14.1)	6 (4.4)	24 (20.5)	7 (6.0)	9 (16.1)	1 (1.8)	9 (24.3)	3 (8.1)	3 (20.0)	1 (6.7)
Excessive activity	PPG	40 (13.6)	10 (3.4)	27 (11.3)	5 (2.1)	15 (8.1)	4 (2.2)	12 (10.5)	3 (2.6)	3 (5.5)	1 (1.8)
	TAG	23 (17.0)	5 (3.7)	29 (24.8)	4 (3.4)	4 (7.1)	0 (0.0)	3 (8.1)	1 (2.7)	2 (13.3)	0 (0.0)
Tendency to keep to itself	PPG	5 (1.7)	0 (0.0)	10 (4.2)	1 (0.4)	9 (4.8)	0 (0.0)	8 (7.0)	0 (0.0)	3 (5.5)	0 (0.0)
	TAG	1 (0.7)	0 (0.0)	2 (1.7)	2 (1.7)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Attention seeking	PPG	80 (27.1)	5 (1.7)	74 (30.8)	4 (1.7)	54 (29.0)	1 (0.5)	25 (21.9)	2 (1.8)	16 (29.1)	3 (5.5)
	TAG	57 (42.2)	4 (3.0)	50 (42.7)	2 (1.7)	13 (23.2)	0 (0.0)	11 (29.7)	1 (2.7)	3 (20.0)	0 (0.0)
Disobedience	PPG	23 (7.8)	10 (3.4)	30 (12.5)	9 (3.8)	10 (5.4)	3 (1.6)	10 (8.8)	5 (4.4)	3 (5.5)	1 (1.8)
	TAG	15 (11.1)	6 (4.4)	11 (9.4)	5 (4.3)	5 (8.9)	0 (0.0)	3 (8.1)	2 (5.4)	1 (6.7)	0 (0.0)
Stealing food	PPG	71 (24.1)	58 (19.7)	66 (27.5)	50 (20.8)	45 (24.4)	25 (13.4)	25 (21.9)	15 (13.2)	12 (21.8)	10 (18.2)
	TAG	39 (28.9)	27 (20.0)	40 (34.2)	26 (22.2)	17 (30.4)	6 (10.7)	14 (37.8)	7 (18.9)	6 (40.0)	3 (20.0)
Excessive coat care	PPG	7 (2.4)	0 (0.0)	9 (3.8)	2 (0.8)	8 (4.3)	2 (1.1)	4 (3.5)	0 (0.0)	1 (1.8)	1 (1.8)
	TAG	3 (2.2)	0 (0.0)	3 (2.6)	0 (0.0)	5 (8.9)	1 (1.8)	2 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)
Inadequate coat care	PPG	1 (0.3)	0 (0.0)	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)
	TAG	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hunting	PPG	123 (41.7)	2 (0.7)	138 (57.5)	1 (0.4)	150 (80.6)	6 (3.2)	82 (71.9)	2 (1.8)	47 (85.5)	3 (5.5)
	TAG	69 (51.1)	0 (0.0)	69 (59.0)	1 (0.9)	45 (80.4)	1 (1.8)	27 (72.9)	3 (8.1)	12 (80.0)	0 (0.0)
Sexual behaviour	PPG	123 (41.7)	0 (0.0)	65 (27.1)	1 (0.4)	83 (44.6)	0 (0.0)	52 (45.6)	0 (0.0)	23 (41.8)	2 (3.6)
	TAG	63 (46.7)	0 (0.0)	46 (39.3)	0 (0.0)	23 (41.1)	0 (0.0)	14 (37.8)	0 (0.0)	5 (33.3)	0 (0.0)

2) Identification of social and environmental factors associated with common (potentially) undesirable behaviours:

Short-term follow-up – Unlike age at gonadectomy, other variables were found to be significantly associated with the occurrence of the potentially undesirable behaviours of interest: inappropriate elimination, fearful behaviour, (non) play-related aggression towards people or animals, and destruction (Table 5). The odds ratios provided an indication of the impact (< 1.0 represents a decrease, > 1.0 an increase) of each class of a specific categorical variable compared to a predefined reference value (for the same categorical variable) on the response under investigation, when all other variables are fixed (average value for continuous variables, reference value for categorical variables). For example, use of physical punishment was associated with a 12-fold increase (OR = 12.242) in inappropriate elimination compared to no use of punishment. Summarizing Table 4, during the first month after adoption, cats whose owners used verbal and/or physical punishment, as opposed to no punishment, had a greater chance to show inappropriate elimination, to display fearful behaviour in response to noises and/or movements, play-related aggressive or destructive behaviour. Secondly, single cats were more likely to behave aggressively during play and fearful in response to noises and/or movements than cats living in a multicat household. Thirdly, compared to those reacting friendly to a stranger (NP) as kittens, kittens responding shy or frightened were more likely to be fearful as well as aggressive to family members in non play-related contexts. Fourthly, spending less time in the proximity of the owner or family members, was associated with being more fearful in response to noises and/or movements, and displaying more inappropriate elimination.

Long-term follow-up – Evolution over time of most undesirable behaviours of interest were not significantly different between PPG and TAG cats. Only for destruction, the interaction between group and time was significant (P = 0.034), implying that the evolution of the occurrence of destruction over time differs between PPG and TAG cats. More specifically, at 8.24 months after adoption (weighted mean based on the time-points and observations at each time-point), PPG cats were more likely to display undesired destructive behaviour than TAG cats, but numerically, this increase was barely different from 1.0 (OR = 1.047 [0.721-1.520]). Non play-related aggression towards animals could not be analyzed because of the very low frequency of this behaviour for both PPG and TAG cats, but various other variables were found to be associated with the occurrence of the undesirable behaviours of interest (Table 6).

Being punished verbally and/or physically was associated with an increase in cats displaying (non) play-related aggression towards people and destructive behaviour compared to cats that were not punished. On the other hand, cats that were punished verbally and/or physically were less likely to behave fearful compared to cats whose owners did not use punishment. Having positive interaction with the owner or other family members at least 2 to 3 times per day was associated with a greater chance of destruction, fearful behaviour and play-related aggression towards people, but a lesser chance of showing non play-related aggression towards the owner or other family members. Also, single cats were more likely to be destructive than cats living in a multicat household. Whereas cats being shy or frightened to a stranger (NP) as kittens were less likely to behave aggressively during play or to be destructive, they were more likely to show inappropriate elimination and fearful behaviour compared to cats who behaved friendly as a kitten.

Table 5. Variables associated ($P < 0.05$ and $0.05 \leq P < 0.10$) with the occurrence of potentially undesirable behaviours in kittens during the first month after adoption from a shelter. The impact of the different variable values compared to the variable reference value (bold) is shown by odds ratios (OR) and their respective 95% confidence limits [95%CL].

Potentially undesirable behaviour	Variable	P-value	Variable values	OR [95%CL]
Inappropriate elimination	Proximity	0.0371	**At least half of the time that family members are home (≥ 50% Dᵃ)**	-
			Less than half of the time that family members are home (< 50% Dᵃ)	1.894 [1.039-3.454]
	Negative interaction (animals)	0.0519	**No or occasionally (< 20% Dᵃ)**	-
			Frequently (at least once per day in ≥ 20% Dᵃ)	1.824 [0.995-3.344]
	Punishment	0.0521	**No punishment**	-
			Verbal	5.002 [1.587-15.770]
			Physical	12.242 [1.481-101.200]
			Verbal and physical	3.485 [1.175-10.340]
	Duration interaction	0.0554	One minute increase	0.997 [0.993-1.000]
Fearful behaviour (people/animals)	Reaction of kitten to stranger	< 0.0001	**Friendly**	-
			Shy	3.771 [1.995-7.128]
			Frightened	5.361 [2.383-12.057]
	Proximity	0.0008	**At least half of the time that family members are home (≥ 50% Dᵃ)**	-
			Less than half of the time that family members are home (< 50% Dᵃ)	2.242 [1.400-3.591]
	Time spent alone	0.0010	**Less frequently 3 hours per day (< 70% Dᵃ)**	-
			Frequently at least 3 hours per day (≥ 70% Dᵃ)	2.300 [1.401-3.776]
	Negative interaction (animals)	0.0065	**No or occasionally (< 20% Dᵃ)**	-
			Frequently (once per day in ≥ 20% Dᵃ)	1.990 [1.212-3.265]
Fearful behaviour (noises /movements)	Punishment	0.0001	**No punishment**	-
			Verbal	2.402 [1.294-4.461]
			Physical	1.851 [0.402-8.531]
			Verbal and physical	3.598 [2.089-6.197]
	Proximity	0.0121	**At least half of the time that family members are home (≥ 50% Dᵃ)**	-
			Less than half of the time that family members are home (< 50% Dᵃ)	1.729 [1.127-2.652]
	Other cat in household	0.0141	**No other cat present**	-
			At least one other cat present	0.619 [0.421-0.908]
	Reaction as kitten to stranger	0.0322	**Friendly**	-
			Shy	2.553 [1.342-4.856]
			Frightened	1.312 [0.589-2.924]

147

Potentially undesirable behaviour	Variable	P-value	Variable values	OR [95%CL]
Non play-related aggression (people)	Reaction of kitten to stranger	0.0027	**Friendly**	-
			Shy	2.704 [1.266-5.775]
			Frightened	3.773 [1.597-8.910]
	Gender	0.0148	**Female**	-
			Male	2.045 [1.150-3.636]
Non play-related aggression (animal)	Negative interaction (animals)	< 0.0001	**No or occasionally (< 20% Dᵃ)**	-
			Frequently (once per day in ≥ 20% Dᵃ)	9.226 [4.452-19.120]
	Other cat in household	0.0070	**No other cat present**	-
			At least one	3.107 [1.364-7.079]
Play-related aggression (people)	Punishment	< 0.0001	**No punishment**	-
			Verbal	2.949 [1.509-5.763]
			Physical	2.706 [0.379-19.305]
			Verbal and physical	5.017 [2.798-8.997]
	Other cat in household	0.0005	**No other cat present**	-
			At least one other cat present	0.422 [0.259-0.688]
	Gender	0.00221	**Female**	-
			Male	1.718 [1.081-2.731]
	Time spent alone	0.0243	**Less frequently 3 hours per day (< 70% Dᵃ)**	-
			Frequently at least 3 hours per day (≥ 70% Dᵃ)	0.554 [0.332-0.926]
	Duration interaction	0.0474	One minute increase	1.003 [1.000-1.005]
	Negative interaction (animals)	0.0499	**No or occasionally (< 20% Dᵃ)**	-
			Frequently (once per day in ≥ 20% Dᵃ)	1.736 [1.000-3.013]
Destruction	Punishment	< 0.0001	**No punishment**	-
			Verbal	4.261 [2.351-7.721]
			Physical	2.625 [0.607-11.349]
			Verbal and physical	6.229 [3.689-10.520]
	Outdoor access	0.0410	**Outdoor access (≥ 25% Dᵃ)**	-
			Rarely outdoor access (< 25% Dᵃ)	1.564 [1.018-2.401]

ᵃD = number of days completed in the diary (during first month after adoption)

Table 6. Variables associated ($P < 0.05$ and $0.05 \leq P < 0.10$) with the occurrence of undesirable behaviours in cats during the long-term follow-up (surveys at 2, 6, 12, 18 and 24 months after adoption from a shelter). The impact of the different variable values compared to the variable reference value (bold) is shown by odds ratios (OR) and their respective 95% confidence limits [95%CL].

Undesirable behaviour	Variable	P-value	Variable values	OR [95%CL]
Inappropriate elimination	Reaction as kitten to stranger	0.0013	**Friendly**	-
			Shy	0.204 [0.028-1.465]
			Frightened	4.099 [1.784-9.417]
	Number of litter boxes compared to number of cats in household	0.0342	**Greater number**	-
			No litter box	0.229 [0.052-1.014]
			Equal number	0.548 [0.155-1.946]
			Smaller number	1.121 [0.316-3.973]
Fearful behaviour	Reaction as kitten to stranger	< 0.0001	**Friendly**	-
			Shy	2.055 [0.544-7.754]
			Frightened	1.724 [0.133-22.409]
	Punishment	< 0.0001	**No punishment**	-
			Verbal	0.813 [0.076-8.738]
			Physical	<0.001 [<0.001-0.023]
			Verbal and physic	0.550 [0.032-9.565]
	Proximity	< 0.0001	**≥ 2 to 3 times per day (active, passive)**	-
			< 2 to 3 times/day	0.005 [0.001-0.023]
			≥ 2 to 3 times/day (active)	2.192 [0.809-5.936].
			≥ 2 to 3 times/day (passive)	2.480 [0.345-17.837]
	Positive interaction	< 0.0001	**≥ 2 to 3 times/day**	-
			< 2 to 3 times/day	0.006 [<0.001-0.046]
			≥ 2 to 3 times/workday	0.015 [0.005-0.047]
			≥ 2 to 3 times/holiday or weekend day	1.763 [0.373-8.325]
	Solely entertainment	< 0.0001	**Regularly**	-
			Rare	6.541 [2.456-17.426]
	Medical condition	< 0.0001	**No medical condition**	-
			At least one	1.144 [0.200-6.549]
	Adaptation to household	0.0104	**Adapted**	-
			Not adapted	20.000 [2.299-166.667]

Undesirable behaviour	Variable	P-value	Variable values	OR [95%CL]
Non play-related aggression (people)	Positive interaction	<0.0001	**≥ 2 to 3 times/day**	-
			< 2 to 3 times/day	1.221 [0.286-5.207]
			≥ 2 to 3 times/workday	< 0.001 [<0.001-<0.001]
			≥ 2 to 3 times/holiday or weekend day	1.034 [0.387-2.764]
	Adaptation to household	0.0014	**Adapted**	-
			Not adapted	10.000 [2.915-34.483]
	Punishment	0.0052	No punishment	-
			Verbal	5.719 [0.715-45.727]
			Physical	6.184 [0.662-57.767]
			Verbal and physic	12.337 [1.592-95.613]
	Play	0.0938	**≥ 1 hour/day**	-
			< 1 hour/day	2.503 [1.059-5.914]
Play-related aggression (people)	Punishment	< 0.0001	**No punishment**	-
			Verbal	73.935 [39.412-138.700]
			Physical	94.666 [34.536-259.488]
			Verbal and physic	257.322 [159.670-414.698]
	Positive interaction	< 0.0001	**≥ 2 to 3 times/day**	-
			< 2 to 3 times/day	0.651 [0.203-2.093]
			≥ 2 to 3 times/workday	0.174 [0.091-0.335]
			≥ 2 to 3 times/holiday or weekend day	0.995 [0.534-1.854]
	Adaptation to household	0.0002	**Adapted**	-
			Not adapted	13.333 [4.348-40.000]
	Medical condition	0.0002	**No medical condition**	-
			At least one	1.003 [0.510-1.974]
	Changes in daily routine	0.0006	**No changes**	-
			At least one	0.0928 [0.464-1.859]
	Reaction as kitten to stranger	0.0011	**Friendly**	-
			Shy	0.196 [0.053-0.727]
			Frightened	0.613 [0.159-2.353]

Undesirable behaviour	Variable	P-value	Variable values	OR [95%CL]
Destruction	Punishment	< 0.0001	**No punishment**	-
			Verbal	10.116 [4.048-25.279]
			Physical	10.771 [3.858-30.075]
			Verbal and physic	14.118 [5.503-36.216]
	Positive interaction	< 0.0001	**≥ 2 to 3 times/day**	-
			< 2 to 3 times/day	0.743 [0.299-1.847]
			≥ 2 to 3 times/workday	< 0.001 [<0.001-< 0.001]
			≥ 2 to 3 times/holiday or weekend day	0.993 [0.615-1.604]
	Outdoor access	0.0022	**Cat's choice**	-
			Owner's decision	1.745 [1.228-2.477]
			No outdoor access	1.770 [1.214-2.580]
	Feeding enrichment	0.0060	**No feeding enrichment**	-
			At least one	0.459 [0.267-0.790]
	Other cat in household	0.0293	**No other cat present**	-
			At least one other cat present	0.667 [0.494-0.963]
	Group*time	0.0335	**TAG (at 8.24 months after adoption)**	-
			PPG (at 8.24 months after adoption)	1.047 [0.721-1.520]
	Reaction as kitten to stranger	0.0673	**Friendly**	-
			Shy	0.620 [0.363-1.059]
			Frightened	0.996 [0.450-2.207]

5. *Discussion*

The present study was designed to provide short- and long-term information regarding behavioural characteristics in shelter cats undergoing PPG compared to TAG. To our knowledge, this is the only prospective study to date, with randomized PPG and TAG groups and short-term and long-term follow-up, spanning the post-adoption developmental stages into social maturity. In shelter cats, age at time of gonadectomy (8 to 12 weeks versus 6 to 8 months of age) did not affect the mean number of potentially undesirable behaviour during the first month after adoption nor the evolution over time of (potentially) undesirable behaviour during 24 months post adoption. The non-significant numerical decrease in mean number of (potentially) undesirable behaviour in PPG and TAG cats around 12 months after adoption could be associated with a post-puberty phase (Beaver 2003a; Beaver 2003b). Also, no association was found between age at gonadectomy and the frequency of in literature commonly reported (potentially) undesirable behaviours during the short- and long-term follow-up (Heidenberger 1997; Fatjo et al. 2006; Shore et al. 2008; Amat et al. 2009).

Notwithstanding the methodological optimization that was attempted in this study, our findings are in agreement with other previously published studies about early age gonadectomy (Stubbs et al. 1996; Howe et al. 2000; Spain et al. 2004; Wright & Amoss 2004). In one study PPG (before 22 weeks of age) compared to TAG was significantly associated with less hyperactivity and more shyness towards strangers when it concerned potentially undesirable behaviours, but the association did not hold for undesirable behaviours (Spain et al. 2004). Most of the potentially undesirable behaviours (e.g. predatory and sexual behaviour, scratching, …) that were frequently reported both in the short- and long-term survey in the current study belong to the normal behavioural repertoire of the cat (Landsberg 1996; Heath 2007; Radosta 2011). Destruction was the most commonly reported potentially undesirable behaviour during the first month after adoption. A thorough behaviour history would be required to discover the underlying causes and motivation, but one reason could be the lively and inquisitive nature of kittens, resulting in exploration using eyes, paws and mouth (Landsberg 1996; Seksel 2008). Another behaviour, play-related aggression may be due to lack of restraint training by conspecifics during the socialization period and beyond (Crowell-Davis 2002; Ramos & Mills 2009; Radosta 2011), but can also be related to the type of play the owner instigates. Play by the owner using hand and feet is said to trigger aggression directed to these body parts and the use of rods and other string toys is advised (Heath 2002; Radosta 2011). Inappropriate elimination and fearful behaviour occurred more

frequently during the first month after adoption in the present study, suggesting that some kittens had difficulties adapting to their new environment (Wright & Amoss 2004).

With regards to the long-term follow-up, inappropriate elimination was always indicated as undesirable. Non play-related aggression towards people, destruction and stealing food were also often reported as undesirable. These findings are in agreement with other studies indicating that inappropriate elimination and aggression were regarded as serious problems for which people would be motivated to seek behavioural advice (Shore et al. 2008) or consulted a veterinarian/behaviourist (Fatjo et al. 2006; Amat et al. 2009). Moreover destruction is frequently considered an undesirable behaviour by cat owners (Morgan & Houpt 1990; Heidenberger 1997; Neidhart & Boyd 2002). Stealing food was only mentioned as an undesirable behaviour problem in one other publication (Heidenberger 1997), possibly because this behaviour is more easily managed (by not leaving food out) compared to aggression, elimination and destruction issues, which often have complex underlying motivations. Aggression involving people was more often reported as being undesirable than aggression towards animals. The latter might impact owners less since they are not the target or because they perceive agonistic interactions between animals as normal and, as such, something that should be tolerated (Fatjo et al. 2006).

Our study indicated that factors other than age at time of gonadectomy were associated with the selected (potentially) undesirable behaviours. Clearly punishment was associated with a number of (potentially) undesirable behaviours: inappropriate elimination (short-term follow-up), fearful behaviour, destruction and play-related aggression to people (short and long-term follow-up), and with non play-related aggression towards people (long-term follow-up). A similar result has been found in dogs: the use of punishment was positively correlated with the incidence of problematic behaviours (Hiby et al. 2004) and associated with a higher number of undesirable behaviours (Blackwell et al. 2008). However, it is important to note that an association does not necessarily also demonstrate causality. In agreement with the reasoning by Blackwell and others for dogs (2008), it is possible that, in the present study, cats showing many undesirable behaviours were more likely to be punished compared to cats rarely showing undesirable behaviours. Additionally, it is likely that undesirable behaviour occurred more in cats associating punishment with the person punishing or with the context in which punishment occurred, rather than with their own behaviour. Furthermore, the remarkable finding in the current study that cats being punished verbally and physically were less likely to behave fearful than cats whose owners did not use punishment or only used verbal punishment could be explained by the possibility that owners of fearful cats might be

inclined to use less severe methods to correct the animal. Another factor that was associated with certain behavioural outcomes during the short-term (fearful behaviour, non-play related aggression to people) as well as the long-term (inappropriate elimination, fearful behaviour, play-related aggression to people) follow-up in the present study, was the response of kittens to a stranger (NP) while at the Faculty of Veterinary Medicine. This response is assumed to be reflective of the degree of socialization of a kitten at time of recruitment. Similarly, other studies indicated that lack of socialization of kittens was associated with an increased risk for showing fearful behaviours later in life (Heidenberger 1997; Heath 2007; Casey & Bradshaw 2008) and for being not friendly towards people (McCune 1995). According to our results and those of a previous study (Amat et al. 2009), housing a single cat was associated with a greater chance for play-related aggression towards people, fearful behaviour in response to noises and/or movements (short-term), and destruction (long-term). Single cats may experience stress associated with living in a more boring social environment (Kendall & Ley 2006) or may not have learned from other cats to moderate their responses in play (Beaver 2004; Overall et al. 2005). According to the short-term follow-up in the current study, cats living in a multicat household were not at a higher risk for one of the most common symptoms of social stress, i.e. inappropriate elimination, unlike reported elsewhere (Kendall & Ley 2006). In the present study during the long-term follow-up, however, an increase in inappropriate elimination was associated with having less litter boxes than the number of cats in the house, which may be indicative of a risk for social stress at a later stage during development.

The results of this study should be interpreted in view of its strengths and a few limitations. The size of the participating shelters might have caused a bias in the sample of adopters since small, usually privately-owned animal shelters might have screened and as such selected adopters more than larger animal shelters. Moreover, owners were informed about the project before adoption of the kitten and the efforts required of them, which might have discouraged some owners. On the other hand, being well informed may have contributed to the response rate remaining high throughout the study in comparison to other longitudinal surveys (Clements et al. 2013). Data were collected by surveys completed by the owner. Without a thorough behavioural history, however, the interpretation of the behaviour by the owner, especially regarding underlying motivation, may not always be correct. We have attempted to minimize such errors by asking questions of descriptive nature. Furthermore, the long-term follow-up relied on recall ability as used previously in behavioural studies with cats (Wright & Amoss 2004; Kendall & Ley 2006; Kendall & Ley 2008; Ramos & Mills 2009) and dogs

(Blackwell et al. 2008). To optimize accuracy of the owner's memory, a daily diary was designed for the first month after adoption and data were gathered at different time-points until 24 months after adoption with recall periods ranging between 1 and 6 months. Finally, the study ended at 24 months after adoption, which does not represent the entire lifespan of a cat. However, it does permit evaluation of behavioural development into social maturity (Horwitz 2001; Overall et al. 2005). The fact that not all cats could be monitored until that time, affected the power of the longitudinal follow-up analysis in that no interactions between variables associated with undesirable behaviours could be investigated. In the present study, no highly detailed measures of intensity (e.g., asking owners to classify potentially undesirable and undesirable behaviors as mild-moderate-severe) or quantity (e.g., daily frequency of behaviors) were included. Instead and for the purpose of not overburdening the respondents, measures of quantity in the short-term follow-up study were based on daily one-zero data (occurring or not) and intensity was measured in the long-term follow-up by asking owners if behaviors were undesirable.

As mentioned by Scarlett et al. (2002) and as suggested during a discussion on early neutering at the WSAVA/FECAVA/BSAVA congress (Clark 2012), neutering cats is not enough to reduce the number of relinquishments. Educating owners and informing them about the normal behaviour of cats, their husbandry needs and the benefits of giving environmental enrichment are very important to prevent unwanted behaviour (Overall et al. 2005; Seksel 2008; Ellis 2009) and essential to ensure a successful adoption (Patronek et al. 1996; Neidhart & Boyd 2002; Wright & Amoss 2004; Shore 2005). The number and variety of significant social and environmental factors identified during short-term and long-term follow-up in our study confirms that the ontogeny of behavioural problems can be the result of a complex process and prevention is definitely better than cure (Hunthausen & Seksel 2002; Scarlett et al. 2002). Veterinarians should routinely inquire owners about animal behaviour during health check-ups to prevent, and to allow early detection and timely management of behavioural problems. If indicated, they should refer owners to a behavioural specialist (Patronek & Dodman 1999; Scarlett et al. 2002; Overall et al. 2005; Fatjo et al. 2006; Lord et al. 2008; Seibert & Landsberg 2008; Seksel 2008; Roshier & McBride 2013a).

6. Conclusion

This study found no evidence that age at time of gonadectomy (PPG versus TAG) in cats has an effect on the mean number of (potentially) undesirable behaviours or on the occurrence of in literature commonly reported behaviours (inappropriate elimination, non play-related aggression, fearful behaviour or destruction) during 24 months after adoption from a shelter. Consequently, also from a behavioural point of view, PPG can be recommended for shelter cats. In addition, various social and environmental factors (use of punishment by the owner, single-cat households, positive interaction with owner or family members, …) associated with the most important (potentially) undesirable behaviours were identified.

7. Funding

8. Acknowledgements

1. The controversy is not about whether to perform gonadectomy in cats, but when …

With cats gaining popularity as household pets but their reproduction often being uncontrolled, the number of cats that are abandoned and/or enter animal shelters also increases. Gonadectomies are by far the most common surgical procedures in small animal practices (Greenfield et al. 2004). Neutering at the traditional age of 6 to 8 months (traditional age gonadectomy, TAG) is a very common practice in cats (Stubbs et al. 1995; Olson et al. 2001; Root Kustritz 2007). The concept of early age neutering (prepubertal gonadectomy, PPG), a suitable answer to the problem of expanding cat populations, on the other hand, has evoked worldwide discussions concerning technical issues related to anaesthesia and surgery and concerning short- and long-term effects on health and behaviour among veterinarians (Spain et al. 2002; Murray et al. 2008; Farnsworth et al. 2013), cat owners (Welsh et al. 2013), breeders and rescue organisations (Pernestal & Axner 2012). To date, in Europe PPG remains uncommon, while it is more widely accepted in the United States, possibly due to the larger body of experimental and clinical studies on PPG. However, the existing research could benefit from additional work that focuses on methodological improvements. Furthermore, the relationship between age at gonadectomy and certain health issues (FLUTD, spontaneous fractures due to delayed physeal closure, obesity, …) has not yet been elucidated.

The general aim of this doctoral dissertation was to investigate to what extent PPG in shelter cats has different effects compared to TAG, with respect to safety during anaesthesia and surgery, and short- and long-term health and behaviour.

1.1. Anaesthetic and surgical protocols to perform prepubertal gonadectomy and comparison with those for traditional age gonadectomy

Important anatomic and physiological considerations while working with kittens are listed in the introduction of this PhD thesis. Anaesthetic and surgical protocols that also emphasize the prevention of hypoglycaemia and hypothermia, proved to be safe and effective for gonadectomy in kittens, as demonstrated in Chapter 1 and 4. These findings are in agreement with earlier reports in the literature (Aronsohn & Faggella 1993; Faggella & Aronsohn 1993; Theran 1993; Howe 1997; Robertson et al. 2003a; Joyce & Yates 2011; Polson et al. 2012).

Although no anaesthetic mortalities were observed (Chapter 1) and all anaesthetic protocols for PPG as described in Chapter 1 provided a surgical plane of anaesthesia and adequate analgesia up to 6 hours postoperatively, our findings were in favour of the intramuscular (IM) administered protocols: 'double' IM protocol (DB-IM: IM administration of the premedication dexmedetomidine combined with buprenorphine, followed by IM injection of ketamine), or the 'single' IM administered medetomidine-buprenorphine-ketamine combination (MBK-IM). Those IM administered protocols provided a more profound sedation with lesser adverse effects compared to the oral transmucosal (DB-OTM) protocol. Regarding time and cost efficacy, the MBK-IM protocol is likely to be the most ideal protocol in shelter kittens, especially if injection, prepping and surgery are well coordinated (Robertson 2005). Similar single injection protocols have been promoted for neutering kittens lately in the literature (Joyce & Yates 2011; Polson et al. 2012; Polson et al. 2014). The only disadvantage of the single injection MBK-IM protocol was the adverse reaction of the kittens at the time of injection (data not shown), and thereby single handler injections in the former are precluded. In contrast, it was mostly possible to administer the double injection IM protocol (DB-IM) without help, since the premedication was administered separately and profound sedation was established before the ketamine injection. Due to the slow onset of the sedative effect following OTM administration observed in the pilot study, it was decided to evaluate the sedative effect 15 minutes after premedication in the DB-IM as well as in the DB-OTM protocol. From personal observation, a profound sedation after IM administration of the premedication was often reached much sooner than 15 minutes. Consequently, it might be preferable to inject ketamine as soon as a profound sedation is reached, probably 5 to 10 minutes after IM injection of the premedication as observed in adult animals (Granholm et al. 2006; Scrollavezza et al. 2009). In this way, peak sedative effect (mostly between 20 and 30 minutes after IM injection) will be achieved during surgery (Granholm et al. 2006; Scrollavezza et al. 2009). As a consequence, it is possible that a lower dosage of ketamine than the one used in the present study might be sufficient to achieve an adequate level of anaesthesia to perform PPG.

Also, as the surgeon gained experience with PPG and the surgical times became shorter towards the end of the project (Chapter 4), it is quite likely that slightly lower dosages of dexmedetomidine and ketamine would have been sufficient. Lower dosages are likely to result in shorter recovery times and thereby reducing the risk for hypothermia and hypoglycemia. Also, the administration of an alpha-2 antagonist shortly after surgery

shortens recovery times without inducing adverse effects (Polson et al. 2014) or compromising pain relief (Polson et al. 2012; Polson et al. 2014). Despite the higher dosage of dexmedetomidine in the OTM group, a slower onset and a lesser extent of sedation and more adverse effects were observed compared to the IM administered protocols in PPG cats (Chapter 1).

To better understand that observation, a pharmacodynamic and a pharmacokinetic study were designed (Chapter 2 and 3).

The clinical findings were indeed backed up with the results in the pharmacokinetic study wherein the pharmacokinetic profile of dexmedetomidine (40 µg/kg) and buprenorphine (20 µg/kg) following OTM and IM administration of their combination was compared in 6 experimental cats (Chapter 3). The smaller area under the plasma concentration-time curve, the lower maximum plasma concentration and the longer time to reach maximum plasma concentration after OTM compared to IM injection suggest a slower and limited absorption of the drugs from the oral mucosa site compared to IM injection site. Many reasons can explain the differences in clinical efficacy and absorption rate following OTM and IM administration. The commercial preparations of dexmedetomidine as well as buprenorphine contain hydrochloride and a preservative. Due to the hydrochloride, the pH of the solution was rather acidic (i.e. 4.838), thereby, resulting in less unionized molecules of the drugs and thus, less absorption. Moreover, small pH reductions of the buccal saliva of the cats are also likely to negatively influence the amount of unionized molecules (Hedges et al. 2013b). In addition, the preservative 'chlorocresol' present in the buprenorphine solution used in the present study, has been linked to an aversive behaviour in cats to OTM administration (Bortolami et al. 2012). The aversive reaction makes an accurate administration more difficult and is also likely to result in drug losses (due to salivation and/or swallowing). Clearly, not only the characteristics of the drug, but also the properties of the solution should be carefully taken into account when choosing a candidate drug for OTM administration.

Notwithstanding the differences in pharmacokinetic profiles and an expected smaller therapeutic window (Chapter 3), the sedative and analgesic effects were not significantly different following IM and OTM administration at every measured time point in the pharmacodynamic study using the same protocol and the same adult cats (Chapter 2). Ideally, a higher number of cats should have been used in the experimental studies to increase statistical power. Whether the small differences in sedation were statistically different or not is prone to discussion. In general, the clinical impression was in accordance with the findings

by Santos and colleagues (2010), who described less sedative effects following OTM administration compared to IM injection of dexmedetomidine (20 µg/kg) plus buprenorphine (20 µg/kg). In the PPG kittens (Chapter 1) differences in antinociceptive effects were also not observed, but this finding can be influenced by the pre-emptive and multimodal approach for pain relief after PPG. Thereby, the analgesic effects of ketamine and an NSAID might dominate the overall analgesic effect. In the literature, studies describing the antinociceptive effect and pharmacokinetic profile of OTM and IM administered dexmedetomidine plus buprenorphine are lacking. Studies investigating the analgesic effect of dexmedetomidine and buprenorphine separately following OTM administration are available, but in particular for buprenorphine, inconsistent findings are reported. Moreover, different dosages were used and therefore, hamper direct comparison (Robertson et al. 2005; Giordano et al. 2010). Recently, Hedges and his colleagues (2013) observed slightly smaller and shorter lasting antinociceptive effects after OTM than after IV administration of buprenorphine (20 µg/kg). (Hedges et al. 2013a). A similar antinociceptive effect for dexmedetomidine (40 µg/kg) following OTM and IM administration has been reported (Slingsby et al. 2009).

From the clinical perspective with regard to the sedative effect and from the findings obtained in the pharmacokinetic study, OTM administration of the combination dexmedetomidine and buprenorphine appears to have a smaller therapeutic efficacy than the IM injection of this sedative-analgesic combination. The clinical usefulness of OTM administration of the combination of dexmedetomidine and buprenorphine in their commercially available solutions containing hydrochloride and preservatives in cats is thus questionable.

Kittens of 8 to 12 weeks of age are much smaller than the average surgical patient generally treated in small animal practices. Nevertheless, the surgical risk for neutering kittens is minimal, providing proper precautions and techniques (Aronsohn & Faggella 1993; Theran 1993; Howe 1997; Joyce & Yates 2011).

During celiotomy procedures in kittens, it is not rare to find excessive free fluid in the abdomen nor to encounter follicular cysts on the ovaries (Aronsohn & Faggella 1993; Root Kustritz 2002; Uchikura et al. 2010). Both findings are clinically irrelevant and did never necessitate changes in the treatment protocol. In female kittens, the spleen seems to be large compared to the small body size (personal observation) and to prevent spleen laceration, an ovariectomy hook should only be used to identify the right uterine horn. The left uterine horn

and ovary should then be approached via the uterine bifurcation, which is easily visible in kittens due to the more caudal ventral incision and elasticity of the tissues (Griffin & Brestle 2010; Bushby & Griffin 2011). Due to the small incision, the use of a spay hook to locate and exteriorize the uterus greatly facilitates the procedure (Griffin & Brestle 2010; Bushby & Griffin 2011).Other older reports did support the use of a spay hook to identify the uterus and ovaries (Goeree 1998b; Haughie 2001), whereas a more recent study did not due to the small and friable nature of the pediaetric tissues (Howe 2006). If no spay hook is used, the uterus can be found between the bladder and the colon by retracting the bladder (Aronsohn & Faggella 1993; Howe 2006; Joyce & Yates 2011).

The classical knot in the spermatic cord is an excellent technique for orchidectomy in kittens, and can be performed without opening the tunic (Howe 2006; Bushby & Griffin 2011). Whereas in adult tomcats the closed technique does not always allow a sufficient length of spermatic cord to be exteriorized, this limitation was not observed in any of the kittens, likely due to the relatively thin immature tunic.

In male and female kittens, all studied surgical techniques proved to be technically feasible, but the use of coagulation or vascular clips for ovarian pedicle haemostasis in female kittens and the spermatic cord knot in male kittens were associated with the shortest surgical times. The use of electrocoagulation has been described for laparoscopic ovariectomy in cats (van Nimwegen & Kirpensteijn 2007; Kim et al. 2011), but it has not been described for open ovariectomy in cats. The use of vascular clips was one of the first techniques to be promoted for PPG since they were believed to be particularly useful for the small and fragile vessels in both male and female kittens (Aronsohn & Faggella 1993). The use of vascular clips for feline gonadectomy never became popular, probably due to their higher cost compared to traditional approach with ligatures, the most conventional and most promoted technique for ovarian pedicle haemostasis in kittens (Aronsohn & Faggella 1993; Goeree 1998b; Haughie 2001; Howe 2006; Bushby 2010; Joyce & Yates 2011). The pedicle tie on the ovarian pedicle is not yet well established in the veterinary world, but the manoeuvre is identical to the creation of a knot in the spermatic cord (Griffin and Brestle 2010, Bushby and Griffin 2011, Bushby 2013).

Regarding time-cost efficacy, feline ovariectomy by coagulation seems the preferred technique in high-volume spay conditions in shelters. If no cautery device is available, the pedicle tie might be a valuable alternative. Although the latter is commonly performed in male cats suggesting that any veterinarian should also be able to easily acquire the pedicle tie

technique in female cats, the relative limited length of the ovarian pedicle and subsequent greater stretching of the tissue can result in technical complications. Hence, this technique might be less appropriate for inexperienced surgeons. Technical difficulties were observed in 2 of 50 kittens treated by the pedicle tie in the current study. If inadvertent tearing did occur, the pedicle was clamped and an encircling ligature was placed to ensure haemostasis.

Few intra- and postoperative complications were observed and all cats (PPG and TAG) recovered uneventfully with the exception of one female kitten in the first month of the project that deceased after excessive blood loss following an iatrogenic spleen laceration.

PPG was significantly faster than TAG, as previously suggested (Howe 1997), probably due to physiological differences like small and elastic gonadal vessels, and/or the limited amount of fat in the subcutis and in the ovarian pedicles (Aronsohn & Faggella 1993; Howe 1999; Bushby & Griffin 2011). In male kittens, the fact that the testes are sometimes harder to stabilize in the scrotal region in preparation for the skin incision (Howe 2006; Bushby & Griffin 2011) did not reverse the mean surgical time benefit in favour of TAG.

In conclusion, the results support the incorporation of PPG as routine procedure in shelter medicine. The findings were in favour of the intramuscular (DB-IM and MBK-IM) anaesthetic protocols, and of the use of electrocoagulation in female PPG and knot placement in male PPG. Clinically relevant differences in any of the anaesthetic or analgesic parameters between PPG and TAG were not observed. PPG was as safe as TAG, yet took less time to be performed and resulted in an identical and low postoperative complication rate.

Anaesthetic and surgical protocols should be tailored to the needs of each specific shelter setting and to the surgeon's experience with PPG.

1.2. Short- and long-term health problems in shelter cats following gonadectomy performed at an early age or at a traditional age

PPG did not evoke an increase in the mortality rate in shelter kittens between the arrival of the kittens at the Faculty of Veterinary Medicine and the end of the first week after the kittens returned to the shelter, neither did it influence the incidence of health issues as reported by the owner during the first month after adoption and during phone interviews with the owners until 24 months of age (Chapter 5).

Notably, mortality rate and health concerns in neutered (PPG) and unneutered (TAG) shelter kittens in the short-term follow-up were in both groups mainly due to infectious diseases. Similar observations were made in previous reports investigating the cause of mortality in (neutered) kittens (Howe 1997; Cave et al. 2002) or the prevalence of health problems in shelter cats one week and one month after adoption (Lord et al. 2008).

Precautions should include excellent management of stress in the shelter as well as in the neuter facility (Howe 1997). Stress induces immunodeficiency and results in vulnerability to various infectious diseases (Pesavento & Murphy 2014). In addition, kittens are more vulnerable to infectious diseases than immunized adult animals, because their response to vaccination is often unknown due to presence of maternal derived antibodies (Day et al. 2010). Infectious disease control is thus essential in shelters (Hurley 2005; Newbury et al. 2010) as well as in the neuter facility (Looney et al. 2008b). Most shelters are intensive housing units where exposure, susceptibility and transmission of infectious diseases are amplified (Bannasch & Foley 2005; Pesavento & Murphy 2014).

It cannot be concluded unambiguously whether the anaesthesia, surgery and the associated stress (Kona-Boun et al. 2005) or the stress associated with the transport, the temporary housing environment (shelter/Faculty), the manipulations and/or the adoption negatively influenced the immune system (Kona-Boun et al. 2005; Pesavento & Murphy 2014). Ideally, surgeries should be performed at the shelter facilities. In the current project, all kittens were moved to allow for standardization, irrespective their immediate or postponed gonadectomy. To better understand the impact of the environmental change, some of the cats should have been left in the shelters while littermates were transported. Such design obviously would not have been workable.

A 48-hours postoperative follow-up period of the kittens in their confident shelter environment may be advisable. In the absence of immediate postoperative complications, the kittens can then be offered for adoption. Adopters should be notified about the vulnerability of kittens to infectious diseases, the high risk environment in the shelter, and the stress associated with the adoption. This information might change the attitude or expectations of owners towards diseases/health-related problems in shelter cats post adoption. It is not rare that animals adopted from animal shelters have mild temporary disease. Mostly, these health problems resolve within one month after adoption (Lord et al. 2008). Veterinarians should use the latter information to reassure people who adopted an animal with mild health issues

(Scarlett et al. 2002), in particular since it is established that disease in the immediate post-adoption period is correlated with animal relinquishment (Wells & Hepper 1999).

Clearly, successfully performing PPG also requires optimal shelter management. Also, it should be remembered that gonadectomy is an elective procedure, it should not be performed in kittens that cannot safely undergo the procedure (Aronsohn & Faggella 1993; Looney et al. 2008b; Root Kustritz 2013).

It is a general concern that PPG might induce several health-related conditions later in life. In the long-term follow-up (6 to 24 months of age), PPG did not affect the incidence of the medical outcomes of interest (Chapter 5). These results are in accordance with the findings of experimental (Root et al. 1996a; Stubbs et al. 1996; Root et al. 1997) and previous clinical long-term studies (Howe et al. 2000; Spain et al. 2004). It should be noted that the occurrence of feline lower urinary tract disease (FLUTD), urethral obstruction, fractures or hypersensitivity skin disorders was rare in the present study ($\leq 13/614$ cats [$\leq 2.1\%$] for each condition). Therefore, the statistical power for detecting significant differences between PPG and TAG cats was rather low.

Whereas PPG compared to TAG did not increase the number of cats with health problems until 24 months of age (Chapter 5), there are indications that PPG is associated with the (earlier) risk on development of overweight. Differences in circulating leptin were observed between gonadectomised and intact cats aged 6 to 8 months (Chapter 6). The increased concentration of plasma leptin in PPG cats is most likely a result of the increased amount of body fat, as described in the literature following gonadectomy in adult cats (Martin et al. 2001; Hoenig & Ferguson 2002; Martin et al. 2006; Belsito et al. 2009). Nevertheless, only female PPG cats were also heavier and more likely to be overweight compared to female unneutered cats (TAG). In male cats, body fat potentially increased after PPG, yet without resulting in significant differences in body weight or in the proportion of overweight cats among male PPG and TAG cats. It is known that gonadectomised cats are at greater risk to become overweight (Robertson 1999; Allan et al. 2000; Courcier et al. 2010; Cave et al. 2012; Courcier et al. 2012). The risk is possibly influenced by age at gonadectomy which has previously been suggested for female kittens (Alexander et al. 2011). Further long-term research, comparing PPG and TAG, should investigate whether the risk to become overweight at a later stage is indeed influenced by the age at gonadectomy or solely by the gonadectomy itself. Ideally, not only BW, BCS and plasma leptin concentration should be

measured, but also body composition (lean tissue, fat tissue, bone mineral) by dual-energy x-ray absorptiometry should be determined (Speakman et al. 2001).

It should be remembered that prevention and early recognition of overweight in cats is essential to increase both quality and quantity of life (Courcier et al. 2010; Zoran 2010). Obesity is correlated with a number of diseases like FLUTD (Willeberg & Priester 1976; Lekcharoensuk et al. 2001; Gunn-Moore 2003; Lund et al. 2005), and lameness and/or fractures (Scarlett & Donoghue 1998; Craig 2001; McNicholas et al. 2002). Veterinarians should warn cat owners of these effects at time of (early age) neutering, and advise energy restrictions (Russell et al. 2000; Hoenig et al. 2003; Colliard et al. 2009; Alexander et al. 2011).

1.3. Behavioural development in shelter cats following gonadectomy performed at an early age or at a traditional age

The main goal of this part of the project was to detect differences in the occurrence of potentially undesirable behaviour during the first month and the evolution of the occurrence of (potentially) undesirable behaviours over time among PPG and TAG cats. A differentiation was made between potentially undesirable and undesirable behaviour. The term potentially undesirable behaviour was used to describe behaviour that was reported by the owner and that might be potentially become troublesome to the owner (Blackwell et al. 2008). The term undesirable behaviour indicates behaviour that is reported by the owner to be truly troublesome or problematic, and thus, considered to be unacceptable for the owner (Blackwell et al. 2008; Amat et al. 2009). The term behaviour problem was avoided since the reported behaviour could not be confirmed by means of an assessment by a behaviourist.

The current findings are for the most part in agreement with previous short- and long-term follow-up studies following PPG in cats (Stubbs et al. 1996; Howe et al. 2000; Spain et al. 2004; Wright & Amoss 2004). Age at time of gonadectomy (PPG and TAG) did not affect the mean number of potentially undesirable behaviour. Similarly, the longitudinal study did not reveal an effect of age at time of gonadectomy (PPG and TAG) on the evolution over time (i.e. 2 until 24 months after adoption) of (potentially) undesirable behaviour.

In the short- as well as the long-term survey, commonly reported potentially undesirable behaviours (e.g. predatory and sexual behaviour, scratching, …) belong to the normal behavioural repertoire of the cat (Landsberg 1996; Heath 2007; Radosta 2011). For example,

in kittens, social play peaks around the third and fourth month of life, and serves to develop and maintain social relations among littermates and acquire social communication skills (West 1974). This type of play involves wrestling, biting and scratching. Kittens need to learn from their playmates, including humans, when their play is too rough. This is probably why a commonly reported behaviour during the first month after adoption was aggression to people during play, a result that is congruent with a study by Lord et al. (2008). Kittens also have a lively and inquisitive nature and a strong predisposition for exploring and exhibiting scratching, chewing and play behaviour (Lord et al. 2008; Seksel 2008), all of which may result in destruction of targeted objects. In the present study, destructive behaviour was indeed commonly reported.

Although the data indicated no effect of PPG (short-term) or age at gonadectomy (long-term) on behaviour, the occurrence of certain (potentially) undesirable behaviours under study (i.e. inappropriate elimination, (non) play-related aggression towards people or animals, destruction and fearful behaviour) were found to be associated with a number of social and environmental factors, the most common being use of punishment by owner, reaction as kitten to a stranger while at the Faculty and housing a single cat.

The finding that punishment was associated with an increase in (potentially) undesirable behaviours is consistent with results in behavioural studies in dogs (Hiby et al. 2004; Blackwell et al. 2008) and cats (Curtis 2008). Although it is impossible to determine causality for any factor other than time at age of gonadectomy (because kittens were only randomly assigned to PPG or TAG groups), the association is indicative of at least some kind of relationship, unidirectional or bidirectional, between punishment and the investigated behaviours.

The response of a kitten to being approached and touched by a stranger partly reflects the socialization status of the kitten. Kittens reacting shy or frightened to a stranger were more likely to display non play-related aggression to humans as kittens, and fearful behaviour in adulthood, whereas a frightened response to a stranger as a kitten was also associated with an increase in inappropriate elimination as an adult. It is known that kittens with a lack of human contact and exposure to environmental stimuli during their socialization period have an increased risk to display fear and stress-related behaviours later on in life (McCune 1995; Overall et al. 2005; Casey & Bradshaw 2008).

Being the only cat in the household was also associated with an increase in play-related aggression towards people and a decrease in fearful behaviour to noises and movements

(short-term; potentially undesirable), and a decrease in destruction (long-term; undesirable). Adding another cat to the household may help prevent boredom (Kendall & Ley 2006) and provide an outlet for a cat's active nature and a substrate for normal and appropriate feline play (Beaver 2004; Overall et al. 2005). However, particularly adult cats do not readily accept other cats as a member of their social group, as evidenced by a greater risk for non play-related aggression in multicat households in the present study. In addition, inappropriate elimination occurred more often in households where the number of litter boxes was smaller compared to the number of cats present, which may be indicative of social stress. If another cat is added to a household with one or more cats, the new cat as well as the resident cats should be sociable (i.e. contact with conspecifics during the socialization period to develop social skills) (Mertens & Schär 1986). In addition, introducing a new cat to another cat or an established group of cats, should be done gradually (Overall et al. 2005) and even then there is no guarantee the new animal will become part of a social group in which members display typical affiliative reactions to each other. In literature it is in fact advised to place two kittens together (if possible, littermates) which are still flexible and easily to adjust to each other (Mertens & Schär 1986).

The number and diversity of significant social and environmental factors with an association with (potentially) undesirable behaviour, identified during short-term and long-term follow-up in the study, confirm that behaviour problems can be multifactorial in origin and that prevention is, here too, better than cure (Hunthausen & Seksel 2002; Scarlett et al. 2002).

Most important, cats need to be socialized in order to be able to identify and communicate with conspecifics, and adapt interaction with other animals and humans (Seksel 2008). Next, cats require an environment that allows them to perform their natural behaviour, particularly behaviours for which high internal motivation exists (a.k.a. "behavioural needs"). Indoor cats, depending entirely on the owner for this, are very vulnerable to experiencing stress and, consequently, to developing problem behaviour if these needs are not met (Crowell-Davis et al. 2004; Seksel 2008).

As owners are largely responsible for the well-being of their pet cats, they should have a good grasp of feline behaviour, including their social behaviour, and social and physical needs (Landsberg 1996; Rochlitz 1999; Jongman 2007). As a result, owner education is essential to prevent unrealistic expectations, the occurrence of unwanted behaviour (Overall et al. 2005; Seksel 2008; Ellis 2009) and to ensure successful adoption (Patronek et al. 1996; Neidhart & Boyd 2002; Wright & Amoss 2004; Shore 2005). The role of the breeder, the

shelter staff and the veterinarian are very important in this respect. In addition, following adoption, veterinarians should routinely ask owners about their animal's behaviour - and any changes therein - during health check-ups to prevent or at least allow early detection and timely management of behaviour problems. If needed, they should refer owners to a behavioural specialist (Patronek & Dodman 1999; Scarlett et al. 2002; Overall et al. 2005; Fatjo et al. 2006; Lord et al. 2008; Seibert & Landsberg 2008; Seksel 2008; Roshier & McBride 2013a).

In summary, the results from Chapters 5 and 7 provide no evidence that PPG increases the risk for health issues until 24 months of age, nor for (potentially) undesirable behaviour until 24 months after adoption. Other social and environmental factors have a greater impact on the ontogeny of (potentially) undesirable behaviour in cats. The findings in Chapter 6 revealed that the risk to develop already overweight at 6 to 8 months of age is likely to be influenced by gonadectomy in kittens. Following (early age) gonadectomy, energy restriction is crucial to prevent excessive weight gain (Martin et al. 2001; Hoenig et al. 2003; Reichler 2009; Alexander et al. 2011). Further long-term research comparing the prevalence of obesity between PPG and TAG cats at an older age should confirm this.

Prevention is better than cure, with regards to overweight and obesity (Zoran 2010) as well as to behavioural problems (Hunthausen & Seksel 2002; Scarlett et al. 2002). Veterinarians should routinely inquire owners about the pet's behaviour at every veterinary visit to prevent and manage behaviour problems, even if it is by referring owners to a behaviourist (Patronek & Dodman 1999; Scarlett et al. 2002; Overall et al. 2005; Seksel 2008; Roshier & McBride 2013b).

2. Limitations and reflections

During the first study year (May 2010 to April 2011), the originally designed anaesthetic and surgical protocols for PPG were gradually adjusted according to the accumulated experience with PPG. This included the gradually increasing dosages of dexmedetomidine and ketamine, due to the observation of inadequate planes of anaesthesia during gonadectomy, and the use of a spay-hook only for lifting the right uterine body due to inadvertent spleen damage leading to one fatal event. In addition, the long postoperative follow-up period (hospitalisation for 5 days, 2 days postoperative follow-up) at the Faculty was reduced to six hours, as there were limited anaesthetic and surgical complications while the stress experienced by the kittens and caused by environmental changes was deemed to increase the likelihood to develop anorexia and upper respiratory tract diseases during hospitalization.

Due to the adaptations made to the anaesthetic and surgical techniques, and in the environmental conditions in the first study year, data of these kittens were not included in the studies described in the Chapters 1 and 4. Before the start of the actual study periods, the surgeon therefore gained already quite some experience in treating kittens. For this reason, not all data (e.g. surgical time, complications) will be completely valid for novice surgeons without any experience with (early age) neutering (Chapter 4). Moreover, by gaining surgical experience and subsequently shortening surgical time (Chapter 4), slightly lower dosages of dexmedetomidine and ketamine might have been sufficient towards the end of the project (Chapter 1).

The pharmacodynamic and –kinetic studies to compare the OTM and IM administration of a sedative-analgesic combination should ideally have been performed in kittens and not in adults, as was the case (Chapters 2 and 3). Yet blood sampling at different time points (e.g. 1, 5 and 10 minutes after administration) was impossible due to the small-size of the kittens and the surgical intervention.

Using a greater sample size in the experimental studies may have increased the reliability of the results, especially in the pharmacodynamic study. Choice of sample size was based on practical and ethical considerations, as well as the numbers of cats used in previous similar pharmacodynamic and -kinetic studies (Selmi et al. 2003; Robertson et al. 2005; Johnson et al. 2007; Hedges et al. 2013a). Results from the pharmacokinetic and -dynamic studies (Chapters 2 and 3) in adult cats can of course not be completely extrapolated to kittens, but

are at most suggestive for underlying trends in the pharmacodynamic and -kinetic profiles of those drugs in kittens.

The size of the participating shelters might have caused a bias in the sample of kittens and cat owners. Firstly, in smaller and usually private-owned shelters (often only housing cats and/or working with foster families) the infectious disease control might be better manageable due to the less intensive housing of animals, the potential decrease in animal stress and the lower turn-over of animals compared to large and public shelters (often housing both cats and dogs) (Pesavento & Murphy 2014). These smaller shelters might also have screened and, as such, selected a particular type of adopters whereas larger animal shelters generally do not do this. Secondly, before deciding on adopting a kitten, owners were informed about the project and the efforts required of them when adopting a kitten included in the study. Therefore, some owners may have been discouraged while others might have been more committed instead. It is likely that being thoroughly informed has contributed to the good response rate of phone interviews (Chapter 5), the health check-up (Chapter 6) and the behavioural surveys (Chapter 7). Unfortunately, it was only possible to organize a health check-up at 6 to 8 months of age of the cats, when TAG cats were still sexually intact (Chapter 6).

Despite the good response rate and the fact that a large sample size was recruited at the beginning of the study, the number of available cats decreased towards the last data collection points during the long-term follow-up (Chapters 5 and 7). This decrease was to be expected as there would be a drop-out of a certain number of respondents (and thus cats) over the course of 24 months. Also, the closing date of the project did not permit a 24-month post adoption follow-up of the kittens recruited in the last study year.

The follow-up period of 24 months, although longer than in most other studies, may not have been sufficient for all parameters, as it does not reflect the lifespan of cats. The time period studied in this project did permit an evaluation of health and behaviour for kittens into the early stages of adulthood (Chapter 5). On the other hand, it should be mentioned, that especially for rare medical conditions like FLUTD with an increased risk between 2 and 6 to 7 years of age (Willeberg & Priester 1976; Lekcharoensuk et al. 2001), a longer follow-up period is needed. Further long-term research, comparing the incidence of FLUTD and obesity between PPG and TAG, is warranted.

Health issues as well as (potentially) undesirable behaviour were reported by owners (Chapters 5 and 7), as previously used in other (behavioural) studies (Howe et al. 2000; Spain et al. 2004; Wright & Amoss 2004; Kendall & Ley 2006; Kendall & Ley 2008; Ramos & Mills 2009). This methodology, however, may not give a true representation of what a behaviour specialist would consider to be behaviour problems This approach, on the other hand, is useful to evaluate the indirect impact on animal welfare since feline behaviour that is perceived by the owner as 'undesirable' or 'unacceptable' can affect human-animal bond (Overall et al. 2005) and thus, increase the risk of relinquishment (Patronek et al. 1996; Salman et al. 1998; Casey et al. 2009). Nevertheless, compared to other long-term studies (Howe et al. 2000; Spain et al. 2004), the validity of the obtained responses is stronger since assessments occurred at different time point with recall periods of maximum 6 months. A single person was responsible for all data collection during the health-check up and phone interviews (Chapters 5 and 6), and if necessary, veterinary records were acquired for health concerns (e.g. FLUTD).

Despite the high prevalence of (early age) gonadectomy in veterinary practices, there is a paucity of research on the effect of age at time of gonadectomy on the physical and behavioural development in cats. The potential to change the timing of gonadectomy will, however, affect the daily practice of many veterinary practitioners and has wide ranging implications on the general cat population and animal welfare. With this comparative study and further related investigations on PPG, the traditional attitudes and the worldwide perception of PPG in cats should be further challenged.

3. Perspectives

Based on the results of this PhD thesis and the current literature, there is no evidence to expect any clinically adverse effects associated with PPG in shelter cats. Within the context of shelter policies, PPG can be recommended for cats. To ensure that PPG would be incorporated as routine procedure in shelters and to change the mentality of the lay and veterinary public about the age at time of gonadectomy in shelter cats, the Federal Public Service should secure the widest possible support regarding financial aspects and scientifically documented information about PPG for all parties involved. Moreover, anaeshetic and surgical protocols to perform PPG as well as the effect of PPG on health and behaviour should be incorporated in the curriculum of the veterinary students.

For client-owned cats, on the other hand, it has been suggested to perform the surgery at 4 to 6 months of age, when cats are fully vaccinated, and yet, before they reach sexual maturity (Goeree 1998a; KNMvD 2009; The Cat Group 2011; Joyce & Yates 2011; Sparkes et al. 2013).

Alongside the strong scientific evidence to support early age neutering of shelter cats, most staff members and veterinarians of the participating shelters were convinced to proceed with PPG in their shelter (data from a survey conducted among the participating shelters towards the end of the research project, not shown). However, they were concerned about the financial feasibility to neuter all shelter cats before adoption, especially since 'no cost' kittens are still available. Respondents wanted cat owners as well as local authorities to take their responsibility to reduce the number of stray and unwanted cats. Furthermore, shelter veterinarians were in favour of developing a policy in the veterinary community regarding mandatory neutering of all shelter cats to avoid conflicts about income amongst veterinarians.

Maybe non-surgical approaches for permanent contraception in cats might become the more practical and more cost-effective method of population control than surgical castration in the future.

A final comment is reserved for feline welfare that will not improve just by neutering cats. Educating owners to improve their knowledge of feline reproduction and behaviour (including basic husbandry and environmental enrichment) remains top priority for a permanent reduction in the number of relinquishments.

Summary

In Belgium, prepubertal gonadectomy (PPG), defined as gonadectomy well before sexual maturity, is not yet routinely performed in cats. Notwithstanding a number of experimental and clinical studies in the United States of America and the United Kingdom about early age neutering, timing of gonadectomy remains controversial. The objections from veterinarians raised against PPG pertain to animal health and welfare and can be summarized as concerns regarding technical challenges, issues of safety during anaesthesia and surgery, and possible short- and long-term complications on health and behaviour.

To better understand the feasibility of PPG and to document short- and long-term effects on health and well-being of cats undergoing early age neutering, the Federal Public Service Health, Food Chain Safety and Environment funded a large-scale project in shelter cats.

For PPG to be fully accepted by the lay and veterinary community, the anaesthetic and surgical procedures should be as safe and efficient as those used for gonadectomy at traditional age (TAG). Moreover, health and behaviour issues should not be more common among PPG cats compared to TAG cats.

The aims of this doctoral dissertation were three-fold: (1) to describe safe and efficient anaesthetic and surgical protocols to perform PPG and to compare those with TAG, (2) to investigate the relationship between age at gonadectomy and short- and long-term health issues in adopted shelter kittens (until 24 months of age), and (3) to assess short- and long-term effects of PPG on the behaviour in shelter cats (until 24 months after adoption).

In this study, 800 shelter kittens were recruited and randomly assigned to two treatment groups using unequal group sizes: 2/3 PPG, between 8 to 12 weeks of age (0.7 to 1.4 kg), and 1/3 TAG, between 6 to 8 months of age.

1. Anaesthetic and surgical protocols to perform prepubertal gonadectomy and comparison with those for traditional age gonadectomy

In the first part of the thesis (Chapter 1), the anaesthetic and analgesic efficacy of three different protocols were studied in kittens (PPG), whereas one protocol was applied in the young adult cats (TAG). The three different injectable protocols for PPG that were compared: (1) an intramuscular (IM) administration of 60 µg/kg dexmedetomidine (D) combined plus 20 µg/kg buprenorphine (B) followed by an IM injection of the anaesthetic agent (20 mg/kg ketamine) (DB-IM), (2) an oral transmucosal (OTM) administration of 80 µg/kg dexmedetomidine plus 20 µg/kg buprenorphine followed by an IM injection of 20 mg/kg ketamine combined with a small dosage of dexmedetomidine (20 µg/kg) (DB-OTM), and (3) an IM injection of a 40 µg/kg medetomidine - 20 µg/kg buprenorphine - 20 mg/kg ketamine combination (MBK-IM). All kittens received additionally a non-steroidal anti-inflammatory drug (DB-IM and DB-OTM: carprofen, MBK-IM: meloxicam). For TAG, only the DB-IM protocol was used, with the same products as in the DB-IM protocol for PPG, but other dosages for dexmedetomidine (40 µg/kg) and ketamine (5 mg/kg). Interestingly, the dosages of dexmedetomidine and ketamine per kg of body weight, required to obtain an adequate plane of anaesthesia, were higher for PPG than for TAG.

The efficacy of each protocol was studied by assessing the sedative effect (numerical rating scale [NRS]), the analgesic effect (dynamic interactive visual analogue scale [DIVAS], a multidimensional pain scale and mechanical threshold testing), the adverse effects (salivation, excitation and vomiting) and the physiologic effects (respiratory rate, heart rate, rectal temperature and peripheral arterial oxygen saturation) until 6 hours postoperatively.

All three studied anaesthetic protocols used in PPG kittens proved to be safe. Regarding the quality of the anaesthesia, some significant differences were observed among the different protocols. Less sedative properties and more adverse reactions were observed after OTM administration (DB-OTM) compared to IM administration (DB-IM) of the premedication. The MBK-IM protocol was associated with the fewest adverse reactions. In general, all protocols provided an adequate plane of anaesthesia to perform the gonadectomy in both sexes. In the recovery period, the sedative effect lasted significantly longer in the DB-OTM group, resulting in longer recovery times. Additionally, more postoperative adverse reactions were observed in the DB-OTM group compared to the DB-IM and the MBK-IM group. Mean pain scores in male and female kittens remained low at 3 and 6 hours postoperatively following all three protocols, suggesting a mild or moderate postoperative pain following

gonadectomy and adequate pain relief. From a clinical perspective, the DB-OTM protocol could not be withheld as a suitable route for premedicating kittens and one of the intramuscular administered protocols are preferential.

Uneventful recovery from anaesthesia was also observed in all TAG cats. Differences between PPG and TAG were few. More PPG cats vomited after premedication compared to TAG cats, however, significance was not reached. Some statistically significant results were clinically less relevant (e.g. DIVAS score of 17 and 9 in female PPG and TAG cats, respectively, or DIVAS score of 2 and 6 in male PPG and TAG cats, respectively).

Although all three anaesthetic protocols appear to be safe in the present study, with a good surgical plane of anaesthesia and an analgesic effect for at least 6 hours postoperatively, the DB-IM and MBK-IM protocols are promoted. In the light of shelter medicine, the MBK-IM protocol (medetomidine 40 µg/kg plus buprenorphine 20 µg/kg plus ketamine 20 mg/kg) will be the most time and cost efficient protocol providing good sedative and anaesthetic effects, a reasonable duration of anaesthesia and/or good analgesic properties.

To circumstantiate the clinical interpretation of the sedative and analgesic efficacy of the OTM and IM administration of the sedative-analgesic combination of dexmedetomidine combined with buprenorphine, a pharmacodynamic (Chapter 2) and a pharmacokinetic (Chapter 3) study were performed in six healthy adult cats.

The pharmacodynamic study (Chapter 2) was designed as a randomized blinded cross-over study with 1 month wash-out between both treatments (OTM or IM administration of the combination dexmedetomidine and buprenorphine). In the latter study three persons evaluated the sedative and analgesic effects using a NRS and DIVAS, respectively, at different time points until 6 hours after administration. In addition, a mechanical threshold device was used to measure antinociception. Adverse reactions (vomiting, salivation), resistance to OTM administration and physiological effects (heart rate, respiratory rate and rectal temperature) were also recorded.

Resistance to OTM administration was observed, as well as vomiting and salivation shortly after administration. No differences in sedative and antinociceptive effects after IM versus OTM administration were found at any of the time points. Both treatments caused comparable changes in sedation score, DIVAS and mechanical threshold values. These experimental results suggested that OTM administration might be an alternative to IM injection to administer this sedative-analgesic combination in (adult) cats.

In a second randomized cross-over study with 1 month wash-out, using the same 6 adult cats, blood samples were collected at different time-points until 24 hours after OTM and IM administration of dexmedetomidine combined with buprenorphine (Chapter 3). Plasma concentrations of both drugs were determined using a high-performance liquid chromatography method with tandem mass spectrometry and pharmacokinetic analysis was performed. Following pharmacokinetic parameters were obtained: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), absorption rate constant (K_a), elimination rate constant (K_e), absorption half-life ($t_{1/2a}$), elimination half-life ($t_{1/2e}$) and mean residence time (MRT). For dexmedetomidine as well as for buprenorphine, the AUC and C_{max} were significantly smaller following OTM compared to IM administration of dexmedetomidine combined with buprenorphine. For buprenorphine, a longer T_{max}, and a shorter $t_{1/2a}$, $t_{1/2e}$ and MRT after OTM administration compared to the IM injection of dexmedetomidine plus buprenorphine were also found.

Pharmacokinetic profiles of dexmedetomidine and buprenorphine following OTM and IM administration of dexmedetomidine combined with buprenorphine indicated a limited absorption of both drugs following OTM administration compared to IM injection (Chapter 3). Consequently, a smaller therapeutic efficacy following OTM administration compared to IM injection of this sedative-analgesic combination would be expected, although that was not reflected in the pharmacodynamic study (Chapter 2). This apparent discrepancy can possibly be explained by a lack of power to demonstrate differences in sedative and analgesic clinical effects because of the limited sample size in the experimental study. After all, in the large-scale study in kittens (Chapter 1) less sedation was observed following OTM administration than after IM injection when using a dexmedetomidine-buprenorphine combination as premedication.

In Chapter 4, the feasibility, surgical time and complications associated with different surgical techniques in PPG kittens were observed and compared. A single technique for male and female TAG cats was used to perform TAG and surgical time and complications were compared to those of the similar technique in PPG kittens.

Female PPG kittens were randomly assigned to one of 4 surgical techniques for prepubertal ovariectomy: placement of ligatures, application of vascular clips, use of a bipolar forceps, or creation of a knot in the ovarian pedicle. A limited ventral celiotomy was performed more caudally than for TAG cats and the ovariectomy hook was exclusively used to identify the

right uterine horn. Male PPG kittens were randomly assigned to 2 groups of prepubertal orchidectomy: creation of a knot by a single throw in the spermatic cord or placement of a single ligature around the spermatic cord, both closed. In TAG cats, the traditional approaches and techniques (ligatures in females, knot in males) were used.

Few intra- and postoperative complications in PPG as well as TAG cats were encountered during the study period. All studied surgical techniques provided easy and reliable haemostasis in kittens and could be withheld for early age gonadectomy, although some required less time than others. In female kittens, the use of coagulation and clips were the fastest techniques, but also more expensive compared to the conventional method using ligatures. The pedicle tie can be an alternative since it is faster than placing ligatures and does not comprise an additional cost. On the other hand, 4 of 7 of the observed intra-operative complications were encountered in the pedicle tie group. Although this difference did not reach statistical significance, the pedicle tie might be less appropriate for inexperienced surgeons. In male PPG kittens, a closed castration with knot placement was the fastest and obviously cheapest technique for gonadectomy. For both sexes, performing gonadectomy in kittens was faster than in young adult cats. Few intra- and postoperative complications were observed without differences between both groups.

In conclusion (Chapters 1-4), PPG can be performed safe and efficient in cats. The MBK-IM protocol appears to be the most appropriate anaesthetic protocol and the coagulation technique in female kittens or the spermatic cord knot in male kittens should be preferred as the most suitable surgical techniques for high volume neuter within a short period of time.

2. Short- and long-term health problems in shelter cats following gonadectomy performed at an early age or at a traditional age

Many veterinarians do not support PPG due to concerns about increased short- and long-term risks on the development of medical conditions (i.e. outbreak of infectious diseases following surgery, and feline lower urinary tract disease (FLUTD; particularly urethral obstruction), lameness, fractures and obesity at long-term). These medical concerns have been addressed in Chapters 5 and 6.

In Chapter 5, mortality rate between the arrival of the kittens at the Faculty of Veterinary Medicine and the end of the first week after the kitten returned to the shelter, and the occurrence of miscellaneous health problems during the first month after adoption were compared between PPG (neutered) and TAG (sexually intact at those time points) cats as short-term follow-up. In the long-term follow-up (i.e. until 24 months of age), the incidence of FLUTD, urethral obstruction, lameness, fractures and hypersensitivity skin disorders were compared among PPG and TAG cats.

During short-term follow-up, mortality rate and the occurrence of miscellaneous health issues were not significantly different among kittens that were neutered during their stay at the Faculty (PPG) and those that were only housed at the Faculty (TAG). Most kittens deceased from infectious diseases (mainly panleukopenia). Upper respiratory tract diseases were the most commonly reported reason to seek veterinary assistance during the first month after adoption.

During the long-term follow-up, no significant differences were detected in the incidence of FLUTD, urethral obstructions, lameness, fractures and hypersensitivity skin disorders between PPG and TAG cats. Most medical conditions were relatively rare ($\leq$ 13/614 cats [$\leq$ 2.1%] for each condition). Consequently, the statistical power might have been too low to detect significant differences.

In Chapter 6, obesity-related results (body weight, body condition score and plasma leptin concentration) collected during health check-up at 6 to 8 months of age were described. At that time-point, TAG cats were still sexually intact.

In female cats, body weight and leptin concentrations were significantly higher in the PPG compared to the TAG group. Furthermore, a greater proportion of female PPG cats were considered overweight compared to female TAG cats. In male cats, only leptin concentrations were significantly higher in the PPG than in the TAG group.

These results indicate that plasma leptin concentrations in cats at 6 to 8 months of age were higher in neutered (PPG) cats compared to sexually intact cats. This might be the result of an increased amount of body fat. In male PPG cats, the potential increase in body fat mass did not (yet) result in a higher body weight than in sexually intact cats. Further long-term research, comparing PPG and TAG, should investigate whether the risk to become overweight at a later stage is indeed influenced by the age at gonadectomy or solely by the gonadectomy itself.

The short-term results showed that the mortality rate and the occurrence of health issues were comparable in PPG and TAG shelter kittens. However, it cannot be precluded that the stress associated with the transport and environmental changes did diminish the immune response to infectious diseases in kittens. The long-term follow-up revealed that PPG was not associated with an increase in risk for the investigated health problems until 24 months of age. PPG might lead to overweight in cats, but further long-term research comparing PPG and TAG is necessary to confirm whether age at gonadecomy or gonadectomy itself is responsible for the increased risk of becoming overweight.

3. Behavioural development in shelter cats following gonadectomy performed at an early age or at a traditional age

The goal of Chapter 7 was to evaluate whether the age at time of gonadectomy affected the behavioural development and the occurrence of (potentially) undesirable behaviour, taking into account social and environmental factors pertaining to the living conditions of the cats. Since PPG is performed during a sensitive period for kitten behavioural development (socialization period), concerns exist that PPG would lead to more behaviour problems than gonadectomy at a later age.

A short- and long-term behavioural follow-up were conducted by means of a 30-day diary immediately after adoption, and surveys at 2, 6, 12, 18 and 24 months after adoption. Over the course of 24 months, owners received email invitations to complete surveys on a dedicated web-based survey platform. The collected behavioural data represented an indirect measure for adopted shelter cat well-being, as behavioural problems are commonly cited as a reason for removing a cat from the household. Consequently, in this study, potentially undesirable behaviour was used to describe behaviour that might be potentially troublesome to the owner, whereas undesirable behaviour refers to behaviour indicated by the owner as truly troublesome or thus, unacceptable.

Results indicated that the mean number of potentially undesirable behaviour per day during the first month after adoption did not differ significantly between neutered (PPG) and unneutered (TAG) cats. Furthermore, no significant difference was found between PPG and TAG cats in the evolution of (potentially) undesirable behaviour over time (2, 6, 12, 18 and 24 months post adoption). The greatest number of potentially undesirable behaviours was

observed during the first months after adoption. Play-related aggressive behaviour towards people, destruction, suckling and fearful behaviour to noises and/or movements were most commonly reported. During the long-term follow-up, the most commonly reported potentially undesirable behaviours were hunting, destruction, sexual behaviour, fearful behaviour and attention seeking. Common undesirable behaviours were destruction, stealing food and (non) play-related aggression to people.

Unlike age at time of gonadectomy, other factors (social and environmental) such as the use of punishment by the owner, having no other cats in the household, the occurrence of positive interaction between the cat and the family, … were found to be significantly associated with a selection of (potentially) undesirable behaviours. This selection was based on behaviour problems that are commonly reported in literature: inappropriate elimination, fearful behaviour, (non) play-related aggression towards people or animals and destruction. It is important to note that the analysis of the social and environmental factors revealed associations, but not causal relationships.

The main conclusion regarding behaviour development was that the results did not show that age at time of gonadectomy affects the mean number of (potentially) undesirable behaviour in shelter cats during 24 months after adoption. However, factors pertaining to the living conditions of the cats were found to have an impact on the likelihood of certain undesirable behaviours.

In this large-scale project, valuable data regarding PPG in cats on Belgian soil were collected. This study distinguishes itself from other comparable studies performed in the USA and UK by the prospective randomized study design, the use of standardized anaesthetic and surgical protocols and the collection of data at different time-points and by a single investigator.

No scientific counter indications for PPG were found, not only during the short-term follow-up, but also during the long-term follow up (24 months). Within the context of shelter policies, PPG can be recommended for cats. To ensure that PPG would be incorporated as a routine procedure in shelters and that PPG would be generally accepted by the lay and veterinary public, the Federal Public Service should secure the widest possible support regarding financial aspects and scientifically documented information about PPG for all parties involved. To further improve feline welfare, particular attention should be paid to the

education of owners about feline reproduction (and the need to neuter) and feline behaviour, including basic husbandry requirements and environmental enrichment to prevent undesirable behaviour and animal relinquishment.

Samenvatting

Samenvatting

In België wordt vroegtijdige castratie, zijnde gonadectomie bij zowel mannelijke als vrouwelijke katten ruim voor de leeftijd van seksuele maturiteit, niet routinematig toegepast. Hoewel er reeds verschillende publicaties over vroegtijdige castratie verschenen zijn in de Verenigde Staten van Amerika en het Verenigd Koninkrijk, blijft het tijdstip van castratie een controversieel onderwerp. Dierenartsen hebben bezwaren tegen vroegtijdige castratie met betrekking tot de gezondheid en het welzijn van de dieren, wegens hun bezorgdheid over de veiligheid en technische haalbaarheid van de anesthesie en chirurgie, en over de mogelijke korte- en langetermijneffecten op de gezondheid en het gedrag.

Om de technische haalbaarheid van vroegcastratie bij kittens te bestuderen en tevens het effect van vroegcastratie op de gezondheid en het welzijn van de katten na te gaan, heeft de Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu een grootschalige studie met asielkatten gefinancierd.

Opdat vroegtijdige castratie bij katten geaccepteerd zou worden bij katteneigenaars en dierenartsen, moet de anesthesie en chirurgie even veilig en efficiënt verlopen als bij castratie op latere leeftijd en mogen gezondheidsproblemen en ongewenste gedragingen niet frequenter voorkomen bij katten die vroegtijdig gecastreerd werden dan bij katten die op de traditionele leeftijd gecastreerd werden.

De doelstellingen van dit doctoraatsproefschrift waren dan ook drievoudig: (1) anesthesie en chirurgie protocollen voor vroegtijdige castratie van katten beschrijven en deze vergelijken met castratie op jongvolwassen leeftijd, (2) de relatie weergeven tussen de leeftijd op het tijdstip van castratie en het voorkomen van gezondheidsproblemen op korte- en lange termijn bij asielkatten (tot 24 maanden leeftijd) beschrijven, en (3) de gedragsontwikkeling tot 24 maanden na adoptie te bestuderen bij asielkittens die ofwel vroegtijdig ofwel op traditionele leeftijd gecastreerd zijn.

Voor het onderzoek werden 800 asielkittens gerekruteerd en at random ingedeeld in 2 behandelingsgroepen met een ongelijke grootte: 2/3 behoorden tot de vroegcastratie groep (VC, tussen 8 en 12 weken leeftijd [0.7-1.4 kg]) en 1/3 tot de laattijdig gecastreerde groep (LC, tussen 6 en 8 maanden leeftijd).

1. Anesthetische en chirurgische protocollen voor vroegtijdige castratie en vergelijking met de protocollen voor laattijdige castratie

In het eerste deel van de thesis (Hoofdstuk 1) werd de anesthetische en analgetische efficiëntie van drie verschillende protocollen voor VC bestudeerd en één protocol werd gebruikt in de LC groep. De drie anesthesieprotocollen voor VC die vergeleken werden, waren: (1) een intramusculaire (IM) toediening van premedicatie (60 µg/kg dexmedetomidine (D) en 20 µg/kg buprenorphine (B)) gevolgd door een IM injectie van het anaestheticum (20 mg/kg ketamine) (DB-IM), (2) een oraal transmucosale (OTM) toediening van 80 µg/kg dexmedetomidine plus 20 µg/kg buprenorphine, gevolgd door een IM injectie van 20mg/kg ketamine gecombineerd met lage dosering dexmedetomidine (20 µg/kg) (DB-OTM), (3) een IM injectie van de combinatie 40 µg/kg medetomidine - 20 µg/kg buprenorphine - 20 mg/kg ketamine (MBK-IM). Alle kittens kregen bijkomend een niet-steroïdale ontstekingsremmer subcutaan toegediend (DB-IM en DB-OTM: carprofen, MBK-IM: meloxicam). Voor castratie op jongvolwassen leeftijd (LC) werd enkel het eerste protocol (DB-IM), met dezelfde producten als het DB-IM protocol voor VC, maar andere doseringen voor dexmedetomidine (40 µg/kg) and ketamine (5 mg/kg). Er diende een duidelijk hogere dosis per kilogram lichaamsgewicht van dexmedetomidine en ketamine gehanteerd te worden in de VC dan in de LC groep om een goede anesthesiediepte te bekomen.

De efficiëntie van elk protocol werd onderzocht op basis van de beoordeling van het sedatief effect (numerieke schaal [NRS]), het analgetische effect (dynamische interactieve analoge schaal [DIVAS], multidimensionale pijn schaal en mechanical threshold test), het voorkomen van bijwerkingen (braken, speekselen, excitatie) en het opvolgen van fysiologische parameters (hart- en ademhalingsfrequentie, rectale temperatuur, perifere weefselsaturatie) tot 6 uur postoperatief.

De drie geteste anesthesieprotocollen voor VC bleken veilig te zijn. Wat de anesthesiekwaliteit betrof, werden enkel verschillen opgemerkt tussen de verschillende anesthesieprotocollen. Minder goede sedatieve eigenschappen en meer bijwerkingen werden opgemerkt na een OTM toediening (DB-OTM) in vergelijking met een IM injectie (DB-IM) van de premedicatie. Bij het MBK-IM protocol traden de minste nevenwerkingen op. Bij alle protocollen was de verkregen anesthesie doorgaans voldoende voor castratie van zowel mannelijke als vrouwelijke kittens. Tijdens de recovery bleek het sedatief effect langer aanwezig te zijn bij de kittens met een OTM toediening van de premedicatie (DB-OTM), wat resulteerde in langere recovery tijden. Bovendien werden meer postoperatieve bijwerkingen

gerapporteerd in de DB-OTM groep in vergelijking met de DB-IM en MBK-IM groepen. De gemiddelde pijnscores bleven op elk onderzocht tijdstip bij mannelijke en vrouwelijke kittens laag, hetgeen een milde of matige pijn na castratie en/of een afdoende analgesie suggereert. Vanuit een klinisch standpunt kan het DB-OTM protocol niet weerhouden worden als een geschikte toedieningsweg voor de premedicatie en is één van de intramusculair toegediende protocollen (DB-IM en MBK-IM) te verkiezen.

Alle LC katten ontwaakten vlot uit de anesthesie. Er werden slechts enkele verschillen waargenomen tussen de VC en LC groep. Het voorkomen van braken na het toedienen van de premedicatie werd frequenter waargenomen in de VC groep dan in de LC groep, hoewel geen statistische significantie werd bekomen. Sommige statistisch significante resultaten zijn dan klinisch minder relevant (bijv. een DIVAS score van 17 en 9 bij respectievelijk vrouwelijke VC en LC katten of een DIVAS score van 2 en 6 bij mannelijke VC en LC katten).

Hoewel alle anesthesieprotocollen veilig bleken, met een goede anesthetische werking en behoorlijke analgetische eigenschappen (tot minimum 6 uur postoperatief), promoten de huidige resultaten het gebruik van het DB-IM of MBK-IM protocol. In asielomstandigheden is een intramusculaire combinatie van medetomidine (40 µg/kg), buprenorphine (20 µg/kg) en ketamine (20 mg/kg), gevolgd door subcutane toediening van meloxicam (4 mg/kg) het meest efficiënte anesthesieprotocol (qua tijd en kosten) met een snelle sedatieve en anesthetische werking, een redelijke anesthesieduur en goede analgetische eigenschappen.

Om de klinische interpretatie van de OTM toediening van de premedicatie (dexmedetomidine gecombineerd met buprenorphine) te onderbouwen, werden een farmacodynamische (Hoofdstuk 2) en een farmacokinetische (Hoofdstuk 3) studie uitgevoerd bij 6 gezonde volwassen katten.

De farmacodynamische studie in Hoofdstuk 2 was opgesteld als een gerandomiseerde, geblindeerde cross-over studie met 1 maand tussen beide behandelingen (OTM of IM toediening van de combinatie dexmedetomidine en buprenorphine). In deze studie evalueerden 3 personen de sedatieve en analgetische eigenschappen aan de hand van respectievelijk een NRS en een DIVAS op verschillende tijdspunten tot 6 uur na de toediening. Ook bijwerkingen (braken, speekselen), weerstand bij OTM toediening, mechanical thresholds en enkele fysiologische parameters (hart- en ademhalingsfrequentie, rectale temperatuur) werden bepaald.

Weerstand bij de OTM toediening, alsook braken en salivatie kort na de toediening werden waargenomen. Een significant verschil in sedatie en/of antinociceptie werd op geen enkel van

de tijdspunten gemeten. Beide behandelingen resulteerden in gelijkaardige veranderingen in sedatiescores, DIVAS pijnscores en "mechanical threshold" waarden. Deze experimentele resultaten suggereerden dat de OTM toediening een alternatieve toedieningsmethode zou kunnen zijn om de sedatief-analgetische premedicatie toe te dienen aan (volwassen) katten.

In de tweede gerandomiseerde cross-over studie met dezelfde 6 katten en met eveneens 1 maand tussen de IM en OTM behandeling, werden op verschillende tijdspunten tot 24 uur na IM of OTM toediening van dexmedetomidine en buprenorphine bloedstalen afgenomen (Hoofdstuk 3). De plasmabepalingen van de concentraties van beide geneesmiddelen gebeurden via vloeistofchromatografie gekoppeld aan tandem massaspectrometrie en een pharmacokinetische analyse werd uitgevoerd. Volgende parameters werden berekend: oppervlakte onder de plasmaconcentratie-tijdscurve (area under the plasmaconcentration/time curve [AUC]), tijd tot maximale plasmaconcentratie (T_{max}), maximale plasmaconcentratie (C_{max}), absorptiesnelheidsconstante (K_a), eliminatiesnelheidsconstante (K_e), absorptie halfwaardetijd ($t_{1/2a}$), eliminatie halfwaardetijd ($t_{1/2e}$) en gemiddelde verblijftijd (MRT).

Voor zowel dexmedetomidine als buprenorphine waren de AUC en de maximale plasmaconcentraties significant lager na OTM toediening dan na IM injectie van dexmedetomidine gecombineerd met buprenorphine. Een langere T_{max} en een kortere $t_{1/2a}$, $t_{1/2e}$ en MRT werden eveneens waargenomen voor buprenorphine na OTM toediening dan na IM injectie van de dexmedetomidine plus buprenorphine.

De farmacokinetische profielen van dexmedetomidine en buprenorphine na IM of OTM toediening van dexmedetomidine gecombineerd met buprenorphine, toonden een verminderde opname aan van beide componenten na een OTM toediening in vergelijking met een IM injectie (Hoofdstuk 3). Er kan bijgevolg een geringere therapeutische werking verwacht worden na OTM toediening van deze sedatief-analgetische combinatie in vergelijking met de IM injectie, hoewel dit niet weerspiegeld wordt in de farmacodynamische studie (Hoofdstuk 2). Mogelijks valt dit te verklaren door een gebrek aan power om klinische verschillen in sedatieve en analgetische effecten aan te tonen, aangezien er slechts 6 katten bestudeerd werden. In de grootschalige studie met de kittens (Hoofdstuk 1) werd namelijk wel een minder goede sedatieve werking waargenomen na de OTM toediening dan na de IM injectie van een dexmedetomidine-buprenorphine combinatie als premedicatie.

In Hoofdstuk 4 werden verschillende chirurgische technieken voor vroegtijdige castratie bij kittens met elkaar vergeleken wat betreft haalbaarheid, operatieduur en het voorkomen van

complicaties. Bij de jongvolwassen katten werd per geslacht slechts één techniek gebruikt en hierbij werd de operatieduur en het voorkomen van complicaties vergeleken met deze bij de analoge techniek uitgevoerd bij kittens

Vrouwelijke kittens werden at random ingedeeld in één van de 4 groepen voor vroegtijdige ovariëctomie: het plaatsen van ligaturen, het aanbrengen van vasculaire clips, het gebruik van elektrocoagulatie of het aanleggen van een knoop in de ovariële stomp (pedicle tie). Een kleine ventrale celiotomie werd uitgevoerd waarbij de incisie meer caudaal gemaakt werd dan bij jongvolwassen katten en het steriliseerhaakje werd enkel gebruikt voor het identificeren van de rechterhoorn van de uterus. Mannelijke kittens werden at random ingedeeld in één van de 2 groepen voor vroegtijdige orchidectomie: het leggen van een knoop in de zaadstreng of het plaatsen van een ligatuur, beiden via de gesloten benadering. Bij de vrouwelijke en mannelijke jongvolwassen katten werd de castratie conventioneel uitgevoerd (ligaturen bij kattinnen, knoop bij katers).

Intra- en postoperatieve complicaties traden slechts zelden op, zowel in de VC als in de LC groep. Alle chirurgische technieken voor vroegcastratie waren haalbaar en resulteerden in een vlotte en veilige hemostase, hoewel sommige technieken sneller waren dan andere. Bij de vrouwelijke kittens bleek het gebruik van elektrocoagulatie of het plaatsen van clips gepaard te gaan met een kortere operatieduur. Uiteraard zijn beide technieken wel duurder dan klassiek ligeren. De 'pedicle tie' kan een alternatief bieden, aangezien deze sneller is dan het ligeren en geen extra kosten met zich meebrengt. Anderzijds dient hierbij wel opgemerkt te worden dat 4 van de 7 waargenomen intra-operatieve complicaties zich voordeden in de groep met de "pedicle tie" als chirurgische techniek. Ondanks het feit dat dit verschil niet significant was, kan de "pedicle tie" minder geschikt zijn voor onervaren chirurgen. Bij mannelijke kittens bleek het leggen van een knoop sneller te zijn dan het ligeren van de zaadstreng, terwijl het uiteraard de goedkoopste techniek is. Het castreren van kittens van beide geslachten verliep sneller dan een castratie op jongvolwassen leeftijd. Voor beide groepen werden weinig intra- en postoperatieve complicaties gerapporteerd en hierbij werden geen significante verschillen tussen de VC en LC groep aangetroffen.

Tot besluit (Hoofdstukken 1-4) kan gesteld worden dat een vroegtijdige castratie efficiënt en veilig uitgevoerd kan worden. Het MBK-IM protocol blijkt uit anesthetisch standpunt het meest geschikte protocol terwijl de elektrocoagulatie voor vrouwelijke kittens en de knoop in de zaadstreng voor mannelijke kittens als de meest geschikte chirurgische technieken naar

voren kunnen geschoven worden om een groot aanbod aan kittens in een korte tijdsspanne te castreren.

2. Complicaties op korte en lange termijn na vroegtijdige castratie of castratie op jongvolwassen leeftijd bij asielkatten

Eén van de frequent aangehaalde argumenten om weigerachtig te staan tegenover VC bij katten, is het vermeende negatieve effect op de gezondheidstoestand van de katten en dit niet alleen op korte maar ook op lange termijn, met name het verhoogde risico op het uitbreken van infectieuze ziekten na de operatie, op urinewegproblemen (in het bijzonder obstructies van de urethra), mankheid, fracturen en overgewicht. Hoofdstukken 5 en 6 spitsten zich hierop toe.

In Hoofdstuk 5 werden de kittensterfte tussen de aankomst van de kittens aan de Faculteit Diergeneeskunde en het einde van de eerste week nadat de kittens teruggebracht waren naar het asiel, en het voorkomen van ziektesymptomen binnen de eerste maand na adoptie als kortetermijneffecten vergeleken tussen geopereerde (VC) en niet-geopereerde (LC) kittens. In de langetermijnopvolging (tot 24 maanden leeftijd) werd de incidentie van urinewegproblemen, obstructie van de urethra, mankheid, fracturen en cutane overgevoeligheidsreacties vergeleken tussen VC en LC katten.

Er werd geen significant verschil gevonden in de kortetermijnsterfte noch in het voorkomen van ziektes gedurende de eerste maand na adoptie tussen de kittens die tijdens hun verblijf aan de Faculteit gecastreerd werden (VC) en deze die enkel aan de Faculteit verbleven (LC). Sterfte werd voornamelijk veroorzaakt door infectieuze aandoeningen (hoofdzakelijk kattenziekte). In de loop van de eerste maand na adoptie was niesziekte de hoofdreden voor de eigenaars om met hun kitten naar de dierenarts te gaan.

De resultaten van de langetermijnopvolging konden geen significant verschil aantonen in het aantal gevallen van urinewegproblemen, obstructies in de urethra, mankheid, fracturen en overgevoeligheidsreacties tussen VC en LC katten. Deze aandoeningen waren meestal zeldzaam ($\leq$ 13/614 katten [$\leq$ 2.1%] per aandoening). Bijgevolg was de statistische power waarschijnlijk te laag om significante verschillen op te sporen. Een beperking was dat niet elke kat in het project tot de leeftijd van 24 maanden kon worden opgevolgd omwille van onvermijdelijke vroegtijdige uitval en de einddatum van het project.

In Hoofdstuk 6 werden de resultaten met betrekking tot overgewicht (lichaamsgewicht, gewichtsscore en plasma concentraties van leptine), die verzameld werden tijdens het controlebezoek op 6 tot 8 maanden, weergegeven. De LC katten waren op dat moment nog seksueel intact.

Bij kattinnen waren zowel het lichaamsgewicht als de plasmaconcentraties van leptine hoger in de VC groep dan in de LC groep. Daarenboven kampte een grotere proportie VC dan LC kattinnen met overgewicht. Bij VC katers werden enkel significant hogere leptine plasmaconcentraties waargenomen dan bij de nog intacte LC katers.

Deze resultaten tonen aan dat de leptine concentraties in het plasma van katten op 6 tot 8 maanden leeftijd hoger is in gecastreerde (VC) katten dan in niet-gecastreerde katten. Dit is waarschijnlijk het gevolg van een toegenomen hoeveelheid vetweefsel bij gecastreerde katten. Bij PPG katers resulteerde deze mogelijks toegenomen vetmassa nog niet in een hoger lichaamsgewicht. Verdere langetermijnstudies die VC en LC vergelijken moeten onderzoeken of het risico op overgewicht op latere leeftijd inderdaad beïnvloed wordt door het tijdstip van castratie dan wel door het castreren zelf.

De kortetermijnresultaten toonden aan dat kittensterfte en het voorkomen van gezondheidsproblemen vergelijkbaar waren tussen VC en LC asielkittens. Er kan echter wel niet uitgesloten worden dat de stress geassocieerd met het transport en de omgevingsveranderingen de weerstand tegen infecties ondermijnde bij de kittens. Uit de langetermijnopvolging bleek dat VC niet geassocieerd was met het frequenter voorkomen van de onderzochte gezondheidsproblemen tot de leeftijd van 24 maanden. Vroegcastratie kan mogelijks leiden tot overgewicht, maar verdere onderzoeken die VC en LC vergelijken zijn nodig om te bevestigen of de leeftijd van castratie of het castreren zelf verantwoordelijk zijn voor het toegenomen risico op overgewicht.

3. Korte- en langetermijneffecten op gedrag en welzijn na castratie van kittens (VC) en jongvolwassen katten (LC)

Het doel van dit luik van het onderzoek (Hoofdstuk 7) was om na te gaan of de leeftijd van castratie een invloed had op de ontwikkeling van het gedrag en dus op het voorkomen van (potentieel) ongewenste gedragingen, rekening houdend met sociale factoren en omgevingsfactoren met betrekking tot de leefomstandigheden van de kat. Aangezien vroegcastratie uitgevoerd wordt tijdens een gevoelige fase voor de gedragsontwikkeling van

het kitten (socialisatieperiode), zijn er bekommernissen dat vroegcastratie zou leiden tot meer gedragsproblemen in vergelijking met castratie op latere leeftijd.

Een korte- en langetermijnopvolging werd uitgevoerd aan de hand van een dagboek gedurende de eerste 30 dagen na adoptie en aan de hand van vragenlijsten op 2, 6, 12, 18 en 24 maanden na adoptie. In de loop van 24 maanden na adoptie ontvingen de eigenaars uitnodigingen per email om de vragenlijsten in te vullen op een online enquête platform. De verzamelde data waren een indirecte maat voor het dierenwelzijn van de geadopteerde asielkatten, aangezien gedragsproblemen vaak aangehaald worden als een reden om de kat te verwijderen uit het huishouden. In deze studie werd bijgevolg enerzijds potentieel ongewenst gedrag gebruikt om gedrag te beschrijven dat mogelijk, maar niet noodzakelijk, een probleem zou kunnen zijn voor een eigenaar. Anderzijds werd ongewenst gedrag gebruikt om gedrag te beschrijven waarvan de eigenaar expliciet had aangegeven dat hij/zij het storend vond, en dus, onaanvaardbaar.

Resultaten toonden aan dat het gemiddeld aantal potentieel ongewenste gedragingen per dag gedurende de eerste maand na adoptie niet verschilden tussen geopereerde (VC) en niet-geopereerde (LC) katten. Bovendien was er geen significant verschil gevonden tussen VC en LC katten in de evolutie van de (potentieel) ongewenste gedragingen over tijd (2, 6, 12, 18 en 24 maanden na adoptie). Het grootste aantal potentieel ongewenste gedragingen werd gedurende de eerste maand na adoptie waargenomen. Agressief gedrag naar mensen toe (spelgerelateerd), vernielzucht, sabbelen en angstig gedrag (voor geluiden en bewegingen) werden het meest gerapporteerd. Bij de evaluatie op 2, 6, 12, 18 en 24 maanden na adoptie werden jachtgedrag, vernielzucht, seksueel gedrag, angstig gedrag en aandacht zoeken vaak als potentieel ongewenst gedrag gerapporteerd. Ongewenste gedragingen die veel door de eigenaar gerapporteerd werden waren vernielzucht, voeding stelen en al dan niet spelgerelateerde agressie naar mensen.

In tegenstelling tot leeftijd van castratie, werden er andere sociale en omgevingsfactoren gevonden zoals het regelmatig gebruik van straf door de eigenaar, de afwezigheid van een andere kat in het huishouden en het voorkomen van positieve interactie, ... die significant geassocieerd waren met een selectie van (potentieel) ongewenste gedragingen. Deze selectie was gebaseerd op gedragsproblemen die in de literatuur vaak gerapporteerd worden: onzindelijkheid, bang zijn (sociaal/niet sociaal), (niet-) spel gerelateerde agressie naar mensen of dieren, vernielzucht. Het is belangrijk op te merken dat enkel associaties en dus geen causale verbanden weergegeven worden bij de analyse van de sociale en omgevingsfactoren.

De belangrijkste conclusie met betrekking tot de gedragsontwikkeling was dat de resultaten aantoonden dat de leeftijd waarop de kat gecastreerd werd geen invloed had op het gemiddeld aantal (potentieel) ongewenste gedragingen bij asielkatten gedurende 24 maanden na adoptie. Er werden daarentegen wel andere factoren geïdentificeerd met betrekking tot de leefomstandigheden van de kat die aldus een grotere impact hadden op het voorkomen van bepaalde ongewenste gedragingen.

In dit onderzoek werden waardevolle gegevens omtrent vroegtijdige castratie bij katten op Belgische bodem verzameld. Deze studie onderscheidt zich van gelijkaardige studies uitgevoerd in de Verenigde Staten van Amerika en het Verenigd Koninkrijk door de gerandomiseerde prospectieve proefopzet, door het gebruik van gestandaardiseerde anesthesie- en chirurgieprotocollen en door de intensieve datacollectie op meerdere tijdstippen, steeds door dezelfde onderzoeker uitgevoerd.

Er werden geen wetenschappelijke tegenaanwijzingen gevonden voor VC van asielkittens, niet op korte maar ook niet op lange termijn (24 maanden). Vroegtijdige castratie van katten kan dus in het kader van asielbeleid aangeraden worden. Opdat VC van asielkatten in alle asielen toegepast zou worden en algemeen aanvaard zou worden, is het belangrijk dat hiervoor vanuit de overheid een draagvlak voor alle betrokken partijen gecreëerd wordt wat betreft de financiële aspecten en de wetenschappelijk onderbouwde informatie over VC. Om het welzijn van katten nog meer te verbeteren, zou er speciale aandacht gegeven moeten worden aan het opleiden van eigenaars over de voortplanting van de kat (en de noodzaak voor castratie) en over kattengedrag, hetgeen de juiste voorzieningen in de huisvesting en omgevingsverrijking impliceert om ongewenste gedragingen en afstand doen van de kat te voorkomen.

REFERENCES

References

References

4AVet (2010) Evaluation Clinique De La Douleur Postoperative Chat, Assocation Vétérinaire pour l'Anesthésie et l'Analgésie Animales (ed). http://www.medvet.umontreal.ca/4avet/ (consulted on April 10, 2010).

Abbo LA, Ko JC, Maxwell LK et al. (2008) Pharmacokinetics of buprenorphine following intravenous and oral transmucosal administration in dogs. Vet Ther 9, 83-93.

ACC&D (2013) Contraception and Fertility Control in Dogs and Cats. Alliance for Contraception in Cats & Dogs. http://www.stray-afp.org/nl/wp-content/uploads/2013/06/Contraception-and-Fertility-Control-in-Dogs-and-Cats-van-Acc-d-febr-2013.pdf (consulted on June 24, 2013).

Adamelli S, Marinelli L, Normando S et al. (2005) Owner and cat features influence the quality of life of the cat. Appl Anim Behav Sci 94, 89-98.

Alexander LG, Salt C, Thomas G et al. (2011) Effects of neutering on food intake, body weight and body composition in growing female kittens. Br J Nutr 106 Suppl 1, S19-23.

Allan FJ, Pfeiffer DU, Jones BR et al. (2000) A cross-sectional study of risk factors for obesity in cats in New Zealand. Prev Vet Med 46, 183-196.

Amat M, de la Torre JLR, Fatjo J et al. (2009) Potential risk factors associated with feline behaviour problems. Appl Anim Behav Sci 121, 134-139.

Ansah OB (2004) Use of the alpha-2-adrenoceptor agonists medetomidine and dexmedetomidine in the sedation of analgesia of domestic cats. In: Department of Veterinary Clinical Sciences, Clinical Pharmacology - Anaesthesia. University of Helsinki, Helsinki, Finland. pp. 1-93. http://ethesis.helsinki.fi/julkaisut/ela/kliin/vk/ansah/

Ansah OB, Raekallio M, Vainio O (1998) Comparison of three doses of dexmedetomidine with medetomidine in cats following intramuscular administration. J Vet Pharmacol Ther 21, 380-387.

Ansah OB, Raekallio M, Vainio O (2000) Correlation between serum concentrations following continuous intravenous infusion of dexmedetomidine or medetomidine in cats and their sedative and analgesic effects. J Vet Pharmacol Ther 23, 1-8.

Anttila M, Penttila J, Helminen A et al. (2003) Bioavailability of dexmedetomidine after extravascular doses in healthy subjects. Br J Clin Pharmacol 56, 691-693.

Appel L, Scarlett J (2013) Pediatric Neutering. In: Shelter Medicine for Veterinarians and Staff, second edition. Miller L & Zawistowski S (eds). John Wiley & Sons, Inc. pp. 647-669.

Appleton DJ, Rand JS, Sunvold GD (2000) Plasma leptin concentrations in cats: reference range, effect of weight gain and relationship with adiposity as measured by dual energy X-ray absorptiometry. J Feline Med Surg 2, 191-199.

Aronsohn MG, Faggella AM (1993) Surgical techniques for neutering 6- to 14-week-old kittens. J Am Vet Med Assoc 202, 53-55.

Aronson LR, Cooper ML (1967) Penile Spines of Domestic Cat - Their Endocrine-Behavior Relations. Anat Rec 157, 71-78.

Aurich J, Becher A (2013) Educating owners--the key to managing cat populations. Vet Rec 172, 576-577.

Backus RC, Havel PJ, Gingerich RL et al. (2000) Relationship between serum leptin immunoreactivity and body fat mass as estimated by use of a novel gas-phase Fourier transform infrared spectroscopy deuterium dilution method in cats. Am J Vet Res 61, 796-801.

References

Bannasch MJ, Foley JE (2005) Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. J Feline Med Surg 7, 109-119.

Batson A (2008) Global Companion Animal Ownership and Trade: Project Summary. World Society for the Protection of Animals: Companion & Working Animals Unit. http://www.wspa.org.uk/Images/Pet%20ownership%20and%20trade%20-%20Global%20report_tcm9-10875.pdf (consulted on February 10 2014)

Beaver BV (2003a) Chapter 5 - Male Feline Sexual Behavior. In: Feline Behavior (Second Edition). W.B. Saunders, Saint Louis. pp. 164-181.

Beaver BV (2003b) Chapter 6 - Female Feline Sexual Behavior. In: Feline Behavior (Second Edition). W.B. Saunders, Saint Louis. pp. 182-211.

Beaver BV (2004) Fractious cats and feline aggression. Journal of Feline Medicine and Surgery 6, 13-18.

Belsito KR, Vester BM, Keel T et al. (2009) Impact of ovariohysterectomy and food intake on body composition, physical activity, and adipose gene expression in cats. J Anim Sci 87, 594-602.

Bermingham EN, Thomas DG, Morris PJ et al. (2010) Energy requirements of adult cats. Br J Nutr 103, 1083-1093.

Blackwell EJ, Twells C, Seawright A et al. (2008) The relationship between training methods and the occurrence of behavior problems, as reported by owners, in a population of domestic dogs. J Vet Behav 3, 207-217.

Bortolami E, Slingsby L, Love EJ (2012) Comparison of two formulations of buprenorphine in cats administered by the oral transmucosal route. J Feline Med Surg 14, 534-539.

Bradshaw JWS, Horsfield GF, Allen JA et al. (1999) Feral cats: their role in the population dynamics of Felis catus. Appl Anim Behav Sci 65, 273-283.

Brown DC, Conzemius MG, Shofer F et al. (1997) Epidemiologic evaluation of postoperative wound infections in dogs and cats. J Am Vet Med Assoc 210, 1302-1306.

BSAVA (2009) BSAVA client questionnaire: behaviour series. In: BSAVA / British Small Animal Veterinary Association: Manual of Canine and Feline Behavioural Medicine, Horwitz DF & Mills DS (eds).

Bullingham RE, McQuay HJ, Dwyer D et al. (1981) Sublingual buprenorphine used postoperatively: clinical observations and preliminary pharmacokinetic analysis. Br J Clin Pharmacol 12, 117-122.

Burkholder WJ, Toll PW (2000) Obesity. In: Small Animal Clinical Nutrition. (4th edn). Hand MS, Thatcher CD, Remillard LR, et al. (eds). Mark Morris Institute, Kansas (USA). pp. 403-430.

Burrow R, Wawra E, Pinchbeck G et al. (2006) Prospective evaluation of postoperative pain in cats undergoing ovariohysterectomy by a midline or flank approach. Vet Rec 158, 657-660.

Bushby PA (2010) Efficient spay/neuter techniques. In: 82nd Annual Western Veterinary Conference, Las Vegas, Nevada.

Bushby PA (2013) Efficient spay/neuter techniques. In: 85th Annual Western Veterinary Conference, Las Vegas, Nevada.

Bushby PA, Griffin B (2011) An overview of pediatric spay and neuter benefits and techniques. Vet Med-Us 106, 83-89.

References

Cambridge AJ, Tobias KM, Newberry RC et al. (2000) Subjective and objective measurements of postoperative pain in cats. J Am Vet Med Assoc 217, 685-690.

Casey R (2002) Fear and stress. In: BSAVA Manual of canine and feline behavioural medicine 1st edition. Horwitz DF, Mills DS & Heath S (eds). British Small Animal Veterinary Association., Gloucester (UK). pp. 144-153.

Casey RA, Bradshaw JWS (2005) The effect of an additional programme of socialisation for kittens (Felis sylvestris) in a rescue centre on behaviour and suitability as a pet after re-homing. Current Issues and Research in Veterinary Behavioural Medicine, 173-178

Casey RA, Bradshaw JWS (2008) The effects of additional socialisation for kittens in a rescue centre on their behaviour and suitability as a pet. Appl Anim Behav Sci 114, 196-205.

Casey RA, Vandenbussche S, Bradshaw JWS et al. (2009) Reasons for Relinquishment and Return of Domestic Cats (Felis Silvestris Catus) to Rescue Shelters in the UK. Anthrozoos 22, 347-358.

Catbagan DL, Quimby JM, Mama KR et al. (2011) Comparison of the efficacy and adverse effects of sustained-release buprenorphine hydrochloride following subcutaneous administration and buprenorphine hydrochloride following oral transmucosal administration in cats undergoing ovariohysterectomy. Am J Vet Res 72, 461-466.

Cave NJ, Allan FJ, Schokkenbroek SL et al. (2012) A cross-sectional study to compare changes in the prevalence and risk factors for feline obesity between 1993 and 2007 in New Zealand. Prev Vet Med 107, 121-133.

Cave TA, Thompson H, Reid SW et al. (2002) Kitten mortality in the United Kingdom: a retrospective analysis of 274 histopathological examinations (1986 to 2000). Vet Rec 151, 497-501.

Chalifoux A, Niemi G, Fanjoy P et al. (1981) Early spay-neutering of dogs and cats. Can Vet J 22, 381.

Chu K, Anderson WM, Rieser MY (2009) Population characteristics and neuter status of cats living in households in the United States. J Am Vet Med Assoc 234, 1023-1030.

Clark K (2012) Neutering: how early is too early? Vet Rec 170, 432-433.

Clements DN, Handel IG, Rose E et al. (2013) Dogslife: a web-based longitudinal study of Labrador Retriever health in the UK. BMC Vet Res. DOI: 10.1186/1746-6148-9-13.

Coe RJ, Grint NJ, Tivers MS et al. (2006) Comparison of flank and midline approaches to the ovariohysterectomy of cats. Vet Rec 159, 309-313.

Colliard L, Paragon BM, Lemuet B et al. (2009) Prevalence and risk factors of obesity in an urban population of healthy cats. J Feline Med Surg 11, 135-140.

Concannon P, Hodgson B, Lein D (1980) Reflex LH release in estrous cats following single and multiple copulations. Biol Reprod 23, 111-117.

Corletto F (2007) Multimodal and balanced analgesia. Vet Res Commun 31 Suppl 1, 59-63.

Corridan C (2010) Doggy Diaries. Personal communication – March 19, 2010.

Courcier EA, Mellor DJ, Pendlebury E et al. (2012) An investigation into the epidemiology of feline obesity in Great Britain: results of a cross-sectional study of 47 companion animal practises. Vet Rec 171, 560-564.

References

Courcier EA, O'Higgins R, Mellor DJ et al. (2010) Prevalence and risk factors for feline obesity in a first opinion practice in Glasgow, Scotland. J Feline Med Surg 12, 746-753.

Craig LE (2001) Physeal dysplasia with slipped capital femoral epiphysis in 13 cats. Vet Pathol 38, 92-97.

Crowell-Davis S (2002) Social behaviour, communication and development of behaviour in the cat. In: BSAVA Manual of Canine and Feline Behavioural Medicine. Horwitz DF, Mills DS & Heath S (eds). British Small Animal Veterinary Association, Gloucester, England. pp. 21-29.

Crowell-Davis S (2007) Cat behaviour: social organization, communication and development. In: The Welfare of Cats. Rochlitz I (ed). Springer, Dordrecht, The Netherlands. pp. 1-22.

Crowell-Davis SL, Curtis TM, Knowles RJ (2004) Social organization in the cat: a modern understanding. J Feline Med Surg 6, 19-28.

Curtis TM (2008) Human-directed aggression in the cat. Vet Clin North Am Small Anim Pract 38, 1131-1143, vii.

Day MJ (2007) Immune System Development in the Dog and Cat. Journal of Comparative Pathology 137, S10-S15.

Day MJ, Horzinek MC, Schultz RD (2010) WSAVA guidelines for the vaccination of dogs and cats. J Small Anim Pract 51, 1-32.

DeTora M, McCarthy RJ (2011) Ovariohysterectomy versus ovariectomy for elective sterilization of female dogs and cats: is removal of the uterus necessary? J Am Vet Med Assoc 239, 1409-1412.

Dixon MJ, Taylor PM, Slingsby L et al. (2010) A small, silent, low friction, linear actuator for mechanical nociceptive testing in veterinary research. Lab Anim 44, 247-253.

Dobbins S, Brown NO, Shofer FS (2002) Comparison of the effects of buprenorphine, oxymorphone hydrochloride, and ketoprofen for postoperative analgesia after onychectomy or onychectomy and sterilization in cats. J Am Anim Hosp Assoc 38, 507-514.

Drasner K, Fields HL (1988) Synergy between the antinociceptive effects of intrathecal clonidine and systemic morphine in the rat. Pain 32, 309-312.

Dyson DH (2008). Perioperative pain management in veterinary patients. Vet Clin North Am Small Anim Pract 38, 1309-1327.

Eidinger D, Garrett TJ (1972) Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. J Exp Med 136, 1098-1116.

Ellis SL (2009) Environmental enrichment: practical strategies for improving feline welfare. J Feline Med Surg 11, 901-912.

Farnsworth MJ, Adams NJ, Seksel K et al. (2013) Veterinary attitudes towards pre-pubertal gonadectomy of cats: a comparison of samples from New Zealand, Australia and the United Kingdom. New Zeal Vet J 61, 226-233.

Favrot C, Steffan J, Seewald W et al. (2012) Establishment of diagnostic criteria for feline nonflea-induced hypersensitivity dermatitis. Vet Dermatol 23, 45-50.

Fatjo J, Ruiz-de-la-Torre JL, Manteca X (2006) The epidemiology of behavioural problems in dogs and cats: a survey of veterinary practitioners. Anim Welfare 15, 179-185.

References

FPS Economics (2008) Dogs and cats of Belgians. http://statbel.fgov.be/nl/binaries/De%20honden%20en%20katten%20van%20de%20Belgen_2008_nl_tcm325-103755.pdf (consulted on February 15, 2014).

FPS (2012) Statistics animal shelters 2012, Federal Public Service Health , Food Chain Safety and Environment (ed). http://health.belgium.be/eportal/AnimalsandPlants/Animal_welfare/Plan_Cats/Communications/press/19080063_NL?ie2Term=Euthanasie&ie2section=83#stat2012 (consulted on February 10, 2014)

Fennell C (1975) Some demographic characteristics of the domestic cat population in Great Britain with particular reference to feeding habits and the incidence of the feline urological syndrome. J Small Anim Pract 16, 775-783.

Ferreira TH, Rezende ML, Mama KR et al. (2011) Plasma concentrations and behavioral, antinociceptive, and physiologic effects of methadone after intravenous and oral transmucosal administration in cats. Am J Vet Res 72, 764-771.

Fettman MJ, Stanton CA, Banks LL et al. (1997) Effects of neutering on bodyweight, metabolic rate and glucose tolerance of domestic cats. Res Vet Sci 62, 131-136.

Fischer HR, Norton J, Kobluk CN et al. (2004) Surgical reduction and stabilization for repair of femoral capital physeal fractures in cats: 13 cases (1998-2002). J Am Vet Med Assoc 224, 1478-1482.

Gaillard RC, Spinedi E (1998) Sex- and stress-steroids interactions and the immune system: Evidence for a neuroendocrine-immunological sexual dimorphism. Domest Anim Endocrin 15, 345-352.

Giordano T, Steagall PV, Ferreira TH et al. (2010) Postoperative analgesic effects of intravenous, intramuscular, subcutaneous or oral transmucosal buprenorphine administered to cats undergoing ovariohysterectomy. Vet Anaesth Analg 37, 357-366.

Goeree G (1998a) A different approach to controlling the cat population. Canadian Veterinary Journal-Revue Veterinaire Canadienne 39, 242-243.

Goeree G (1998b) Pediatric neuters can be technically challenging. Can Vet J 39, 244.

Gorman SP, Levy JK, Hampton AL et al. (2002) Evaluation of a porcine zona pellucida vaccine for the immunocontraception of domestic kittens (Felis catus). Theriogenology 58, 135-149.

Gourkow N, Lawson JH, Hamon SC et al. (2013) Descriptive epidemiology of upper respiratory disease and associated risk factors in cats in an animal shelter in coastal western Canada. Can Vet J 54, 132-138.

Granholm M, McKusick BC, Westerholm FC et al. (2006) Evaluation of the clinical efficacy and safety of dexmedetomidine or medetomidine in cats and their reversal with atipamezole. Vet Anaesth Analg 33, 214-223.

Greenfield CL, Johnson AL, Schaeffer DJ (2004) Frequency of use of various procedures, skills, and areas of knowledge among veterinarians in private small animal exclusive or predominant practice and proficiency expected of new veterinary school graduates. J Am Vet Med Assoc 224, 1780-1787.

Griffin B, Brestle K (2010) Early surgical neutering in female cats: theory and wet lab. In: Congrès de médecine féline, Lyon (France).

Grint NJ, Burford J, Dugdale AH (2009) Investigating medetomidine-buprenorphine as preanaesthetic medication in cats. J Small Anim Pract 50, 73-81.

References

Grint NJ, Murison PJ, Coe RJ et al. (2006) Assessment of the influence of surgical technique on postoperative pain and wound tenderness in cats following ovariohysterectomy. J Feline Med Surg 8, 15-21.

Grossman CJ (1985) Interactions between the Gonadal-Steroids and the Immune System. Science 227, 257-261.

Grove DM, Ramsay EC (2000) Sedative and physiologic effects of orally administered alpha(2)-adrenoceptor agonists and ketamine in cats. J Am Vet Med Assoc 216, 1929-1932.

Gunn-Moore DA (2003) Feline lower urinary tract disease. J Feline Med Surg 5, 133-138.

Harper EJ, Stack DM, Watson TD et al. (2001) Effects of feeding regimens on bodyweight, composition and condition score in cats following ovariohysterectomy. J Small Anim Pract 42, 433-438.

Harris D, Robinson JR (1992) Drug delivery via the mucous membranes of the oral cavity. J Pharm Sci 81, 1-10.

Harrison KA, Robertson SA, Levy JK et al. (2011) Evaluation of medetomidine, ketamine and buprenorphine for neutering feral cats. J Feline Med Surg 13, 896-902.

Hart BL, Barrett RE (1973) Effects of castration on fighting, roaming, and urine spraying in adult male cats. J Am Vet Med Assoc 163, 290-292.

Hart BL, Cooper L (1984) Factors relating to urine spraying and fighting in prepubertally gonadectomised cats. J Am Vet Med Assoc 184, 1255-1258.

Haughie A (2001) Early Age Neutering. World Society for the Protection of Animals. http://www.lese.lt/data/dokumentai/WSPA%20info/Early_Age_Neutering.pdf (consulted on May, 2010).

Heath SE (2002) Feline aggression. In: BSAVA Manual of Canine and Feline Behavioural Medicine 1st edition. Horwitz DF, Mills DS & Heath S (eds). British Small Animal Veterinary Association, Gloucester (UK). pp. 216-228.

Heath SE (2007) Behaviour problems and welfare. In: The Welfare of Cats. Rochlitz I (ed). Springer. pp. 91-119.

Hedges AR, Pypendop BH, Shilo Y et al. (2013b) Impact of the blood sampling site on time–concentration drug profiles following intravenous or buccal drug administration. J Vet Pharmacol Ther, 37, 145-150.

Hedges AR, Pypendop BH, Shilo-Benjamini Y et al. (2013a) Pharmacokinetics of buprenorphine following intravenous and buccal administration in cats, and effects on thermal threshold. J Vet Pharmacol Ther, DOI: 10.1111/jvp.12084

Hedlund CS (2002) Surgery of the Reproductive and Genital Systems. In: Small Animal Surgery. Fossum TW (ed). Mosby, Missouri. pp. 610-674.

Heidenberger E (1997) Housing conditions and behavioural problems of indoor cats as assessed by their owners. Appl Anim Behav Sci 52, 345-364.

Hellyer P, Rodan I, Brunt J et al. (2007) AAHA/AAFP pain management guidelines for dogs and cats. J Feline Med Surg 9, 466-480.

Herron MA (1972) The effect of prepubertal castration on the penile urethra of the cat. J Am Vet Med Assoc 160, 208-211.

References

Hiby EF, Rooney NJ, Bradshaw JWS (2004) Dog training methods: their use, effectiveness and interaction with behaviour and welfare. Anim Welfare 13, 63-69.

Hobi S, Linek M, Marignac G, Olivry T et al. (2011) Clinical characteristics and causes of pruritus in cats: a multicentre study on feline hypersensitivity-associated dermatoses. Vet Dermatol, 22, 406-13.

Hoenig M, Ferguson DC (2002) Effects of neutering on hormonal concentrations and energy requirements in male and female cats. Am J Vet Res 63, 634-639.

Holden D (2007a) Paediatric Patients. In: BSAVA: Manual of canine and feline anaesthesia and analgesia. Seymour C & Duke-Novakovski T (eds). British Small Animal Veterinary Association, Gloucester (UK). pp. 296-302.

Horwitz DF (2001) Feline Aggression. Recent Advances in Companion Animal Behaviour Problems, International Veterinary Information Service. http://www.ivis.org/advances/Behaviour_Houpt/horwitz/chapter.asp?LA=1

Hosgood G, Hoskins JD, Davidson JR et al. (1998) Small animal paediatric medicine and surgery. Butterworth-Heinemann Ltd., Oxford

Hoskins JD (1999) Pediatric health care and management. Vet Clin North Am Small Anim Pract 29, 837-852.

Houlton JE, McGlennon NJ (1992) Castration and physeal closure in the cat. Vet Rec 131, 466-467.

Howe LM (1997) Short-term results and complications of prepubertal gonadectomy in cats and dogs. J Am Vet Med Assoc 211, 56-62.

Howe LM (1999) Prepubertal gonadectomy in dogs and cats - Part I. Comp Cont Educ Pract 21, 103-111.

Howe LM (2006) Surgical methods of contraception and sterilization. Theriogenology 66, 500-509.

Howe LM, Slater MR, Boothe HW et al. (2000) Long-term outcome of gonadectomy performed at an early age or traditional age in cats. J Am Vet Med Assoc 217, 1661-1665.

Hoyumpa Vogt A, Rodan I, Brown M et al. (2010) AAFP-AAHA: feline life stage guidelines. J Feline Med Surg 12, 43-54.

Hunt JR, Grint NJ, Taylor PM et al. (2013) Sedative and analgesic effects of buprenorphine, combined with either acepromazine or dexmedetomidine, for premedication prior to elective surgery in cats and dogs. Vet Anaesth Analg 40, 297-307.

Hunthausen W, Seksel K (2002) Preventive behavioural medicine. In: BSAVA Manual of Canine and Feline Behavioural Medicine. Horwitz DF, Mills DS & Heath S (eds). British Small Animal Veterinary Association, Quedgeley, Gloucester. pp. 49-60.

Hurley KF (2005) Feline infectious disease control in shelters. Vet Clin North Am Small Anim Pract 35, 21-37.

Jackson EK (1984) Contraception in the dog and cat. Br Vet J 140, 132-137.

Jana K, Samanta PK (2011) Clinical evaluation of non-surgical sterilization of male cats with single intra-testicular injection of calcium chloride. BMC Vet Res 7, 39.

Jemmett JE, Evans JM (1977) A survey of sexual behaviour and reproduction of female cats. J Small Anim Pract 18, 31-37.

Jessup DA (2004) The welfare of feral cats and wildlife. J Am Vet Med Assoc 225, 1377-1383.

References

Johnson JA, Robertson SA, Pypendop BH (2007) Antinociceptive effects of butorphanol, buprenorphine, or both, administered intramuscularly in cats. Am J Vet Res 68, 699-703.

Jongman EC (2007) Adaptation of domestic cats to confinement. J. Vet. Behav 2, 193-196.

Joyce A, Yates D (2011) Help stop teenage pregnancy! Early-age neutering in cats. J Feline Med Surg 13, 3-10.

Juul A (2001) The effects of oestrogens on linear bone growth. Hum Reprod Update 7, 303-313.

Kanchuk ML, Backus RC, Calvert CC et al. (2002) Neutering induces changes in food intake, body weight, plasma insulin and leptin concentrations in normal and lipoprotein lipase-deficient male cats. J Nutr 132, 1730S-1732S.

Karsh EB, Turner DC (1986) The human-cat relationship. In: The domestic cat: the biology of its behaviour. Turner DC & Bateson P (eds). Cambridge University Press, Cambridge (UK). pp. 159-177.

Kendall K, Ley J (2006) Cat ownership in Australia: Barriers to ownership and behavior. J. Vet. Behav 1, 5-16.

Kendall K, Ley JQ (2008) Owner observations can provide data for constructive behavior analysis in normal pet cats in Australia. J Vet Behav 3, 244-247.

Kim YK, Lee SY, Park SJ et al. (2011) Feasibility of single-portal access laparoscopic ovariectomy in 17 cats. Vet Rec 169, 179.

KNMvD - Koninklijke Nederlandse Maatschappij voor Diergeneeskunde (2009) Standpoint KNMvD on the early age gonadectomy in puppies and kittens. https://hondenbescherming.nl//media/cms_page_media/67/KNMvD_standpunt%20vroegcastratie.pdf (consulted on June, 2012).

Ko JC, Austin BR, Barletta M et al. (2011) Evaluation of dexmedetomidine and ketamine in combination with various opioids as injectable anaesthetic combinations for castration in cats. J Am Vet Med Assoc 239, 1453-1462.

Ko JC, Berman AG (2010) Anesthesia in shelter medicine. Top Companion Anim Med 25, 92-97.

Kona-Boun JJ, Silim A, Troncy E (2005) Immunologic aspects of veterinary anesthesia and analgesia. J Am Vet Med Assoc 226, 355-363.

Kuhlman JJ, Jr., Lalani S, Magluilo J, Jr. et al. (1996) Human pharmacokinetics of intravenous, sublingual, and buccal buprenorphine. J Anal Toxicol 20, 369-378.

Kutzler M, Wood A (2006) Non-surgical methods of contraception and sterilization. Theriogenology 66, 514-525.

Kutzler MA (2007) Estrus induction and synchronization in canids and felids. Theriogenology 68, 354-374.

Kuusela E, Raekallio M, Anttila M et al. (2000) Clinical effects and pharmacokinetics of medetomidine and its enantiomers in dogs. J Vet Pharmacol Ther 23, 15-20.

Laflamme DP (1997) Development and validation of a body condition score system for cats: a clinical tool. Feline Pract 25, 13-18.

Laflamme DP (2006) Understanding and managing obesity in dogs and cats. Vet Clin North Am Small Anim Pract 36, 1283-1295, vii.

References

Lainesse C, Frank D, Meucci V et al. (2006) Pharmacokinetics of clomipramine and desmethylclomipramine after single-dose intravenous and oral administrations in cats. J Vet Pharmacol Ther 29, 271-278.

Lamont LA (2002) Feline perioperative pain management. Veterinary Clinics of North America: Small Animal Practice 32, 747-763.

Lamont LA (2008) Adjunctive analgesic therapy in veterinary medicine. Vet Clin North Am Small Anim Pract 38, 1187-1203.

Landsberg G (1996) Feline behavior and welfare. J Am Vet Med Assoc 208, 502-505.

Landsberg G, Hunthausen W, Ackerman L (1997) Puppy and kitten development. In: Handbook of behavior problems of the dog and cat. Saunders Elsevier, London (UK). pp. 15-27.

Landsberg G, Hunthausen W, Ackerman L (2003) Development, social, and communicative behavior. In: Behavior Problems of the Dog and the Cat. (2nd edition edn). Saunders Elsevier, China. pp. 20-26.

Lascelles BD, Waterman AE, Cripps PJ et al. (1995) Central sensitization as a result of surgical pain: investigation of the pre-emptive value of pethidine for ovariohysterectomy in the rat. Pain 62, 201-212.

Lawler DF (2008) Neonatal and pediatric care of the puppy and kitten. Theriogenology 70, 384-392.

Lawler DF, Sjolin DW, Collins JE (1985) Incidence Rates of Feline Lower Urinary-Tract Disease in the United-States. Feline Pract 15, 13-16.

Lehmann EL (2006) Nonparametrics: Statistical Methods Based on Ranks, Revised. Springer Science + Business Media, LLC, New York, USA. pp. 76-81.

Lekcharoensuk C, Osborne CA, Lulich JP (2001) Epidemiologic study of risk factors for lower urinary tract diseases in cats. J Am Vet Med Assoc 218, 1429-1435.

Levine ED (2008) Feline fear and anxiety. Vet Clin North Am Small Anim Pract 38, 1065-1079, vii.

Levy JK (2011) Contraceptive vaccines for the humane control of community cat populations. Am J Reprod Immunol 66, 63-70.

Levy JK, Crawford PC (2004) Humane strategies for controlling feral cat populations. J Am Vet Med Assoc 225, 1354-1360.

Levy JK, Friary JA, Miller LA et al. (2011) Long-term fertility control in female cats with GonaCon, a GnRH immunocontraceptive. Theriogenology 76, 1517-1525.

Levy JK, Gale DW, Gale LA (2003) Evaluation of the effect of a long-term trap-neuter-return and adoption program on a free-roaming cat population. J Am Vet Med Assoc 222, 42-46.

Liberg O, Sandell M (1986) Spatial organisation and reproductive tactics in the domestic cat and other felids. In: The domestic cat: the biology of its behaviour. Turner DC & Bateson P (eds). Cambrigde University Press, New York (USA). pp. 83-99.

Lieberman LL (1987) A case for neutering pups and kittens at two months of age. J Am Vet Med Assoc 191, 518-521.

Lin H-C (2007) Dissociative Anesthetics. In: Lumb & Jones' Veterinary Anaesthesia and Analgesia. (4th edition edn). Tranquilli WJ, Thurmon JC & Grimm KA (eds). Blackwell Publishing, Iowa, USA. pp. p.300-307.

Little S (2008) Early age altering of kittens. In: CVC in Kansas City Proceedings, Kansas City. pp. 3.

References

Little S (2013) Preventive healthcare: a life-stage approach. In: BSAVA Manual of Feline Practice. Harvey A & Tasker S (eds). British Small Animal Veterinary Association, Gloucester; UK. pp. 32-52.

Little S, Sears W, Lachtara J et al. (2009) Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in Canada. Can Vet J 50, 644-648.

Looney AL, Appel LD, Bohling MW et al. (2008a) Anesthesia and Analgesia Guidelines for High Quality, High Volume Spay/Neuter Initiatives Dempsey JE & MFA (eds). http://sheltervet.org/wp-content/uploads/2012/08/VTFASN_AnesthesiaAnalgesia.pdf.

Looney AL, Bohling MW, Bushby PA et al. (2008b) The Association of Shelter Veterinarians veterinary medical care guidelines for spay-neuter programs. J Am Vet Med Assoc 233, 74-86.

Lord LK, Reider L, Herron ME et al. (2008) Health and behavior problems in dogs and cats one week and one month after adoption from animal shelters. J Am Vet Med Assoc 233, 1715-1722.

Lowe SE, Bradshaw JWS (2002) Responses of pet cats to being held by an unfamiliar person, from weaning to three years of age. Anthrozoos 15, 69-79.

Luke C (1996) Animal shelter issues. J Am Vet Med Assoc 208, 524-527.

Lund EM, Armstrong PJ, Kirk CA et al. (2005) Prevalence and risk factors for obesity in adult cats from US veterinary practices. Intern J Appl Res Vet Med 3, 88-96.

Madhav NVS, Shakya AK, Shakya P et al. (2009) Orotransmucosal drug delivery systems: A review. Journal of Controlled Release 140, 2-11.

Martin L, Siliart B, Dumon H et al. (2001) Leptin, body fat content and energy expenditure in intact and gonadectomized adult cats: a preliminary study. J Anim Physiol Anim Nutr (Berl) 85, 195-199.

Martin LJ, Siliart B, Dumon HJ et al. (2006) Spontaneous hormonal variations in male cats following gonadectomy. J Feline Med Surg 8, 309-314.

Martin P, Bateson P (1986) Behavioural development in the cat. In: The domestic cat: the biology of its behaviour. Turner DC & Bateson P (eds). Cambrigde University Press, New York (USA). pp. 9-22.

Mathews KA (2000) Pain Assessment and General Approach to Management. Veterinary Clinics of North America: Small Animal Practice 30, 729-755.

May C, Bennett D, Downham DY (1991) Delayed physeal closure associated with castration in cats. Journal of Small Animal Practice 32, 326-328.

McCune S (1995) The impact of paternity and early socialisation on the development of cats' behaviour to people and novel objects. Appl Anim Behav Sci 45, 109-124.

McInnes F, Clear N, James G et al. (2008) Evaluation of the clearance of a sublingual buprenorphine spray in the beagle dog using gamma scintigraphy. Pharm Res 25, 869-874.

McNeil J, Constandy E (2006) Addressing the problem of pet overpopulation: The Experience of New Hanover County Animal Control Services. J Public Health Man 12, 452-455.

McNicholas WT, Jr., Wilkens BE, Blevins WE et al. (2002) Spontaneous femoral capital physeal fractures in adult cats: 26 cases (1996-2001). J Am Vet Med Assoc 221, 1731-1736.

References

Mendelson J, Upton RA, Everhart ET et al. (1997) Bioavailibility of sublingual buprenorphine. J. Clin Pharmacol 37, 31-37.

Mertens C, Schär R (1986) Practical aspects of research on cats. In: The domestic cat: the biology of its behaviour. Turner DC & Bateson P (eds). Cambridge University Press, New York (UK). pp. 179-193.

Meyer RE (2007) Anesthesia of pediatric small animal patients. In: Recent advances in veterinary anesthesia and analgesia: companion animals. Gleed RD & Ludders JW (eds). Ivis, Ithaca, NY.

Moore GE, DeSantis-Kerr AC, Guptill LF et al. (2007) Adverse events after vaccine administration in cats: 2,560 cases (2002-2005). J Am Vet Med Assoc 231, 94-100.

Morgan M, Houpt KA (1990) Feline behaviour problems: the influence of declawing. Antrozöos 3, 50-53.

Munson L (2006) Contraception in felids. Theriogenology 66, 126-134.

Murray JK, Roberts MA, Whitmarsh A et al. (2009) Survey of the characteristics of cats owned by households in the UK and factors affecting their neutered status. Vet Rec 164, 137-141.

Murray JK, Skillings E, Gruffydd-Jones TJ (2008) Opinions of veterinarians about the age at which kittens should be neutered. Vet Rec 163, 381-385.

Murray PG, Clayton PE (2013) Endocrine control of growth. Am J Med Genet C Semin Med Genet 163C, 76-85.

Murrell J (2007) Choice of premedicants in cats and dogs. In Practice 29, 100-106.

Neidhart L, Boyd R (2002) Companion animal adoption study. J Appl Anim Welf Sci 5, 175-192.

New JC, Jr., Salman MD, Scarlett JM et al. (1999) Moving: characteristics of dogs and cats and those relinquishing them to 12 U.S. animal shelters. J Appl Anim Welf Sci 2, 83-95.

Newbury S, Blinn MK, Bushby PA et al. (2010) Guidelines for Standards of Care in Animal Shelters. http://oacu.od.nih.gov/disaster/ShelterGuide.pdf

Nicholson M, Beal M, Shofer F et al. (2002) Epidemiologic evaluation of postoperative wound infection in clean-contaminated wounds: A retrospective study of 239 dogs and cats. Vet Surg 31, 577-581.

Nilsson O, Marino R, De Luca F et al. (2005) Endocrine regulation of the growth plate. Horm Res 64, 157-165.

Nutter FB, Levine JF, Stoskopf MK (2004) Reproductive capacity of free-roaming domestic cats and kitten survival rate. J Am Vet Med Assoc 225, 1399-1402.

Odendaal JSJ (1997) A diagnostic classification of problem behaviour in dogs and cats. Vet Clin N Am-Small 27, 427-443.

Ohlsson C, Isgaard J, Tornell J et al. (1993) Endocrine regulation of longitudinal bone growth. Acta Paediatr Suppl 82 Suppl 391, 33-40; discussion 41.

Oliveira EC, Fagundes AK, Melo CC et al. (2013) Intratesticular injection of a zinc-based solution for contraception of domestic cats: A randomized clinical trial of efficacy and safety. Vet J 197, 307-310.

Olm DD, Houpt KA (1988) Feline house-soiling problems. Appl Anim Behav Sci 20, 335-345.

Olson PN, Kustritz MV, Johnston SD (2001) Early-age neutering of dogs and cats in the United States (a review). J Reprod Fertil Suppl 57, 223-232.

References

Ossipov MH, Suarez LJ, Spaulding TC (1989) Antinociceptive interactions between alpha 2-adrenergic and opiate agonists at the spinal level in rodents. Anesth Analg 68, 194-200.

Overall KL, Rodan I, Beaver BV et al. (2005) Feline behavior guidelines from the American Association of Feline Practitioners. J Am Vet Med Assoc 227, 70-84.

Overley B, Shofer FS, Goldschmidt MH et al. (2005) Association between ovarihysterectomy and feline mammary carcinoma. J Vet Intern Med 19, 560-563.

Pascoe PJ (2000a) Opioid analgesics. Vet Clin North Am Small Anim Pract 30, 757-772.

Pascoe PJ (2000b) Perioperative pain management. Vet Clin North Am Small Anim Pract 30, 917-932.

Patronek GJ, Dodman NH (1999) Attitudes, procedures, and delivery of behavior services by veterinarians in small animal practice. J Am Vet Med Assoc 215, 1606-1611.

Patronek GJ, Glickman LT, Beck AM et al. (1996) Risk factors for relinquishment of cats to an animal shelter. J Am Vet Med Assoc 209, 582-588.

Pernestal K, Axner E (2012) An internet survey of breeders' and cat rescue organisations' opinions about early castration of cats. J Feline Med Surg 14, 849-856.

Perry KL, Fordham A, Arthurs GI (2014) Effect of neutering and breed on femoral and tibial physeal closure times in male and female domestic cats. J Feline Med Surg 16, 149-156.

Pesavento PA, Murphy BG (2014) Common and emerging infectious diseases in the animal shelter. Vet Pathol 51, 478-491.

Polson S, Taylor PM, Yates D (2012) Analgesia after feline ovariohysterectomy under midazolam-medetomidine-ketamine anaesthesia with buprenorphine or butorphanol, and carprofen or meloxicam: a prospective, randomised clinical trial. J Feline Med Surg 14, 553-559.

Polson S, Taylor PM, Yates D (2014) Effects of age and reproductive status on postoperative pain after routine ovariohysterectomy in cats. J Feline Med Surg 16, 170-176.

Porters N, Bosmans T, Debille M et al. (2014a) Sedative and antinociceptive effects of dexmedetomidine and buprenorphine after oral transmucosal or intramuscular administration in cats. Vet Anaesth Analg 41, 90-96.

Porters N, de Rooster H, Bosmans T et al. (2014b) Pharmacokinetics of oral transmucosal and intramuscular dexmedetomidine combined with buprenorphine in cats. Manuscript submitted.

Porters N, de Rooster H, Moons CPH et al. (2014c) Prepubertal gonadectomy in cats: different injectable anaesthetic combinations and comparison with gonadectomy at traditional age. Manuscript in revision.

Porters N, Polis I, Moons CPH et al. (2014d) Prepubertal gonadectomy in cats: different surgical techniques and comparision with gonadectomy at traditional age. Vet Rec. DOI 10.1136/vr.102337.

Powers J (1990) Statistical analysis of pharmacokinetic data. J Vet Pharmacol Ther 13, 113-120.

Proost JH, Meijer DK (1992) MW/Pharm, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. Comput Biol Med 22, 155-163.

Radosta L (2011) Chapter 12 - Feline Behavioral Development. In: Small Animal Pediatrics. Peterson MR & Kutzler MA (eds). W.B. Saunders, Saint Louis. pp. 88-96.

References

Ramos D, Mills DS (2009) Human directed aggression in Brazilian domestic cats: owner reported prevalence, contexts and risk factors. J Feline Med Surg 11, 835-841.

Rathbone MJ, Drummond BK, Tucker IG (1994) The oral cavity as a site for systemic drug delivery. Advanced Drug Delivery Reviews 13, 1-22.

Reichler IM (2009) Gonadectomy in cats and dogs: a review of risks and benefits. Reprod Domest Anim 44 Suppl 2, 29-35.

Robertson ID (1999) The influence of diet and other factors on owner-perceived obesity in privately owned cats from metropolitan Perth, Western Australia. Prev Vet Med 40, 75-85.

Robertson SA (2005) Anesthesia protocols for early kitten sterilization and feral cat clinics (oral presentation). In: 77th Annual West Vet Conf, Las Vegas, USA.

Robertson SA (2007) Anaesthesia and analgesia for kittens and puppies. In: Voorjaarsdagen, Amsterdam, Netherlands. pp. 46-47.

Robertson SA (2008) Managing Pain in Feline Patients. Vet Clin North Am Small Anim Pract 38, 1267-1290.

Robertson SA, Lascelles BD, Taylor PM et al. (2005) PK-PD modeling of buprenorphine in cats: intravenous and oral transmucosal administration. J Vet Pharmacol Ther 28, 453-460.

Robertson SA, Levy J, Gunkel C et al. (2003a) Comparison of isoflurane and butorphanol with medetomidine, ketamine and buprenoprhine for anesthesia of 7-12 week old kittens for surgical sterilization. In: Association of Veterinary Anaesthetists, Spring Meeting: Pain Assessement and Pain Control, Doorwerth, Netherlands.

Robertson SA, Taylor PM (2004) Pain management in cats--past, present and future. Part 2. Treatment of pain--clinical pharmacology. J Feline Med Surg 6, 321-333.

Robertson SA, Taylor PM, Sear JW (2003b) Systemic uptake of buprenorphine by cats after oral mucosal administration. Vet Rec 152, 675-678.

Rochlitz I (1999) Recommendations for the housing of cats in the home, in catteries and animal shelters, in laboratories and in veterinary surgeries. J Feline Med Surg 1, 181-191.

Rochlitz I (2005) A review of the housing requirements of domestic cats (Felis silvestris ccitus) kept in the home. Appl Anim Behav Sci 93, 97-109.

Root Kustritz MV (1999) Early spay-neuter in the dog and cat. Vet Clin North Am Small Anim Pract 29, 935-943, vii.

Root Kustritz MV, Johnston SD, Lieberman LL (2000) Availability of training for prepuberal gonadectomy at North American veterinary colleges. J Am Vet Med Assoc 216, 1566-1567.

Root Kustritz MV (2002) Early spay-neuter: Clinical considerations. Clinical Techniques in Small Animal Practice 17, 124-128.

Root Kustritz MV (2007) Determining the optimal age for gonadectomy of dogs and cats. J Am Vet Med Assoc 231, 1665-1675.

Root Kustritz MV (2012) Effects of surgical sterilization on canine and feline health and on society. Reprod Domest Anim 47 Suppl 4, 214-222.

References

Root Kustritz MV (2014) Pros, Cons, and Techniques of Pediatric Neutering. Vet Clin North Am Small Anim Pract 44, 221-233.

Root MV, Johnston SD, Johnston GR et al. (1996a) The effect of prepuberal and postpuberal gonadectomy on penile extrusion and urethral diameter in the domestic cat. Vet Radiol Ultrasoun 37, 363-366.

Root MV, Johnston SD, Olson PN (1995) Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and juvenile mortality in the domestic cat. J Am Anim Hosp Assoc 31, 429-433.

Root MV, Johnston SD, Olson PN et al. (1996b) The effect of prepuberal and postpuberal gonadectomy on heat production measured by indirect calorimetry in male and female domestic cats. Am J Vet Res 57, 371-374.

Root MV, Johnston SD, Olson PN (1997) The effect of prepuberal and postpuberal gonadectomy on radial physeal closure in male and female domestic cats. Vet Radiol Ultrasound 38, 42-47.

Rosen DA, Daume JT (2006) Short duration large dose dexmedetomidine in a pediatric patient during procedural sedation. Anesth Analg 103, 68-69, table of contents.

Rosen JB, Schulkin J (1998) From normal fear to pathological anxiety. Psychol Rev 105, 325-350.

Roshier AL, McBride EA (2013a) Canine behaviour problems: discussions between veterinarians and dog owners during annual booster consultations. Vet Rec 172, 235.

Roshier AL, McBride EA (2013b) Veterinarians' perceptions of behaviour support in small-animal practice. Vet Rec 172, 267.

Russell K, Sabin R, Holt S et al. (2000) Influence of feeding regimen on body condition in the cat. J Small Anim Pract 41, 12-17.

Salman MD, New JG, Jr., Scarlett JM et al. (1998) Human and animal factors related to relinquishment of dogs and cats in 12 selected animal shelters in the United States. J Appl Anim Welf Sci 1, 207-226.

Salmeri KR, Bloomberg MS, Scruggs SL et al. (1991) Gonadectomy in immature dogs: effects on skeletal, physical, and behavioral development. J Am Vet Med Assoc 198, 1193-1203.

Salonen JS (1989) Pharmacokinetics of medetomidine. Acta Vet Scand Suppl 85, 49-54.

Santos LC, Ludders JW, Erb HN et al. (2010) Sedative and cardiorespiratory effects of dexmedetomidine and buprenorphine administered to cats via oral transmucosal or intramuscular routes. Vet Anaesth Analg 37, 417-424.

Savola JM, Virtanen R (1991) Central alpha 2-adrenoceptors are highly stereoselective for dexmedetomidine, the dextro enantiomer of medetomidine. Eur J Pharmacol 195, 193-199.

Scarlett JM, Donoghue S (1998) Associations between body condition and disease in cats. J Am Vet Med Assoc 212, 1725-1731.

Scarlett JM, Salman MD, New JG et al. (2002) The role of veterinary practitioners in reducing dog and cat relinquishments and euthanasias. J Am Vet Med Assoc 220, 306-311.

Scrollavezza P, Tambella AM, Vullo C et al. (2009) Evaluation of the muscular relaxant effect of dexmedetomidine or medetomidine in cats. Vet Res Commun 33 Suppl 1, 213-215.

Seibert LM, Landsberg GM (2008) Diagnosis and management of patients presenting with behavior problems. Vet Clin North Am Small Anim Pract 38, 937-950, v.

References

Seksel K (2008) Preventing behavior problems in puppies and kittens. Vet Clin North Am Small Anim Pract 38, 971-982, v-vi.

Selmi AL, Mendes GM, Lins BT et al. (2003) Evaluation of the sedative and cardiorespiratory effects of dexmedetomidine, dexmedetomidine-butorphanol, and dexmedetomidine-ketamine in cats. J Am Vet Med Assoc 222, 37-41.

Serpell JA (1986) The domestication and history of the cat. In: The domestic cat: the biology of its behaviour. Turner DC & Bateson P (eds). Cambridge University Press, New York (USA). pp. 151-159.

Shore ER (2005) Returning a recently adopted companion animal: adopters' reasons for and reactions to the failed adoption experience. J Appl Anim Welf Sci 8, 187-198.

Shore ER, Burdsal C, Douglas DK (2008) Pet Owners' Views of Pet Behavior Problems and Willingness to Consult Experts for Assistance. J Appl Anim Welf Sci 11, 63-73.

Shore ER, Petersen CL, Douglas DK (2003) Moving as a reason for pet relinquishment: a closer look. J Appl Anim Welf Sci 6, 39-52.

Slingsby LS, Lane EC, Mears ER et al. (1998) Postoperative pain after ovariohysterectomy in the cat: a comparison of two anaesthetic regimens. Vet Rec 143, 589-590.

Slingsby LS, Murrell JC, Taylor PM (2010) Combination of dexmedetomidine with buprenorphine enhances the antinociceptive effect to a thermal stimulus in the cat compared with either agent alone. Vet Anaesth Analg 37, 162-170.

Slingsby LS, Taylor PM (2008) Thermal antinociception after dexmedetomidine administration in cats: a dose-finding study. J Vet Pharmacol Ther 31, 135-142.

Slingsby LS, Taylor PM, Monroe T (2009) Thermal antinociception after dexmedetomidine administration in cats: a comparison between intramuscular and oral transmucosal administration. J Feline Med Surg 11, 829-834.

Slingsby LS, Taylor PM, Waterman Pearson AE (2006) Efficacy of buccal compared to intramuscular dexmedetomidine for antinociception to a thermal nociceptive stimulus in the cat. In: Proceedings of the 9th World Congress of Veterinary Anaesthesia, Santos, Brazil. pp. 163.

Slingsby LS, Waterman-Pearson AE (2000) Postoperative analgesia in the cat after ovariohysterectomy by use of carprofen, ketoprofen, meloxicam or tolfenamic acid. J Small Anim Pract 41, 447-450.

Slingsby LS, Waterman-Pearson AE (2002) Comparison between meloxicam and carprofen for postoperative analgesia after feline ovariohysterectomy. J Small Anim Pract 43, 286-289.

Smith RN (1969) Fusion of ossification centres in the cat. J Small Anim Pract 10, 523-530.

Spain CV, Scarlett JM, Cully SM (2002) When to neuter dogs and cats: a survey of New York state veterinarians' practices and beliefs. J Am Anim Hosp Assoc 38, 482-488.

Spain CV, Scarlett JM, Houpt KA (2004) Long-term risks and benefits of early-age gonadectomy in cats. J Am Vet Med Assoc 224, 372-379.

Sparkes A (2011) Neutering cats - assessing attitudes and challenging conventions. J Feline Med Surg 13, 1-2.

Speakman JR, Booles D, Butterwick R (2001) Validation of dual energy X-ray absorptiometry (DXA) by comparison with chemical analysis of dogs and cats. Int J Obes Relat Metab Disord 25, 439-447.

References

Stavisky J, Brennan ML, Downes M et al. (2012) Demographics and economic burden of un-owned cats and dogs in the UK: results of a 2010 census. BMC Vet Res 8, 163-175.

Steagall PV, Mantovani FB, Taylor PM et al. (2009a) Dose-related antinociceptive effects of intravenous buprenorphine in cats. Vet J 182, 203-209.

Steagall PV, Taylor PM, Brondani JT et al. (2007) Effects of buprenorphine, carprofen and saline on thermal and mechanical nociceptive thresholds in cats. Vet Anaesth Analg 34, 344-350.

Steagall PV, Taylor PM, Rodrigues LC et al. (2009b) Analgesia for cats after ovariohysterectomy with either buprenorphine or carprofen alone or in combination. Vet Rec 164, 359-363.

Stubbs WP, Bloomberg MS (1995) Implications of early neutering in the dog and cat. Semin Vet Med Surg (Small Anim) 10, 8-12.

Stubbs WP, Bloomberg MS, Scruggs SL et al. (1996) Effects of prepubertal gonadectomy on physical and behavioral development in cats. J Am Vet Med Assoc 209, 1864-1871.

Stubbs WP, Salmeri KR, Bloomberg MS (1995) Early neutering of the dog and cat. In: Kirk's Current Veterinary Therapy XII Small Animal Practice. Bonagura JD & Kirk RW (eds). WB Saunders & co, Philadelphia. pp. 1037-1040.

Thatcher CD, Hand MS, Remillard LR (2000) Small Animal Clinical Nutrition: An Iterative Process. In: Small Animal Clinical Nutrition. (4th edition edn). Hand MS, Thatcher CD, R.L. R, et al. (eds). Walsworth Publishing Company, Missouri. pp. 1-19.

The Cat Group (2011) Cat neutering practices in the UK. J Feline Med Surg. http://jfm.sagepub.com/content/13/1/56.full.pdf (consulted on November 6, 2013)

Theran P (1993) Animal welfare forum: overpopulation of unwanted dogs and cats. Early-age neutering of dogs and cats. J Am Vet Med Assoc 202, 914-917.

Trevejo R, Yang M, Lund EM (2011) Epidemiology of surgical castration of dogs and cats in the United States. J Am Vet Med Assoc 238, 898-904.

Uchikura K, Nagano M, Hishinuma M (2010) Evaluation of follicular development and oocyte quality in pre-pubertal cats. Reprod Domest Anim 45, 405-411.

Vaisanen MA, Tuomikoski SK, Vainio OM (2007). Behavioral alterations and severity of pain in cats recovering at home following elective ovariohysterectomy or castration. J Am Vet Med Assoc 231, 236-242.

van Goethem B, Schaefers-Okkens A, Kirpensteijn J (2006) Making a rational choice between ovariectomy and ovariohysterectomy in the dog: a discussion of the benefits of either technique. Vet Surg 35, 136-143.

van Nimwegen SA, Kirpensteijn J (2007) Laparoscopic ovariectomy in cats: comparison of laser and bipolar electrocoagulation. J Feline Med Surg 9, 397-403.

Verthelyi D (2001) Sex hormones as immunomodulators in health and disease. Int Immunopharmacol 1, 983-993.

Walker AD, Weaver AD, Anderson RS et al. (1977) An epidemiological survey of the feline urological syndrome. J Small Anim Pract 18, 283-301.

References

Weinberg DS, Inturrisi CE, Reidenberg B et al. (1988) Sublingual absorption of selected opioid analgesics. Clin Pharmacol Ther 44, 335-342.

Weiss JM (1972) Psychological factors in stress and disease. Sci Am 226, 104-113.

Wells DL, Hepper PG (1999) Prevalence of disease in dogs purchased from an animal rescue shelter. Vet Rec 144, 35-38.

Welsh CP, Gruffydd-Jones TJ, Murray JK (2013) The neuter status of cats at four and six months of age is strongly associated with the owners' intended age of neutering. Vet Rec 172, 578.

West M (1974) Social Play in Domestic Cat. Am Zool 14, 427-436.

Wiese AJ, Muir WW (2007) Anaesthetic and cardiopulmonary effects of intramuscular morphine, medetomidine and ketamine administered to telemetered cats. J Feline Med Surg 9, 150-156.

Willeberg P, Priester WA (1976) Feline urological syndrome: associations with some time, space, and individual patient factors. Am J Vet Res 37, 975-978.

Windmill KF, Lee VW (1998) Effects of castration on the lymphocytes of the thymus, spleen and lymph nodes. Tissue Cell 30, 104-111.

Windmill KF, Lee VWK (1999) Influences of surgical castration on the thymus of male rats. J Reprod Immunol 44, 29-39.

Windmill KF, Meade BJ, Lee VW (1993) Effect of prepubertal gonadectomy and sex steroid treatment on the growth and lymphocyte populations of the rat thymus. Reprod Fertil Dev 5, 73-81.

Woolf CJ, Chong MS (1993) Preemptive analgesia--treating postoperative pain by preventing the establishment of central sensitization. Anesth Analg 77, 362-379.

Wright BD (2002) Clinical pain management techniques for cats. Clinical Techniques in Small Animal Practice 17, 151-157.

Wright JC, Amoss RT (2004) Prevalence of house soiling and aggression in kittens during the first year after adoption from a humane society. J Am Vet Med Assoc 224, 1790-1795.

Zoran DL (2010) Obesity in dogs and cats: a metabolic and endocrine disorder. Vet Clin North Am Small Anim Pract 40, 221-239.

Zub D, Berkenbosch JW, Tobias JD (2005) Preliminary experience with oral dexmedetomidine for procedural and anaesthetic premedication. Paediatr Anaesth 15, 932-938.

CURRICULUM VITAE

Curriculum Vitae

Nathalie Porters werd geboren op 3 augustus 1983 in Wilrijk. Ze behaalde haar diploma secundair onderwijs (Latijn-Wiskunde) aan het Sint Ursula Instituut in Onze-Lieve-Vrouw Waver en startte hierna in 2002 met de studies Diergeneeskunde aan de UGent. In 2008 behaalde ze het diploma van dierenarts met grote onderscheiding, en in 2009 het diploma van geaggregeerde voor het hoger secundair onderwijs.

In het daaropvolgende anderhalf jaar werkte zij halftijds als zelfstandige dierenarts in een eerstelijnspraktijk voor gezelschapsdieren en halftijds als lerares wetenschappen in het secundair onderwijs. Geboeid door het wetenschappelijk onderzoek, startte zij in april 2010 een doctoraatstudie bij de vakgroep Geneeskunde en Klinische Biologie van de Kleine Huisdieren, waar zij gedurende 4 jaar onderzoek verrichtte aangaande vroegtijdige castratie van katten. De eerste 3 jaren werden gefinancieerd door de Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselgezondheid en Leefmilieu. Het vierde onderzoeksjaar werd betaald met beurzen van de Facultaire Commissie Wetenschappelijk Onderzoek (FCWO) en het Bijzonder Onderzoeksfonds (Ugent BOF).

Nathalie Porters is auteur en mede-auteur van verschillende wetenschappelijke publicaties in nationale en internationale tijdschriften. Ze nam deel aan verschillende nationale en internationale congressen. Recent behaalde zij het getuigschrift van de Doctoral Schools of Life Sciences and Medicine van de Universiteit Gent.

Publications in Journals

Porters N, de Rooster H, Haesebrouck F (2009). Prophylactic and therapeutic use and abuse of antibiotics in small animal surgery. Vlaams Diergeneeskundig Tijdschrift 78, 315-325.

Peeters EHKA, Porters N, Bols PEJ, Nelissen M, Moons CHP, de Rooster H, Polis I (2012) Anaesthesia in kittens a review of the literature with stress on the possibilities in Belgium. Vlaams Diergeneeskundig Tijdschrift 81**,** 129-137.

Porters N, Bosmans T, Debille M, de Rooster H, Duchateau L, Polis I (2014) Sedative and antinociceptive effects of dexmedetomidine and buprenorphine after oral transmucosal or intramuscular administration in cats. Veterinary Anaesthesia and Analgesia 41**,** 90-96.

Porters N, Polis I, Moons CPH, Duchateau L, Goethals K, Huyghe S, de Rooster H (2014). Prepubertal gonadectomy in cats: different surgical techniques and comparision with gonadectomy at traditional age. Veterinary Record, DOI: 10.1136/vr.102337.

Porters N, de Rooster H, Bosmans T, Baert K, Cherlet M, Croubels S, De Backer P, Polis I. Pharmacokinetics of oral transmucosal and intramuscular dexmedetomidine combined with buprenorphine in cats. Manuscript submitted.

Porters N, de Rooster H, Moons CPH, Duchateau L, Goethals K, Bosmans T, Polis I. Prepubertal gonadectomy in cats: different injectable anaesthetic combinations and comparison with gonadectomy at traditional age. Manuscript in revision.

Porters N, de Rooster H, Verschueren K, Polis I, Moons CHP. Development of behavior in adopted shelter kittens following gonadectomy performed at an early age or at a traditional age. Manuscript in revision.

Porters N, Polis I, Moons CPH, Van de Maele I, Ducatelle R, Goethals K, Duchateau L, de Rooster H. Relationship between age at time of gonadectomy and health problems in adopted shelter kittens. Manuscript submitted.

Porters N, Moons CPH, Polis I, Dewulf J, de Rooster H. The Sterycat project: what's next? - the opinion of participating shelter veterinarians and caretakers on early age neutering in cats. Manuscript in preparation.

Conference Contributions

<u>Porters N</u>, Bosmans T, Debille M, de Rooster H, Duchateau L, Polis I. Oral transmucosal and intramuscular administration of dexmedetomidine and buprenorphine in healthy adult cats. Accepted for poster presentation, Association of Veterinary Anaesthetists Spring Meeting, 22-23 March 2012, Davos, Switzerland.

<u>Porters N</u>, de Rooster H, Bosmans T, Baert K, Cherlet M, Croubels S, De Backer P, Polis I. Feline pharmacokinetics of oral transmucosal and intramuscular dexmedetomidine and buprenorphine. Accepted for poster presentation, Voorjaarsdagen, 18-20 April 2013, Amsterdam, Nederland.

<u>Porters N</u>, de Rooster H, Polis I, Verschueren K, Moons CHP. Influence of prepubertal gonadectomy on the behaviour of shelter kittens at one month after adoption. Abstract accepted for poster presentation, Voorjaarsdagen, 17-19 April 2014, Amsterdam, Nederland.

DANKWOORD

Dankwoord

Waar begint en eindigt een dankwoord…

Eerst en vooral, aan iedereen die zijn steentje heeft bijgedragen tot dit mooie eindresultaat, dankuwel!

En dat zijn een heleboel mensen: promotors, collega's van onze vakgroep en andere vakgroepen, asielmedewerkers en –dierenartsen, katteneigenaars, studenten van de hoge school, leden van de begeleidingscommissie, het FOD, sponsors, ...

Het Sterycat project was er ééntje waarbij vele partijen betrokken waren, en dat heeft mij op organisatorisch en communicatief vlak veel kennis bijgebracht.

Mijn hoofdpromotor, Prof. de Rooster, Hilde, dankzij jou tegen te komen op een bijscholing met je enthousiasme over het Sterycat-project, heb ik de overstap gemaakt van de praktijk naar het wetenschappelijk onderzoek. Je was reeds mijn promotor van mijn thesis in het laatste jaar diergeneeskunde, en het was superfijn om deze samenwerking door te zetten. Doorheen de kliniek-uren, de operaties, de lessen, …. maakte je steeds tijd voor me vrij. Als ik je nodig had, dan weet ik dat ik op je kon rekenen voor de begeleiding en het nodige advies. Je bleef ook steevast geloven in het project, want in het 1^e jaar leefde er wel wat commotie over het onderwerp 'vroegcastratie van asielkatten' in de diergeneeskundige wereld. Zelfs prof. Gasthuys wil ik nog even bedanken om dit onderwerp te verdedigen bij de Orde der Dierenartsen. Gelukkig is die storm over vroegcastratie van katten doorheen het project gaan liggen, en kon ik van in het begin de sympathie en de medewerking ervaren van menig asielmedewerkers en asieldierenartsen in Vlaanderen. Dankzij deze fantastische mensen heb ik veel geleerd over asielbeleid en heb ik het Sterycat-project met 800 kittens tot een mooi einde kunnen brengen, nogmaals een heel dikke dankuwel en keep up the good work!

Mijn co-promotoren, prof. Polis en Dr. Moons, ook jullie wil ik graag bedanken voor de ondersteuning end de input bij het verwezenlijken van deze thesis.

Prof. Polis, dankuwel voor de 'anaesthetische en analgetische' ondersteuning tijdens het project. U was altijd enthousiast over het project en over meerdere ideeën die ik erbij betrok, namelijk de aankoop van een analgesie-meter (de befaamde 'prod-plus') en de bijkomende pharmacodynamische en –kinetische studie over dexmedetomidine plus buprenorphine. De vrijheid die ik kreeg om mijn ideeën uit te voeren hebben me erg gestimuleerd.

Dr. Moons, Christel, dankjewel voor al het 'gedrags'advies. In het begin heb ik mij nog serieus moeten inwerken op vlak van gedrag bij katten, want ik geef toe, daar was ik niet zo vertrouwd mee. Doorheen de loop van het project, heb ik hierover erg veel bijgeleerd en mede door jouw enthousiasme, wilde ik hier zelf ook echt veel meer over weten. Ik bekijk de 'Felidae' alvast met andere ogen en vind het zalig om het gedrag van mijn eigen kat Nutella nu ook (bijna) helemaal te begrijpen.

Onze meetings waren altijd gezellig en productief: samen brain-stormen over ideeën, de analyses en resultaten bediscussiëren, … En als ik even het noorden kwijt was met onze gigantische data-set, dan werd ik snel weer op het juiste pad gewezen met jouw constructieve feedback. Slechts een deel van de data is gebruikt in deze publicatie, en ik hoop alvast dat alle beschrijvende data nog verder-bestaan mogen krijgen in het ethologie labo. Ik heb het ook enorm gewaardeerd dat jij een trouwe supporter was bij mijn presentaties voor de asielmedewerkers en –dierenartsen, tijdens de kattendagen van de KaHo en tijdens het symposium van het FOD.

Kortom, aan alle promotoren, een dikke dankuwel voor hun goede ondersteuning en begeleiding!

Ik wil ook graag iedereen van onze vakgroep bedanken voor de leuke momenten en gezellige babbeltjes de afgelopen jaren, en ook voor hun interesse in het onderzoek (dat werkte erg motiverend en motivatie is heel belangrijk bij een doctoraat). Ik denk dat bijna iedereen wel eens een sterycatje in zijn/haar handen gehad heeft of op hun eigen manier bijgedragen hebben aan het Sterycat project (o.a. Cindy en Dominique, thanks voor het financiële gedeelte van het onderzoek).

Ik denk ook nog graag even terug aan de vele gezellige momenten achteraan in de hospi. Elien en Bieke, de maatjes van het FIV-project, de vele grappige en leuke babbeltjes, en hilarische kitten/katten-momentjes zal ik missen. Samen met Ann, waren jullie altijd bereid om even te assisteren … . Dankjewel!

Dan de container, een uniek eiland op zich. Stéphanie, ik hoor je al denken, wat zal ze nu schrijven over de 'container'… . Ik geef toe dat ik de tropische temperaturen in de zomer en de vriesmomenten in de winter, af en toe nog met een heerlijke 'aroma', niet echt zal missen.

De leuke mensen en de gezellige rommel in de container daarentegen, dat zal ik wel erg missen! Stéphanie, Hilde, Alessandra, Alejandro, Adriaan, Marjan, Matam en Ilona, … jullie waren heel fijne collega's en ik wens jullie allemaal nog het allerbeste toe!

Stéphanie, jij bent uiteraard meer dan een bureau-genoot. Nog eens een dikke merci voor alle babbels, voor al je hulp tijdens het onderzoek en tijdens het schrijven, en alle lekkere taartjes die je meebracht om te proeven, … . Je nuchtere en tegelijk enthousiaste kijk op de zaken werkte erg stimulerend! Ik wens je nog een super zwangerschap toe en ben benieuwd om Boebel in september te komen bewonderen (maar uiteraard spreken we daartussen ook nog af). Trouwens, je weet me ook te vinden als ik ook iets voor jouw doctoraat moet nalezen hé.

Antita, Mariëlla, Valentine, Liesbeth, … de gezellige resto-momentjes waren altijd heel ontspannend en deugddoend. Antita en Mariëlla, onze pizza/pasta-avondjes en Martini/ Porto momentjes mogen we zeker niet verloren laten gaan! En dan zijn er nog vele andere mooie herinneringen… De congres momenten met Antita, Tony, Tim B en Tim W, Sanne en Bram… het waren goede tijden in Davos. De voorjaarsdagen in Amsterdam, Katrien, het congres was interessant, maar onze mini city-trip in Amsterdam en de vele trein-uurtjes waren legendarisch. Het was een gezellige trip en leuke afsluiter op het einde van mijn doctoraat!

Thanks for all the talks! En veel geluk in jullie verdere carrière!

Graag dank ik ook alle leden van de begeleidingscomité tijdens het Sterycat project, en het FOD voor de financiële ondersteuning gedurende 3 onderzoeksjaren. Anneleen, ik ben blij dat ik jou leren kennen heb via het Sterycat-project en wens je nog enorm veel succes met Felinova, goed bezig! In het bijzonder wil ik ook graag Mr. Van Tilburgh bedanken, die zich ook steeds het reilen en zeilen van het project aangetrokken heeft en ook meermaals asielen aangemoedigd heeft om mee te werken aan het project. Ook vandaag bent u aanwezig als lid van de leescommissie, hetgeen ik erg waardeer.

Natuurlijk ben ik ook Professor Croubels, Professor Martens, Professor Diederich, Dr. Rijsselaere en Dr. Bosmans heel erg dankbaar voor het nalezen van mijn thesis en voor jullie constructieve feedback. Dankzij jullie opmerkingen en inzichten kon ik steeds weer het doctoraat vanuit een andere invalshoek bekijken en over-denken.

Last, but not least, my dear friends and family.

Mijn liefste vrienden van Waver/Mechelen en van diergeneeskunde/Gent, mijn liefste (schoon)familie, ik ben jullie erg dankbaaar voor alle ontspannende en fleurige momenten samen! Jullie oprechte interesse in het verloop van mijn doctoraatsonderzoek heb ik altijd gewaardeerd en hebben mij zeker geholpen om dit werk tot een mooi einde te brengen. Ik vind het trouwens heel fijn dat jullie vandaag tijd vrijgemaakt hebben om deze verdediging bij te wonen!

Elke, voor jou tikt de klok niet meer verder, maar jij, als katten-liefhebber, zou hier anders zeker op de 1^e rij gezeten hebben (zoals je voor Sophie en mij tijdens onze halve-marathons supporterde). Eveneens bobonne en moeke, jullie mogen deze dag niet meer meemaken, maar ik weet zeker dat jullie fier geweest zouden zijn.

Mijn ouders, mamsie en papsie, ik dank jullie graag nogmaals om mij de mogelijkheid te geven om te studeren en om mij ter aller tijde te steunen. En wees gerust, nu stop ik met 'studeren' en ga ik 'echt werk' zoeken.

En mijn allerliefste snoepie/bollie Christian en mijn allerliefste poppemie Magali, mijn twee oogappels, mijn gezinnetje, … . Jullie waren mijn toeverlaat ter aller tijde, vooral het laatste jaar met de nodige stress-momenten waren niet altijd even gemakkelijk. Magali, met je lieve gebaren en je schaterlach, doe je me altijd even alle zorgen vergeten en word ik er terug aan herinnerd om echt van alle kleine zaken in het leven te genieten. Wat is het zalig om mama te zijn … .
Bollie, mijn rots in de branding, … ik weet dat je een hekel hebt aan te lange (publieke) dankwoorden, dus hou ik het hier kort, maar mag jij nog wel een langere versie in jouw boekje lezen. Dank je voor alle hulp en steun tijdens het doctoraat, ik zie je zooooooooo graag! Oh, en ik zou nog bijna de mascotte van ons gezinnetje vergeten: Nutella… jij bent de echte levende herinnering van mijn doctoraat in ons gezinnetje! Dat je nog vele jaren bij ons mag vertoeven!

<div align="right">

Nathalie

19 mei 2014

</div>