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A prospective study to investigate the impact of neutering bitches prepubertally or post-pubertally

By

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Abstract

Contradictory findings in the literature relating to the effects of timing of neutering on bitch behaviour and health make decision-making regarding neutering timing difficult. Most literature that examines the effect of neutering timing has used age rather than the onset of puberty as the primary variable. The aims of this study were to 1) conduct scoping reviews to identify and chart the evidence on the effects of neutering timing in relation to puberty on the behaviour and health of female dogs, and 2) conduct a prospective study to investigate the effects of neutering timing in relation to puberty on physical development, behaviour, progression through an assistance dog training programme and health for female dogs.

The scoping reviews identified only one study that considered behaviour. The study examined behaviour retrospectively and reported no differences between bitches neutered pre or post-pubertally. For health, one study was identified that examined neoplasia. In that case-control study malignant mammary neoplasia was less likely in bitches neutered before puberty. Additionally, six studies were identified that considered urogenital disease related to pubertal status at neutering, and three reported significant results. Two studies presented conflicting results for urinary incontinence, and one study suggested that vulval development may be negatively affected by prepubertal neutering. No studies were found that considered atopic dermatitis, developmental orthopaedic disease or obesity.

For the prospective study, 306 Labrador and Golden Retriever crossbreed bitches were neutered before (at six months of age, PPN, n=155) or after puberty (after the first oestrus, Control, n=151). Data on growth and physical development were collected from assessments conducted at six and 17 months of age. Behavioural data were collected using questionnaires completed at

one and three years of age. Data for progression through training and for health were extracted from an electronic database.

PPN bitches had greater changes in height (mean difference=2.039 cm, P<0.001) and smaller changes in vulval size (length: mean difference=−0.377 cm, P<0.001, width: mean difference=−0.221 cm, P<0.001) between six and 17 months of age than Control bitches. At 17-months, more PPN bitches had vulvas that appeared juvenile (P<0.001) and recessed (P=0.005).

Pubertal status at neutering was not associated with behavioural factor scores at one or three years of age, and there was no difference in the number of PPN (n=97) and Control (n=98) bitches that qualified as guide dogs. Examination of health data suggested detrimental associations between prepubertal neutering and musculoskeletal disease and immune disease. Cruciate disease occurred in significantly more PPN (n=11) than Control bitches (n=1; P=0.009). For osteoarthritis (P=0.016) and having at least one musculoskeletal disease (P=0.023) there were significant differences indicated between survival curves. The first diagnosis of atopy was reported at significantly older ages for PPN (3.9 ± 0.6 years) than Control bitches (1.6 ± 0.3 years; P=0.008), and for otitis (P=0.008) there was a significant difference between the survival curves.

Based on the findings presented, neutering after puberty is advised for bitches of the breeds studied, and potentially after 12 months of age based on the work of others.

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Ethical approval

The study received ethical approval on 06/01/2012 by the School of Veterinary Medicine and Science, Ethical Review Committee. The Ethical Review Number is 501 120106.

Publications arising from thesis

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Chapter 1. Introduction

1.1 NEUTERING IN DOGS

The neutering of dogs, including whether to neuter, the best age to neuter and which neutering option to choose (medical, surgical, and surgery type), has been the subject of research and debate for decades (van Goethem *et al.*, 2006; Root-Kustritz, 2007; Reichler, 2009; McKenzie, 2010; Palmer *et al.*, 2012; Root-Kustritz, 2014; Howe, 2015; Oberbauer *et al.*, 2019; Urfer and Kaeberlein, 2019). Neutering dogs is common practice in the UK with 70% of dogs, and 75% of female dogs, reported to be neutered (PDSA, 2022). While the UK public generally supports routine neutering (Wongsaengchan and McKeegan, 2019), the ethical considerations of neutering dogs based on the associated benefits and risks have been discussed (Palmer *et al.*, 2012; Sandøe *et al.*, 2015), with some countries considering neutering to be a form of sexual mutilation (Adams, 2016; Marsh, 2019).

The age at which dogs reach sexual maturity can be as early as six to seven months (Stubbs *et al.*, 1995; Kilborn *et al.*, 2002; Feldman and Nelson, 2004). Neutering prior to sexual maturity and in some cases early neutering (six to 14 weeks of age, Kustritz, 2002) is recommended by some authors and organisations (Griffin *et al.*, 2016). Others have suggested that a more individual approach to neutering and the age at which to neuter is encouraged that takes into account circumstances (e.g. controlled pet home compared to in shelter for adoption), breed, gender and associated disease risk (Spain *et al.*, 2004; Root-Kustritz, 2007; Sanborn, 2007; McKenzie, 2010; Palmer *et al.*, 2012; Hart *et al.*, 2014; Houlihan, 2017; Hart and Hart, 2021).

The variation within the veterinary literature is mirrored by the disparity in veterinary advice and practice with regard neutering and the optimal age to neuter (Diesel *et al.*, 2010). Numerous factors are often considered when veterinarians are advising clients, and

when pet dog owners are deciding whether, and when, to neuter their dogs. These include the risks of surgery and anaesthesia, effects on growth and physical development, risk of disease, future reproductive capability, longevity and effect on behaviour.

1.2 RISKS OF SURGERY

Perioperative outcomes are important to owners when making neutering decisions and have been investigated by various authors. Bitches may experience stress and pain with surgical neutering (Väisänen, 2006; Palmer *et al.*, 2012), and there are short-term surgical and anaesthetic risks (Yates and Leedham, 2019). Reported peri- and postoperative complication rates following ovariohysterectomy vary between 4 and 35% (Pollari and Bonnett, 1996; Pollari *et al.*, 1996; Burrow *et al.*, 2005; Muraro and White, 2014), although retrospective examinations of data from veterinary records rather than standardised prospective studies may lead to underreporting of complications in some cases. Variation in reported complication rates between studies may also be influenced by differences in study methodology and inclusion criteria, breeds and populations of dogs used, surgeon experience and surgical technique (Burrow *et al.*, 2005; Blacklock *et al.*, 2016; Benavides Melo *et al.*, 2018).

Intraoperative haemorrhage is reported to be the most common complication during ovariohysterectomy, occurring in between 1 and 79% of cases, the latter higher incidence being reported in an early study (Berzon, 1979; Burrow *et al.*, 2005; Muraro and White, 2014; Belluzzi *et al.*, 2021). Postoperative complications are most commonly related to the surgical wound and are suggested to occur more frequently than intraoperative problems, and include swelling, discharge, seroma, dehiscence, granulomatous reaction, and self-inflicted trauma (Berzon, 1979; Pollari and Bonnett, 1996; Howe, 1997; Burrow *et al.*, 2005; Adin, 2011; Akinrinmade and Eyarefe, 2012; Muraro and White, 2014, Blacklock *et al.*, 2016).

Many factors have been suggested to influence the occurrence of intra and postoperative complications relating to ovariohysterectomy in bitches. These include surgical and anaesthesia times, bitch age at the time of surgery, and surgeon experience and technique (Howe, 1997; Beal *et al.*, 2000; Nicholson *et al.*, 2002; Burrow *et al.*, 2005; Howe, 2006; Pollari *et al.*, 2006; Peeters and Kirpensteijn, 2011; Blacklock *et al.*, 2016; Airikkala-Otter *et al.*, 2018).

The complication rate reported following gonadectomy in dogs and cats that underwent surgery at ≥24 weeks of age (733 animals, 10.8%) was significantly higher than that for dogs and cats that underwent surgery at <12 weeks of age (723 animals, 6.5%; Howe, 1997). Howe (1997) proposed that these results suggest that dogs can be neutered before puberty without an increased risk of complications during or in the seven days following surgery. However, whether the animals in the study had actually reached puberty was not confirmed. Therefore, it is possible that the group that were neutered at ≥24 weeks of age could have included prepubertal animals.

No previous studies have investigated the incidence of peri- or postoperative complications for ovariohysterectomy in bitches neutered before or after known puberty. Published studies have been based on age rather than known stage of reproductive development at the time of neutering. As part of the project reported in this thesis, but not reported here, a study was undertaken to compare complication rates during surgery, in the immediate postoperative period and during the 14 days following surgery, in bitches neutered either before or after puberty (Moxon *et al.*, 2023a). In that study, a small but significant difference in surgery duration was reported (mean of 17.4 minutes) for bitches neutered prepubertally than for those neutered after puberty (mean of 18.9 minutes). There were no significant differences in the incidence of any perioperative complications, although just over

three times the number of bitches neutered post-pubertally had intraoperative complications compared to those neutered prepubertally. In the 14-day postoperative period, bitches neutered post-pubertally had a higher incidence of wound discharge and a wound healing score of '2' (both wound redness or swelling and wound discharge reported). Additionally, more bitches neutered post-pubertally showed inappetence. Considering outcomes relating to the perioperative period in isolation, the results suggest that the prepubertal neutering of bitches could be a suitable strategy for assistance dog organisations. However, the effects on longer term outcomes, such as future growth, health and behaviour should also be considered.

1.3 EFFECT ON GROWTH AND DEVELOPMENT

In dogs, longitudinal skeletal growth is regulated by a combination of genetic, hormonal, environmental and biomechanical factors (Nap and Hazewinkel, 1994; Van der Eerden *et al.*, 2003; Von Pfeil *et al.*, 2009b). Oestrogen is associated with inhibition of the growth hormone–insulin-like growth factor axis and with closure of the physes at skeletal maturity (potentially acting directly to influence growth plate chondrocytes) (Van der Eerden *et al.*, 2003; Makielski *et al.*, 2019). Dogs can reach sexual maturity as early as six to seven months of age (Stubbs *et al.*, 1995; Hewitt and England, 1999; Kilborn *et al.*, 2002; Feldman and Nelson, 2004). Early removal of the gonads has been associated with delayed closure of the radial and ulna physes, extended time to reach a growth plateau and longer radial and ulna bone length in male and female dogs (Salmeri *et al.*, 1991) and cats (May *et al.*, 1991; Stubbs *et al.*, 1996; Root-Kustritz *et al.*, 1997). While radiographic measurements were undertaken in these studies, therefore could be considered reliable, there were commonly less than 10 animals in each study group. The work that is commonly referenced in the dog (Salmeri *et al.*, 1991) compared neutered versus entire mixed-breed bitches and bitches neutered at different ages. The authors reported a delay in the closure of the distal radial growth plate for those neutered at

seven weeks (seven bitches) and seven months (four bitches) of age compared to bitches left entire (six bitches). Furthermore, the results suggested that closure was delayed for longer in bitches neutered at seven weeks compared to seven months of age, with longer radial and ulna length in bitches neutered at seven weeks of age. Whether or not the differences in time of growth plate closure and radial length between neutered and entire females are of clinical significance is unclear. Bitches neutered at seven months by Salmeri *et al.* (1991) may not have reached sexual maturity, therefore the effects on bitches neutered before and after puberty remain unknown. In contrast, when radial lengths were compared between 11 mixed-breed bitches ovariohysterectomised at 10 weeks of age and 10 bitches that underwent sham surgery by Sontas and Ekici (2007), there were no significant differences and the authors concluded that skeletal development was not impacted by prepubertal gonadectomy. However, final measurements in this study were taken at 24 weeks of age, by which time growth may not have plateaued.

Oestrogen is also essential for the development of the external genitalia in female dogs (Verstegen-Onclin and Verstegen, 2006; Morgan, 2008; Schafer-Somi *et al.*, 2014). Reduction of oestrogen due to gonadectomy may prohibit normal vulval development, particularly if removal of the ovaries occurs prior to puberty (Verstegen-Onclin and Verstegen, 2006; Nelson and Couto, 2009). Salmeri *et al.* (1991) measured dorsoventral length of the vulvar commissure and reported that subjectively, vulvas were smaller for bitches neutered at seven weeks (n=7) and seven months of age (n=4) than in entire bitches (n=6), although numbers of bitches were small and statistical analysis was not performed. This raises questions regarding the effect that length of exposure to ovarian hormones has on vulval development. Verstegen-Onclin and Verstegen (2006) presented data for 27 bitches with a history of recurrent urinary tract infection (UTI), vaginitis and/or perivulvar dermatitis. On examination, 85% of the bitches had a recessed or

hypoplastic vulval appearance and 40% had a perivulvar skin fold. Twenty-five of the bitches were neutered and 21 were neutered before or around the time of puberty. The authors hypothesised that neutering prior to puberty decreases the release of oestrogen and prevented normal development of external genitalia.

In bitches, vulval development and conformation, the prevalence of vulval abnormalities and the impact of surgical neutering on these factors, as well as on physical height, is poorly documented. Although neutering had been suggested as a possible causal factor for developmental abnormalities in some studies, there have been no prospective studies investigating how neutering bitches before or after known puberty may affect these outcomes. Further research is required into relationships between neutering, age and pubertal status at the time of neutering and physical development. Such information would be useful to assist with decision making on the optimum time for neutering bitches.

1.4 RISK OF DISEASE

There are numerous, commonly retrospective, studies investigating the effect of neutering (and age at neutering) on the occurrence of diseases including neoplasia, musculoskeletal disease, urinary incontinence (UI), diseases affecting the genitourinary tract and obesity. Commonly veterinarians suggest a relationship between health outcomes and pubertal status at neutering, but there are few studies that categorise bitches by pubertal status at the time of neutering. Therefore, the evidence to support veterinarians is lacking. One factor often mentioned is mammary neoplasia, however there is only one study that considers pubertal status at neutering and future incidence of the disease (Schneider et al., 1969). Studies investigating the effect of neutering (and age at neutering) have considered both large populations of dogs of many breeds and single breeds, presenting complex age- and breed-specific findings that infer both benefits and adverse effects of neutering, dependant on the population studied and disease

investigated. In some cases, differing results have been presented for the same breed and sex of dog regarding the incidence of the same disease, these disparities will be presented within the following section.

1.4.1 Neoplasia

Neoplasia is common and is a leading cause of death in dogs. They are multifactorial in origin and have been suggested to be associated with age at neutering (Houlihan, 2017). In a study of the cause of death of more than 40,000 dogs in a veterinary teaching hospital, neutered dogs were reported to be more likely to die from neoplasia than entire dogs; however, the age at neutering, length of time between puberty and neutering and reproductive history of the dogs was not reported (Hoffman *et al.*, 2013). Studying the length of exposure to gonadal hormones has shown different conclusions compared to categorising the dogs as neutered or entire when analysing data for the effect of neutering (Waters *et al.*, 2011).

Neutered compared to entire female dogs have been reported to be at increased risk for some cancers, such as cardiac neoplasia, mast cell tumour, lymphoma/lymphosarcoma, and osteosarcoma (Ru *et al.*, 1998; Ware and Hopper, 1999; Hart *et al.*, 2014; Zink *et al.*, 2014; Belanger *et al.*, 2017). Others have reported decreased risk of certain cancers for neutered bitches, such as for mammary neoplasia where there are suggested protective effects of neutering, especially earlier neutering (Schneider *et al.*, 1969; Sonnenschein *et al.*, 1991; McKenzie, 2010; Beaudu-Lange *et al.*, 2021). A systematic review of the literature by Beauvais *et al.* (2012a) identified only weak evidence linking neutering and a reduction in the occurrence of mammary neoplasia, although the evidence did suggest that neutering before 2.5 years of age was related to a decrease in malignant mammary neoplasia. Since the latter review was undertaken, further published work has examined the incidence of mammary neoplasia in bitches. For entire female German Shepherds, Golden Retrievers and Labradors, the occurrence of

mammary neoplasia was low (4.1%, 0% and 1.4% respectively), mammary neoplasia was not present in any Golden Retriever or Labrador bitches neutered <2 years of age and in only 1.5% of German Shepherd bitches neutered <2 years of age. For bitches neutered between two and eight years of age the incidence of mammary neoplasia was 3.9% in German Shepherds, 3.5% in Golden Retrievers and 2.0% in Labradors (Hart *et al.*, 2014; Hart *et al.*, 2016).

The effect of neutering on other types of neoplasia has been less well described. Transitional cell carcinoma (also called urothelial carcinoma) is rare in dogs, but female dogs are reportedly at four times greater risk if neutered (Reichler, 2009). Neoplasia affecting the reproductive organs, including uterine, cervical and ovarian neoplasia, are again rare in female dogs, but are reportedly reduced with neutering, and while lower urinary tract neoplasia may be more common in neutered bitches, the low incidence (<1%) may mean they are less likely to influence neutering decisions (Sanborn, 2007; Houlihan, 2017). Some authors have shown no association between neutering and the risk of certain cancers, and results can vary between breeds and sexes of dog (Hart *et al.*, 2016; Hart *et al.*, 2020a,b).

Considering the timing of neutering, associations with development of other types of neoplasia have been reported. Torres de la Riva *et al.* (2013) reported more favourable outcomes for earlier neutering for haemangiosarcoma and mast cell tumour. In that study, 242 female Golden Retriever bitches were neutered before (n=172) or at/over 12 months of age (n=70) and were studied to nine years of age. They found no significant difference between age at neuter groups in the incidence of lymphoma, although for this cancer, a higher percentage of bitches in the early (5.9%) rather than late neuter group (1.4%) were diagnosed. Cooley *et al.* (2002) found increased risk for earlier neutering; Rottweilers with the shortest time entire (earliest age at gonadectomy) were at higher risk of

developing bone sarcoma, with a 1.4% increase in risk for each month reduction in being entire (calculated for males and females combined, n=683). Female Rottweilers neutered at <1 year of age were three times more likely than entire females to develop bone sarcoma (Cooley *et al.*, 2002). However, whether there were any dogs with bone sarcoma diagnosed before neutering and whether such cases were excluded from analysis was not clear.

The differences between breeds should be considered when examining the effect of neutering and neutering timing on the development of neoplasia. For instance, in Golden Retrievers and Labradors admitted to a veterinary teaching hospital, the incidence of haemangiosarcoma, lymphosarcoma and mast cell neoplasia was affected by neutering and neutering age (Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014). However, the incidence of these types of neoplasia in female German Shepherds admitted to the same hospital was similar in those that were neutered at any age compared to those left entire (Hart *et al.*, 2016). The risk of having at least one neoplasia in entire Golden Retrievers and Labradors was 3%, this increased to between 8% and 14% dependant on age at neutering for Golden Retrievers and to 5.6% for Labradors neutered between two and eight years of age (Hart *et al.*, 2014). In female Golden Retrievers, the incidence of haemangiosarcoma increased in females neutered at >1 year of age (7.4%) compared to <1 year of age (1.8%) and entire females (1.6%) in a study by Torres de la Riva (2013). However, the same significant effect was not present when data for a larger sample of Golden Retrievers from the same database was examined, considering age of neutering as <6 months of age, six to 11 months of age, 12 to 24 months of age, two to eight years of age or entire (Hart *et al.*, 2014). A study in a different population of dogs, which only examined the effect of neutering, reported an increased incidence of haemangiosarcoma in neutered German Shepherds, Golden Retrievers and Labradors, and an increased incidence of aortic body neoplasia in German Shepherds (Ware and Hopper, 1999). For mast cell neoplasia, there

was a significant effect of age at neutering for female Golden Retrievers neutered at <1 year (2.3%) and >1 year (5.7%) (Torres de la Riva *et al.*, 2013), and neutered at <6 months (2.9%), 12 to 24 months (3.1%) and two to eight years of age (5.7%); all had significantly higher incidences mast cell neoplasia than entire females (approx. 0%, Hart *et al.*, 2014). While no significant effect of neutering on the incidence of lymphosarcoma in female Golden Retrievers was found by Torres de la Riva *et al.* (2013), female Golden Retrievers neutered at six to 11 months of age had significantly higher incidence of lymphosarcoma (11.0%) than entire bitches (1.8%) in a larger study published one year later (Hart *et al.*, 2014). For female Labradors, despite the overall incidence of neoplasia being affected by age at neutering, no one type of neoplasia for bitches neutered in any age group was significantly increased compared to entire bitches (Hart *et al.*, 2014). Data for these studies were only examined up to nine years of age, therefore some types of neoplasia may have occurred later in life. Nevertheless, the differences between and within studies demonstrate the complicated and inconsistent nature of findings related to the effect of neutering and age at neutering on the likelihood of having different types of neoplasia in the female dog. It is likely that there is some effect, but that other genetic and environmental factors are also influential.

1.4.2 Musculoskeletal disease

The effect of neutering on musculoskeletal disease has been considered in many studies, with inconsistencies and complexities within the results complicated by the multifactorial nature of the diseases and differences between breeds. In addition, interpretation of the results when considered alongside data for other diseases, such as the likelihood of developing neoplasia, can be complex.

One of the most commonly studied diseases is cranial cruciate ligament (CCL) rupture, and an association with neutering has been demonstrated (Slauterbeck *et al.*, 2004; Witsberger *et al.*, 2008;

Belanger *et al.*, 2017). For female Golden Retrievers and German Shepherds neutered at <1 year of age, the incidence of CCL rupture was between 4.6 and 10.9% compared to 0% to 0.6% in entire females (P<0.05; Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014; Hart *et al.*, 2016). However, in female Labradors neutered at <1 year of age, there was no significant difference in incidence of CCL rupture (5% in neutered and 2.3% in entire; Hart *et al.*, 2014). Spain *et al.* (2004) found no effect of age at neutering on incidence of CCL rupture, however this study included many breeds, and the breed-specific findings suggested by others, had breed not been accounted for in the analysis, may have masked any effect. In a study of 3,218 dogs seen at an orthopaedic veterinary practice, 3.5% had a rupture of the anterior cruciate ligament and the incidence was greater in neutered (5.2%) than entire females (2.4%, odds ratio 2.19, Slauterbeck *et al.*, 2004).

In German Shepherds, the risk of having at least one joint disorder (elbow dysplasia, hip dysplasia or CCL rupture) was increased significantly in females neutered at <1 year of age (16.4%) compared to entire females (5.1%; Hart *et al.*, 2016). The same relationship was evident for Labradors and Golden Retrievers, with a 5% risk of at least one joint disorder in entire females compared to between 10% and 20% in females neutered before 12 months of age (Hart *et al.*, 2014). In the German Shepherd and Golden Retriever, the main cause for this difference was the increase in incidence of CCL rupture. In the Labrador, the increased incidence of hip dysplasia in neutered females was the factor. There was no significant increase in the incidence of hip dysplasia in neutered female Golden Retrievers or elbow or hip dysplasia in German Shepherds neutered in any age group (Hart *et al.*, 2014; Hart *et al.*, 2016).

Howe *et al.* (2001) studied the long term outcome of neutering 269 dogs at <24 weeks of age (n=175) or ≥24 weeks of age (n=94). There was no significant difference in the incidence of

musculoskeletal problems or hip dysplasia between the two groups, however the dogs were followed for approximately four years, therefore disease may not yet have developed. In addition, the groups were not separated by reproductive development and puberty but by age at neuter, which was estimated in some cases. In contrast, in a study of dogs neutered at similar ages (<5.5 months of age, n=948, or ≥5.5 months of age, n=894) the incidence of hip dysplasia was increased in dogs neutered in the younger age group (6.7% compared to 4.7%). Furthermore, dogs with hip dysplasia that were neutered ≥5.5 months of age were reportedly three times more likely to be euthanised as a result, suggesting a milder form of the disease may be present in dogs neutered in the younger age group (Spain *et al.*, 2004).

1.4.3 Urogenital disease

Evidence for a relationship between urinary incontinence (UI) and neutering is inconsistent and inconclusive. The results from some studies have indicated that an association may exist; in 77 female dogs with UI, signs were reportedly present in 26% prior to neutering while signs developed after neutering in 74% (Nickel *et al.*, 1999). The relative risk of acquired UI in neutered bitches compared to entire bitches was reported as 7.8 by Thrusfield *et al.* (1998), with confidence intervals indicating that between 62% and 96% of cases may be caused by neutering. Neutering timing has also been proposed to affect the incidence of UI but the direction of the effect is unclear; some authors have suggested that the risk is reduced in bitches neutered at an earlier age or before puberty (Stocklin-Gautschi *et al.*, 2001; Veronesi *et al.*, 2009) whilst others suggest that the risk is increased for earlier neutered bitches (Spain *et al.*, 2004; Lutz *et al.*, 2019; Pegram *et al.*, 2019a). A systematic review of the literature by Beauvais *et al.* (2012b) identified some evidence of an association and an effect of age, with early neutered bitches perhaps being at greater risk. They concluded that the evidence for a relationship between neutering and UI was weak.

The risk of pyometra is removed in neutered bitches (Sanborn, 2007), while in entire bitches it has been reported to be 2.5% in German Shepherds, 1.8% in Golden Retrievers and <4% in Labradors followed to eight years of age (Hart *et al.*, 2014; Hart *et al.*, 2016). The risk of cystitis has been considered rarely by authors examining the effects of neutering, however Spain *et al.* (2004) reported a higher incidence in 499 female dogs neutered at <5.5 months of age compared to 484 female dogs neutered at ≥5.5 months of age (hazard ratio 2.8).

1.4.4 Obesity

Several studies have reported an association between neutering and obesity in dogs (McGreevy *et al.*, 2005; Colliard *et al.*, 2006; Lund *et al.*, 2006; Courcier *et al.*, 2010). There are few studies that consider the impact of age at neuter on future weight status in dogs. Spain *et al.* (2004) demonstrated decreasing risk of overweight body condition with decreasing age at neuter for 1,659 male and female dogs of various breeds, raising the possibility that earlier neutering can reduce the risk becoming overweight. However, Simpson *et al.* (2019) suggested that dogs neutered at 6-12 months of age had an increased risk for overweight/obesity compared to those neutered at >12 months of age, but found no difference in age at neuter between dogs neutered at <6 and 6-12, or >12 months of age. Obesity is a major and growing concern in the dog (German, 2022), thus it is important to understand whether the likelihood of becoming overweight or having obesity differs for dogs that were neutered before or after puberty.

1.5 LONGEVITY

There are few studies investigating the impact of neutering on lifespan in dogs and no study considers age or pubertal status at neutering and effect on lifespan (Urfer and Kaeberlein, 2019). As with other variables, there are contrasting reports from studies with differing methodologies regarding the effect of neutering female dogs on longevity (McKenzie, 2010). Given the potential for

neutered dogs to be more likely to develop certain diseases that can impact quality and length of life, it seems plausible that longevity in neutered dogs may be reduced. However, it is also possible that an increase in longevity could result in higher numbers of dogs alive at older ages available to be diagnosed with disease (Root-Kustritz, 2014). Entire female dogs may have had different roles and functions, or may belong to owners with varying financial circumstances or beliefs regarding neutering or animal welfare. All these factors may themselves impact longevity.

Studies have suggested that neutered bitches have increased longevity compared to those that are entire (Root-Kustritz, 2007; Hoffman *et al.*, 2013), but these studies commonly consider reproductive status at the time of death rather than length of exposure to gonadal hormones and age at neutering. Reichler (2009) suggested that increased longevity may be attributable to decreased incidence of disease, a lower risk of associated behaviours that may result in injury, also suggested by Hoffman *et al.* (2013), or enhanced care of neutered dogs. Life expectancy was reported to be increased by 26.3% in neutered female dogs in an investigation of life span and cause of death in 40,000 dogs that died during a 20-year period in a veterinary teaching hospital. The mean age reached by neutered dogs (male and female) was 9.4 years compared to 7.9 years for entire dogs (Hoffman *et al.*, 2013). However, in such studies data may be skewed because dogs that are neutered have reached an age at which they can be neutered, whereas more dogs that are entire at death may have died at younger ages (Urfer and Kaeberlein, 2019).

A study in female Rottweilers investigated the effect of lifetime ovary exposure on longevity and the likelihood of reaching an exceptional age (≥13 years of age). Rather than considering whether or not the dogs were neutered at the time of death, the investigators compared the duration of "ovary exposure" in the first eight years of life. Female Rottweilers with the longest ovary

exposure were over three times more likely to reach an exceptional age compared with those with the shortest ovary exposure (Waters *et al.*, 2009). The impact of considering reproductive status at the time of death when investigating longevity, instead of the length of exposure to gonadal hormones was highlighted when the authors published a second study where the same female dogs were classified as neutered or entire at the time of death. Only 4.8% of the bitches that reached exceptional age were entire when they died. By classifying bitches as neutered or entire, the data would suggest that neutered bitches were more likely to reach an exceptional age, when actually those with the longest ovary exposure were the most likely (Waters *et al.*, 2011). A similar study conducted in Labradors considered the effect of neutering age on age at death but found no relationship. However, only 12 male and 27 female dogs were included (Adams *et al.*, 2015). Finally, in two separate studies, no difference in longevity was reported between neutered and entire Vizslas and no effect on mortality of age at neutering was observed for dogs neutered <5.5 or ≥5.5 months of age (Spain *et al.*, 2004; Zink *et al.*, 2014).

1.6 EFFECT ON BEHAVIOUR

The effects of neutering on dog behaviour are less well studied than the effects on health, but have revealed results that are as complex and unclear. Many studies have suggested behavioural differences between entire and neutered female dogs (O'Farrell and Peachey, 1990; Salmeri *et al.*, 1991; Guy *et al.*, 2001; Serpell and Hsu, 2005; Duffy and Serpell, 2006; Kim *et al.*, 2006; Maejima *et al.*, 2007; Kubinyi *et al.*, 2009; Duffy and Serpell, 2012; Starling *et al.*, 2013; Casey *et al.*, 2014; Balint *et al.*, 2017; Farhoody *et al.*, 2018). However, many studies are retrospective and rely on owner-reports of behaviour rather than direct observations, there is considerable variation between studies in populations and methodology, and contradictory findings make associations unclear for many behaviours. Other authors have reported no differences in behaviour (Serpell and Hsu, 2005; Doring *et al.*, 2009; Lisberg and

Snowdon, 2011; Flint *et al.*, 2017; Jacobs *et al.*, 2018a; van Rooy *et al.*, 2018; McGuire, 2019).

In addition to studies comparing behaviour between neutered and entire dogs, the effects of the timing of neutering, most commonly related to the age at neutering, on behaviour have been examined. Some authors suggest no effect of age at neutering on behaviour for female dogs (Salmeri *et al.*, 1991 [seven weeks versus seven months of age]; Howe *et al.*, 2001 [less or greater than 24 weeks of age]; Edwards *et al.*, 2019). While others have reported differences in the prevalence and onset of behavioural problems, including withdrawal from service dog training programmes for behavioural reasons, that are related to age at neutering (O'Farrell and Peachey, 1990; Spain *et al.*, 2004; Zink *et al.*, 2014; Zlotnick *et al.*, 2019). Studies have also described differences for some but not all behaviours and within behavioural categories, for instance displays of aggression towards different stimuli. For aggression measured using the Canine Behavioral Assessment and Research Questionnaire (C-BARQ), no relationship between age at neutering and aggression towards familiar people or dogs was identified by Farhoody *et al.* (2018) or by Starling *et al.* (2019), while effects were reported for aggression towards strangers and delivery people. Similar to findings related to examinations of behaviour in entire and neutered dogs, the reported effect of age at neutering is not consistent across behaviours or between sexes (Salmeri *et al.*, 1991; Spain *et al.*, 2004; Kubinyi *et al.*, 2009).

Authors of review papers, editorials, conference papers and proceedings have suggested differences in behaviour related to neutering before or after puberty (Kustritz, 2002; Thrusfield, 2011; Borstad, 2015; De Cramer and May, 2015; Romagnoli, 2017; Warnes, 2018) whilst other authors claim there to be no effect on behaviour (Stubbs and Bloomberg, 1995; Houlihan, 2017). These comments imply that studies have been conducted, however it appears that there may be potential misinterpretation of studies

that investigate the impacts of neutering at different ages rather than the effects of prepubertal neutering. Only one retrospective study has been identified that considered the pubertal status of bitches at neutering. In that study, Balogh *et al.* (2018) reported no differences in owner-reported behaviour or in urination behaviour between bitches neutered before or after puberty.

A potential relationship between cognitive impairment and neutering has been suggested in dogs. Neutered dogs have been reported to be more likely to have signs of canine cognitive dysfunction and to show faster progression in terms of cognitive decline (Azkona *et al.*, 2009; Hart, 2001). Further studies with larger numbers of dogs are required to confirm an effect of neutering, and any association with neutering timing and the effect of gonadal hormones (Urfer and Kaeberlein, 2019).

1.7 NEUTERING WITHIN GUIDE DOGS

Within the UK, assistance dogs are routinely neutered; hearing dogs are neutered at approximately 10 months of age (Guest *et al.*, 2007). Guide Dogs UK neuter male dogs at approximately 11-12 months of age and females usually after their first season, between nine and 18 months of age. Current practice within Guide Dogs UK is for bitches to be neutered by ovariohysterectomy after the end of their season (Peacock, personal communication, 2009). Neutering preferentially takes place during the puppy raising stage, prior to the bitches entering training because of anecdotal suggestions that neutering during early training can result in a four-week delay - including two weeks' convalescence and then a further two weeks for some bitches to 'settle' (Miller, personal communication, 2010). Furthermore, Asher *et al.* (2016) identified a significantly increased likelihood of being withdrawn from the guide dog training programme if bitches were neutered while in early training (40%), compared to bitches neutered while in puppy raising (27%). This supported the practice of neutering during the puppy raising stage.

Guide Dogs' electronic database (GDIR) was searched for 'routine spay' health codes; in 2009 and 2010, 136 bitches were neutered during early training and 812 were neutered in puppy raising. For some bitches, the 'spay window' encroaches on the time for commencing guide dog training, which can delay entry for training. Anecdotal evidence has suggested that the restriction around timing of neutering has prevented some bitches from commencing training at the optimum time, which negatively affects production targets (Miller, personal communication, 2010). Further health code searches were performed, establishing that in 2009 and 2010, 64 bitches were neutered when they were over 13 months of age, but still in the puppy raising stage.

Some published papers have mentioned in their discussions the effects of neutering assistance dogs and guide dogs at different ages. However, these are commonly the result of personal communications with the authors rather than referencing the findings from published studies. Kustritz (2002) presented a personal communication from Dr. Patricia Olson (2002), which stated that dogs neutered prior to puberty had higher success rates for becoming guide dogs. Contrastingly, Zink *et al.* (2014) reported a personal communication from Rogahn regarding a prospective study by Canine Companions for Independence that neutered dogs prepubertally. They found a decrease in success for dogs that were neutered, although the group used for comparison was not stated. A study in male guide dogs was presented at the International Working Dog Breeding Conference, 2005, and was described in the proceedings. For male guide dogs, no differences in behaviour or qualification rates were observed between those neutered between seven to eight weeks, six to eight months or 10 to 14 months of age (De Sapio *et al.*, 2005). More recently, Zlotnick *et al.* (2019) reported the findings of a study in which 245 male and female dogs in a service dog training programme were retrospectively categorised into neutering age groups (<7 months, 7-11 months, >11 months). Dismissals from the programme for health and

behavioural reasons were compared between neuter age groups. The authors reported that behavioural withdrawals were approximately 40% less likely in dogs neutered between 7 and 11 months of age than at <7 months, and 30% less likely than at >11 months of age. Additionally, dogs neutered at <7 months were more than twice as likely to be dismissed for orthopaedic problems as dogs neutered at any older age. While results for females were not reported separately, the authors stated that health-related dismissals did not differ by sex or breed. However, it is important to note that these data related to dismissals from the service dog training programme and not diagnoses.

1.8 IDENTIFYING THE GAP IN EXISTING KNOWLEDGE

Studies conducted into the effects of neutering at different ages, such as before or after 5.5, 6 or 12 months of age (Howe *et al.*, 2001; Spain *et al.*, 2004; Torres de la Riva *et al.*, 2013), whether retrospective or prospective, and in single or multiple breeds, do not provide valid evidence of the effect of neutering pre- or post-pubertally on dog health, behaviour or physical development. The pubertal status of the dogs at neuter, if mentioned at all, is commonly implied based on the age at neutering, which itself has been estimated in some cases. Whether or not bitches have experienced puberty and an oestrus is either not known or not reported in most studies. This adds additional complications when attempting to review the literature. Some of the problem is that the impacts can be multifactorial; numerous health and behavioural issues can be impacted and the potential for breed type to further confuse results does not help. It is likely that the findings of one study in one breed will not be transferable to other breeds, as evidenced by the differences in the findings for Labradors and Golden Retrievers (Hart et al., 2020a,b), breeds that are quite similar. Many controlled studies, with large numbers of dogs of many breeds, followed for a long time with extensive comprehensive health and behavioural data, are required. While this may help understanding, it could further complicate the decisions

needed to be made in veterinary practice. In addition, pubertal status at neutering and length of ovary exposure, or exposure to ovarian hormones, should be considered to fully understand the relationship between neutering before or after puberty and the occurrence of different conditions.

There is a paucity of evidence documenting the effect of neutering at different ages, or indeed neutering pre- and post-puberty, on the impacts on dogs that are being trained to be assistance dogs. As well as the effects on health and behaviour, it is important to consider whether there are any effects on the likelihood of progression to qualification and longevity in the working role.

1.9 CONCLUSION

Considerable literature exists reporting comparisons between neutered and entire dogs, and the impacts on health and to a lesser extent, behavioural outcomes. However, the evidence relating to the impacts of neutering age on these outcomes is confusing, and there is an apparent lack of evidence relating to the effect of neutering bitches before or after puberty on physical development, behaviour, health, and the impacts on progression within an assistance dog training programme.

This thesis will attempt to provide an answer to the question of the best time to neuter female dogs for the breeds studied; either prepubertally or post-pubertally. The study will document any health and behaviour differences between pre- or post-pubertally neutered bitches by conducting a scoping review to identify and chart the existing literature, and by conducting a prospective study. The results will be relevant not only to Guide Dogs in the UK and guide dog schools internationally, but to other assistance and working dog organisations as well as pet dog owners and veterinarians.

Chapter 2. Effect of timing of neutering in relation to puberty on behaviour in the female dog – a scoping review

2.1 INTRODUCTION

Dog behaviour is influenced by genetic and environmental factors (Mackenzie *et al.*, 1986). Neutering has frequently been reported to influence behaviour in female dogs and published studies have implied differences in behaviour between neutered and entire bitches (Duffy and Serpell, 2006; Kim *et al.*, 2006; Starling *et al.*, 2013; Casey *et al.*, 2014; Farhoody *et al.*, 2018). Other studies have reported that neutering had no effect on bitch behaviour (Serpell and Hsu, 2005; Flint *et al.*, 2017; Jacobs *et al.*, 2018a,b; van Rooy *et al.*, 2018;). Additionally, differences in behaviour between bitches neutered at different ages have been reported (Spain *et al.*, 2004; Kubinyi *et al.*, 2009; Zink *et al.*, 2014; Starling *et al.*, 2019). Authors of literature reviews, editorials and conference papers have suggested differences in the behaviour of dogs attributable to neutering before or after puberty (Kustritz, 2002; Thrusfield, 2011; De Cramer and May, 2015; Romagnoli, 2017; Warnes, 2018). On closer inspection, the authors of the aforementioned studies have suggested relationships based on studies that have examined behaviour for dogs neutered at different ages, rather than before or after puberty. Others have reported there to be no effect on behaviour related to neutering bitches prepubertally (Stubbs and Bloomberg, 1995; Le Bars, 2006; Houlihan, 2017). These are narrative reviews without the methodical and systematic search methods that would be used in a scoping review. To the author's knowledge, no systematic review or research synthesis have yet been published on the impact of neutering bitches before or after puberty on behaviour. Therefore, decision making for veterinarians and those attempting to identify the optimum time to neuter bitches remains challenging.

Due to the potential for the timing of neutering to impact behaviour in bitches, and the apparent lack of clarity in the existing literature, a scoping review was designed. A scoping review allows for a systematic search and appraisal of the existing evidence related to the impacts of neutering before or after puberty on bitch behaviour, in order to evaluate the evidence in a reliable, robust and repeatable way. Unlike a systematic review, which is designed to methodically search and analyse the literature related to a specific question to report a finding or inform clinical practice, a scoping review is used to identify and chart the currently available literature and can therefore identify gaps in knowledge. Scoping reviews can provide a broad overview of literature relating to a subject including an examination of study designs in published work on a topic. Scoping reviews are a useful first step in collating the evidence on a subject and can be used prior to a systematic review (Peters *et al.*, 2015; Munn *et al.*, 2018; Lockwood *et al.*, 2019; Sucharew and Macaluso, 2019).

The aim of this study was to systematically search the available literature to identify and chart the current evidence on the effect of the timing of neutering in relation to puberty on the behaviour of female domesticated dogs, and to highlight areas where knowledge is lacking.

The objectives of the study were to:

- Conduct a systematic search of databases using published methodology to identify current literature on neutering in female dogs.
- Review and appraise the identified literature on the effects of neutering bitches pre or post-pubertally on behaviour.
- Chart and summarise evidence on associations between behavioural development and behavioural problems in bitches neutered before puberty compared to bitches neutered after their first oestrus.

- Chart study methodology from existing publications to identify knowledge gaps and construct recommendations for future studies to improve the evidence base.

2.2 METHODOLOGY

2.2.1 Protocol and registration

A search has been conducted for existing scoping and systematic reviews on the impact on behaviour of neutering bitches before and after puberty. JBI Evidence Synthesis, PROSPERO and PubMed were searched on 10/09/2021 and no reviews were identified.

A Preferred Reporting Items for Systematic reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR; Tricco *et al.*, 2018) was used for this scoping review. The scoping review protocol was developed before data extraction and was registered with the Open Science Framework (Freeman and Moxon, 2020). The project was reviewed and approved by the Ethics Committee, School of Veterinary Medicine and Science, University of Nottingham.

2.2.2 Search strategy

Primary literature searches were conducted on 26/03/2020 in three scientific databases; CAB Abstracts (1910 to present), Medline (1910 to present) and Web of Science (1950 to present) (Grindlay *et al.*, 2012). The search was repeated on 02/02/2022. The results from 02/02/2022 are presented in this chapter.

Search terms were developed and reviewed by a team of five people, including the authors. The search terms were:

Neuter OR neuters OR neutering OR neutered OR spay OR spays OR spayed OR spaying OR ovariohysterectomy OR ovariohysterectomies OR sterilize OR sterilizes OR sterilization OR sterilise OR sterilises OR sterilisation OR gonadectomy OR gonadectomise OR gonadectomize OR ovariectomy OR ovariectomies OR de-sexing OR

desex OR desexing OR de-sex OR castration OR castrate OR castrating OR sterilized OR sterilised

AND
Bitch OR bitches OR dog OR dogs OR canine OR canines OR canid OR canids OR canis

AND
Behaviour OR behavior

Additionally, BestBETs and RCVS Knowledge Summaries were searched. Papers were also included that were identified from a previous literature search by the primary author and were related to the search terms but not identified elsewhere.

2.2.3 Study selection

The search results were exported into EndnoteX8 (Clarivate Analytics[1]). Duplicate records were identified and deleted before publications were assessed against the inclusion criteria (Table 2.1). A three-stage systematic review and exclusion process was completed independently by two researchers involving 1) review of publication titles, 2) review of publication abstracts and 3) review of the full-text for the remaining publications. At title and abstract review, ambiguous publications were retained for the next stage of review. Publications where abstracts could not be identified were retained for full-text review. For publications where full-text was not available, individual authors and journals were contacted to attempt to obtain the full-text. A study was excluded if English language full-text was not available from University of Nottingham libraries or e-libraries, from free online Open Access and legal deposit libraries,

[1] https://endnote.com/

from online searches, or from direct contact with authors or journals.

Table 2.1. The inclusion and exclusion criteria for a scoping review of the literature to identify and chart the current evidence on the effect of the timing of neutering in relation to puberty on the behaviour of female domesticated dogs.

CRITERIA	INCLUSION	EXCLUSION
POPULATION	Female domesticated dogs	Male dogs Other species
EXPOSURES	Entire female dogs presented for surgical neutering	Papers comparing entire bitches to neutered bitches Papers not relating neutering to age Papers relating to the treatment of dogs with behaviour problems
INTERVENTION	Neutering procedure including chemical techniques and open and laparoscopic techniques which involve removal of the ovaries	Papers relating to neutering by hysterectomy
OUTCOME	Papers including assessments or measures of dog behaviour Owner observations or survey of dog behaviour	Health outcomes Surgical outcomes 14-day perioperative period and behaviour relating to recovery following surgery Pseudopregnancy Student or owner opinions, perceptions or attitudes without behaviour observation or measure Behaviour relating specifically to the time of mating Papers only examining effect of neutering on control of reproduction
LANGUAGE	English or papers with translation available	English translation not available
STUDY DESIGN	Case series, cohort, case control, cross sectional	Individual case reports, qualitative studies
PUBLICATION TYPE	Peer reviewed journals Conference proceedings	Narrative, textbook chapters, reviews (reference lists from systematic reviews were used to crosscheck we had all relevant studies but were not used themselves) Unable to obtain full study details (full text) Non-peer reviewed journals

2.2.4 Charting process

The remaining final full-text publications were read and assessed independently by two researchers to ensure eligibility for inclusion. Characteristics and relevant information were extracted and presented.

2.2.4.1 Data extraction

Following full text review, key information was extracted from each of the remaining 13 publications retained for the scoping review using a standardised form (Appendix 1) and tabulated. Contents included in the table presenting study characteristics were: author, publication year, data source or country, study design, sample size, study aims, details of the intervention, behavioural outcomes and how outcomes were measured and whether dogs were randomly or retrospectively allocated to study groups. Additional details were extracted and presented for participant characteristics. These were: how the sample was sourced, the breed, age and sex of dogs, whether dogs neutered for a behaviour problem were excluded, whether previous breeding history was considered and how puberty was defined. This was carried out independently by one researcher.

2.2.4.2 Summarising the available evidence

The 13 publications were assessed by two researchers. Significant effects of timing of neutering on behaviour were reported for either bitches or dogs and bitches (where studies did not present results for sexes of dogs separately). Studies that reported significant effects only in male dogs and studies that reported no significant effects were not included in the charting tables. The evidence was presented in two ways considering 1) impacts on behavioural outcomes and 2) impacts relating to the timing of neutering. Where possible for the impacts on behavioural outcomes, behaviours were grouped into Fear and anxiety related behaviours, Aggression, Excitability, Training and obedience and Social behaviours. A 'Favourable or detrimental impact summary' was included to show more clearly whether the difference reported in behavioural

outcomes resulted in behaviours that were more or less favourable for dogs neutered at certain times.

2.3 RESULTS

2.3.1 Study selection

The primary literature search identified 1,048 publications following the removal of duplicates. Six hundred and eighty were excluded on title review, 248 were excluded following abstract review and 107 were excluded on full-text review, including 14 that were not available in English, 24 that did not have full text available, 28 for publication type, 40 for concept and one for study population. Therefore 13 publications remained for assessment (Figure 2.1).

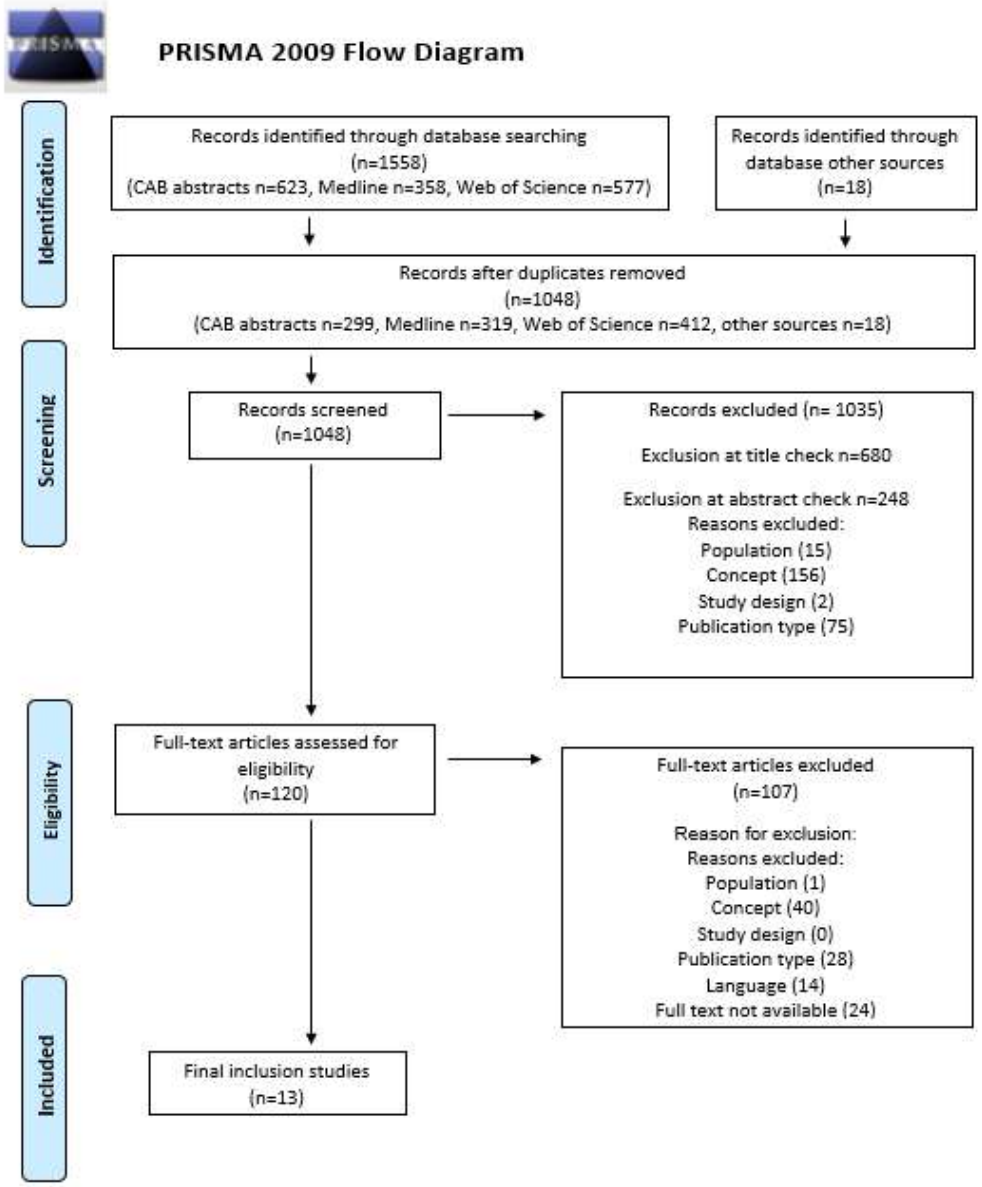


Figure 2.1 The PRISMA 2009 flow diagram showing the number of publications that were identified, reviewed, and excluded at title, abstract and full-text review in a scoping review to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on the behaviour of female domesticated dogs. The reasons for exclusion at abstract and full-text review are presented along with the number of publications included in the final assessment.

2.3.2 Study characteristics

The final 13 papers were from studies conducted on dogs in the USA (4/13) or various or multiple countries (5/13) published between 1990 and 2021. Two studies did not report the location. Six of the 13 studies were cross-sectional, four were prospective cohort studies and three were retrospective cohort studies. Dogs were most commonly retrospectively grouped into neuter groups based on age (7/13 studies) or pubertal status at neutering (2/13 studies). One study randomly allocated dogs to neutering groups and three prospective studies grouped dogs based on age when presented for neutering. Eight studies did not report the type of neutering surgery performed and one study included bitches that had received deslorelin acetate implants for oestrus suppression.

Behaviours were frequently measured using owner questionnaires (11/13 studies) and five of these used C-BARQ questions. Two of the studies that used owner questionnaires also used additional collection methods; one study used direct observations of behaviour and one used data from veterinary records. One study used subjective assessments of behaviour made by primary caregivers and one used data from service dog training programme records (Table 2.2).

Table 2.2. The study characteristics for 13 publications identified for inclusion in a scoping review of the literature undertaken to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on the behaviour of female domesticated dogs.

Author and year	**Source / country**	**Study design**	**Sample size**	**Aims***	**Intervention**	**Behavioural outcomes***	**Outcome measurements**	**Allocated randomly to neutering groups or retrospectively grouped**	**Comparisons reported between dogs neutered in different groups or just to the entire group?**
Balogh *et al.*, 2018	Not reported	CS	58	To determine whether behaviour differences in urination and general behaviour exist between entire bitches and those neutered before or after puberty	12 entire bitches, 17 bitches neutered before puberty, 29 neutered after puberty Method of neutering was not specified	Observations: urination frequency, posture, ground-scratching; owner questionnaire: urination behaviour, over-marking, urination frequency unknown environments; C-BARQ behaviour	Owner questionnaires and behavioural observations	Retrospectively grouped	Before and after puberty compared
Brandli *et al.*, 2021	Switzerland	RC	32	To evaluate the side effects of repeated deslorelin acetate application for sustained	4 bitches first implant before puberty, 28 bitches after puberty, deslorelin acetate implant	Changes in aggression or affection toward people or dogs, playfulness, anxiety toward objects,	Owner questionnaires	Retrospectively grouped	No comparisons reported between dogs neutered at

				gonadal suppression	for oestrus suppression	people or dogs and obedience			different ages
Edwards et al., 2019	Various – data from C-BARQ database	CS	26,555 (12,801 female)	To understand dog and owner characteristics that influence fear or stress response at the veterinary clinic	For all 20,095 neutered dogs, breakdown by sex not provided: 1,665 neutered <6 months, 4,849 neutered 6-12 months, 1,374 neutered 12-18 months, 3,677 neutered >18 months. The method or type of surgery was not reported.	C-BARQ responses to Q43 'fear of veterinary examination' and Q47 'fear of unfamiliar' C-BARQ behavioural subscales, touch sensitivity and non-social fear	Owner questionnaires	Retrospectively grouped	No comparisons reported between dogs neutered at different ages
Farhoody *et al.*, 2018	Various – data from C-BARQ database	CS	13,795, 13,498 and 13,237 dogs for each behaviour measured. Number of female dogs for	To determine whether aggressive behaviour toward familiar people, strangers, or other dogs was significantly different in dogs neutered at different ages	Dogs neutered at 6 weeks to 6 months, >6-12 months, >12-18 months, >18 months. Exact numbers for each analysis not reported. The method or type	C-BARQ data for behaviours measured: aggression towards familiar people, aggression towards strangers, aggression towards other dogs	Owner questionnaires	Retrospectively grouped	No comparisons reported between dogs neutered at different ages

			each not reported.	compared to entire dogs.	of surgery was not reported.				
Howe *et al.*, 2001	USA	PC	269 (153 female)	To determine long-term results and complications of neutering performed at an early age (prepubertal) or at the traditional age in dogs.	94 dogs neutered ≥24 weeks of age and 175 neutered <24 weeks of age. Ovariohysterectomy or castration.	Behavioural information, not stated	Owner telephone questionnaires 41-64 months after adoption	Grouped based on age at surgery	Between age group comparisons reported
O'Farrell and Peachey, 1990	Not reported	PC	300	To examine the behaviour of bitches at the time of and after neutering	Age at neuter for 150 bitches: 45 bitches <12 months, 42 bitches 12-24 months, 63 bitches ≥24 months). Ovariohysterectomy.	Aggression; fear; activity level; excitability; destructive chewing; reactions to separation from owner; behaviour in the car; roaming; feeding; urination and defecation; scavenging	Owner questionnaires six months after neutering	Grouped based on age at surgery	Between age group comparisons reported
Palestrini *et al.*, 2021	Italy	PC	96 (64 female)	To evaluate the effects of gonadectomy on dog behaviour across time, comparing behaviours with	33 neutered bitches: 0-6 months = 2, 7-12 months = 7, 1-5 years = 13, 6-10 years = 8, >10 years	Grooming, Sleeping, Exploration, Drinking, Play, Mounting, Aggression towards dogs, strangers or	Owner questionnaires at time 0 before neutering and nine months later	Grouped by age when presented for neutering	No comparisons reported between dogs neutered at

				a control group of healthy, entire dogs	= 3. Ventral midline ovariohysterectomy.	owner, Barking, Eating, Greeting, Chewing object, Destruction, Inappropriate Elimination, Pica, Coprophagia, Repetitive behaviours, Roaming, Restlessness, Pull on the leash, Thunderstorm and noise phobia			different ages
Salmeri *et al.*, 1991	USA	PC	32 (17 female)	To determine how behaviour was affected by neutering at 7 weeks versus 7 months of age	14 females neutered at 7 weeks (7 females), 8 neutered at 7 months (4 females), 10 entire (6 females). Females were neutered via ovariohysterectomy.	Excitability, general activity, barking, playfulness, aggression toward other dogs, affection toward people, outgoing nature	Subjectively assessed by four primary care providers during the 15 months	Randomly allocated	Between age group comparisons reported
Spain *et al.*, 2004	USA	RC	1,659 (number of females for	Evaluate the long-term risks and benefits of early-age	Neutered <5.5 months or ≥5.5 months, number	Aggression towards people, hyperactivity, aggression towards	Owner questionnaire and	Retrospectively grouped	Between age group comparisons reported

			behaviour analysis not reported)	(before 5.5 months) versus traditional-age gonadectomy	breakdown for behaviour analysis not available. The type of surgery was not reported.	animals, destructive behaviours that persisted past 1 year of age, digging holes in the garden, escaping while owner was home, excessive barking, excitement around visitors, excessive jumping, excessive licking or chewing of skin, noise phobias, playful behaviours, separation anxiety, sexual behaviours, shyness, urination in the house when frightened or excited, aggression towards the veterinarian or veterinary staff and pica	veterinary records		
Starling *et al.*, 2019	Various – data from C-BARQ database	CS	8,981	Examine relationships between behaviour and neutering timing considered as	Females neutered before 10 years of age (median 6.9 months, mean	100 C-BARQ behaviours	Owner questionnaires	Retrospectively grouped	Relationships with age at neuter reported

				age at neuter and percentage lifetime exposure to gonadal hormones	14.5 months). The method or type of surgery was not reported.				
Stellato *et al.*, 2021	38 countries, 55.3% Canada, 35.3% USA, 9.4% remaining countries	CS	1,346 for fear 1,776 for aggression. Number of females for each analysis not reported.	To identify risk-factors associated with fear and aggression within veterinary clinics	Neutered at <6 months, 6-11 months, 1 year, 2 years or more than 2 years. The method or type of surgery was not reported.	Veterinary fear Veterinary aggression (including fear and aggression portions of the C-BARQ, and researcher-generated questions on fear and aggression)	Owner survey	Retrospectively grouped	No comparisons reported between dogs neutered at different ages
Zink *et al.*, 2014	25 different countries, majority USA, UK, Canada and Australia (USA 86.7%)	CS	2,505 (1,360 female)	Investigate associations between age at gonadectomy and sex of gonadectomised dogs and risk and age of onset of behavioural disorders	Owners selected age at gonadectomy as ≤6 months (209 female), 7-12 months (157 female) and >12 months (459 female), or entire. The type of surgery was not reported.	Various behaviour, not described	Owner survey	Retrospectively grouped	No comparisons reported between dogs neutered at different ages

Zlotnick *et al.*, 2019	USA	RC	245 (77 female)	Investigate differences in the incidence of health or behavioural problems and completion of service dog training in dogs neutered at different ages	Neutered at <7 months (n=110) Neutered at 7-11 months (n=58) Neutered at >11 months (n=77). The type of surgery was not reported.	Dismissal from service dog training programme for behavioural reasons	From service dog records	Retrospectively grouped	Between age group comparisons reported

*In some papers, behaviour is one of many aims and outcomes measured. For the purposes of reporting within this scoping review, only relevant behavioural aims and outcomes are included.
Abbreviations: RC – retrospective cohort, PC – prospective cohort, CS – cross-sectional.

2.3.3 Participant characteristics

In nine of the 13 studies, animals were pet dogs, two of these included dogs that were neutered while in an animal shelter. Two studies used data extracted from the C-BARQ database, which could include dogs in other roles and from various sources, including shelters. Other dogs were from a research kennel facility (1/13), a service dog training programme (1/13), and one study included dogs of various types including working, show and breeding. One study did not state the types of dogs used (Stellato *et al.*, 2021). Eleven of the 13 studies included dogs of various breeds and usually did not identify individual breeds. Four studies included only bitches. Nine studies included dogs and bitches, six of these did not report results for each sex by neuter group independently, one reported some results by sex and two did report results for male and female dogs separately. Ten studies did not consider previous breeding history for the dogs and for the three that mentioned breeding history, one included only nulliparous bitches, one only reported it for entire dogs and the last did not include details of breeding history in the results. Four of the 13 studies excluded dogs that were neutered for behavioural reasons; one only excluded dogs that were neutered for dominance aggression, and nine did not exclude dogs neutered for behavioural reasons; one of these did include neutering reason in statistical analysis. Only one study examined behaviour for bitches that were surgically neutered before or after puberty, albeit with pubertal status at neutering based on retrospective owner recollection. One study examined behaviour for bitches treated with deslorelin acetate to suppress oestrus, however data were not analysed statistically and numbers were too small for comparative analysis between neuter age groups. The remaining 11 studies either did not mention puberty (10/13) or based conclusions regarding puberty on age at neutering (1/13) (Table 2.3).

Table 2.3. Participant characteristics for 13 publications identified for inclusion in a scoping review of the literature undertaken to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on the behaviour of female domesticated dogs.

Author and year	**Sample source**	**Breed**	**Age at behavioural measurement**	**Sex**	**Excluded dogs neutered due to a behavioural problem?**	**Previous breeding history considered?**	**How puberty was defined**
Balogh *et al.*, 2018	Pet dogs identified from veterinary hospital databases	Labradors	Four to 10 years. Median age of Entire = 71 months, Prepubertally neutered = 76 months, Post-pubertally neutered 80 months	All female	No	No	Based on retrospective owner reports of whether the dog was neutered before or after puberty. No definition of puberty provided.
Brandli *et al.*, 2021	Pet dogs identified from clinical records from the Clinic for Small Animals, University of Zurich	31 purebred (14 different breeds) one mixed-breed	4 prepubertal bitches (mean age 4.8 +/- 1.3 months) 28 bitches post-pubertal (mean age 17.1 +/- 10.7 months)	All female	No	Yes – all nulliparous	Not reported
Edwards *et al.*, 2019	Pet, working, show and breeding	Various breeds and crossbreeds	Mean age = 4.5 ±3.5 years 583 <0.5 years, 12,033 0.5 to 3	13,754 male 12,801 female, results for impacts of	No, but reason for neutering	No	N/A, puberty was not considered within the study, only age

	dog data from the C-BARQ database		years, 13,939 >3 years	neuter age not reported by sex	considered for analysis		
Farhoody *et al.*, 2018	Pet dog data from the C-BARQ database	Various breeds, not stated	Aggressive behaviour toward familiar people: mean=226 to 235 days (SD 168 to 182 days) Aggressive behaviour toward strangers: mean=224 to 242 days (SD 163 to 181 days) Aggressive behaviour toward other dogs: mean=200 to 251 days (SD 164 to 177 days)	Male and female, results not reported by sex	Yes	No	N/A, puberty was not considered within the study, only age
Howe *et al.*, 2001	Pet dogs adopted from two shelters	Breeds not stated	Age at evaluation not stated.	153 female, 116 male, sex ratio in each neuter age group not reported,	No	No	Based on age at neutering, ≥24 or <24 weeks of age

				results not reported by sex			
O'Farrell and Peachey, 1990	An unselected sample of pet dogs recruited at the time of neutering surgery via veterinary practices	Breeds not stated	Neutered mean = 33.5 months Control mean = 33.3 months	All female, 150 neutered and 150 entire age and breed matched controls	Only for dominance aggression	Yes, but not mentioned in statistics or results	N/A, puberty was not considered within the study.
Palestrini *et al.*, 2021	Pet dogs recruited through veterinary hospital	Pure and mixed breed	Neutered bitches: 0-6 months = 2, 7-12 months = 7, 1-5 years = 13, 6-10 years = 8, >10 years = 3 Entire bitches: 0-6 months = 3, 7-12 months = 2, 1-5 years = 16, 6-10 years = 8, >10 years = 2	Both male and female, reported separately	No	No	N/A, puberty was not considered within the study.
Salmeri *et al.*, 1991	Dogs in a research kennel facility	Mixed-breed	Dogs followed from 0-15 months of age	Seven-week neuter: 7 male 7 female 7-month neuter: 4 male 4 female	No	No	N/A, puberty was not considered within the study, only age

				Entire: 4 male 6 female. Results for males and females reported separately			
Spain *et al.*, 2004	Pet dogs adopted from a shelter	Various breeds, not stated	Median age at end of follow up 5.0 years (range 0.5 to 12.2 years)	Male and female, sex ratio in each age at neuter group for behaviour analysis not reported. Some results reported separately for males and females.	No	No (but maximum age at neuter was 12 months so breeding unlikely)	N/A, puberty was not considered within the study, only age
Starling *et al.*, 2019	Pet dog data from the C-BARQ database	Various breeds, not stated	Not stated	All female	Yes	No	N/A, puberty was not considered within the study, only age and percentage lifetime exposure to gonadal hormones
Stellato *et al.*, 2021	Not stated, convenience sampling to recruit participants through	Various pure and mixed breeds	1-5 years, mean = 3.2 ± 1.4 years	Male and female, numbers of each sex not reported, impacts of	No	No	N/A, puberty was not considered within the study, only age

	social media platforms			neuter age not reported by sex			
Zink *et al.*, 2014	Pet dog data gathered from an anonymous online survey	Viszlas	0-16 years	1,145 male and 1,360 female (535 entire). Results for males and females by neuter group not reported separately.	Yes except for fear of storms	Yes for entire (53%), not mentioned for neutered	N/A, puberty was not considered within the study, only age
Zlotnick *et al.*, 2019	Dogs in a service dog programme	Labradors, Golden Retrievers, various other breeds and crosses	Not stated	168 male and 77 female Neutered <7 months: 110 Neutered 7 to 11 months: 58 Neutered after 11 months: 77 Sex breakdown by neuter group not reported, results not reported by sex	No	No	N/A, puberty was not considered within the study, only age

2.3.4 Charting the studies against the aims of the scoping review

2.3.4.1 Behavioural outcomes

Fifteen different behaviours were reported to be significantly impacted by timing of neutering in the 13 studies, along with withdrawals from an assistance dog programme for behavioural reasons (Table 2.4). There was one study where specific behaviours were not considered independently and were grouped as 'behavioural disorders'. Four studies reported no significant impact of timing of neutering in relation to age or puberty on the behavioural outcomes measured (Salmeri *et al.*, 1991; Howe *et al.*, 2001; Balogh *et al.*, 2018; Edwards *et al.*, 2019), and two did not analyse behavioural data due to small numbers of dogs (Brandli *et al.*, 2021; Palestrini *et al.*, 2021). These were not included in the charting table.

The behaviours that were impacted were commonly those related to aggressive behaviour, fear and anxiety or social behaviour. Three studies made no comparisons between dogs neutered in different age groups and only compared each age group to entire dogs. The effects of neutering at younger and older ages were mixed, with some behaviours being reported as more favourable in dogs that were neutered at younger ages and some being reported as less favourable. This is apparent within behaviour groups, such as fear and anxiety related behaviours; the odds of displaying urination when frightened and separation anxiety behaviours were lower for dogs neutered when less than 5.5 months of age, whereas the odds of noise phobia increased for every month decrease in age at neutering. The charting of study results highlights the inconsistencies in classification of timing of neutering between studies and the lack of information concerning the timing of neutering in relation to puberty.

Table 2.4. The behavioural outcomes that were impacted by timing of neutering identified in a scoping review undertaken to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on the behaviour of female domesticated dogs.

Behavioural outcome	**Author**	**Study design**	**Sex**	**Relationship with timing of neutering**	**Favourable or detrimental impact of neutering summary**
All behaviour disorders grouped	Zink *et al.*, 2014	CS	Both male and female	Neutering at ≤6 months of age significantly increased the odds (1.8x, P<0.001) of developing a behavioural disorder compared to entire dogs. No results reported for differences between dogs neutered in different age groups.	Detrimental for dogs neutered at ≤6 months of age compared to entire dogs
Fear and anxiety related behaviours	Zink *et al.*, 2014	CS	Both male and female	Fear of storms: Dogs neutered at ≤6 months (OR=3.9, P<0.001), between 7 and 12 months (OR=4.7, P<0.001), or at >12 months of age (OR=4.0, P<0.001) had increased odds of developing fear of storms compared with entire dogs. No results reported for differences between dogs neutered in different age groups.	Detrimental for dogs neutered in all age groups compared to entire dogs
	Stellato *et al.*, 2021	CS	Both male and female	Higher fear score at veterinary clinics for dogs neutered at ≤1 year of age than older age groups when compared to entire dogs (<6 months P=0.015, 6-11 months P<0.001, 1 year P=0.008).	Detrimental for dogs neutered at ≤1 year of age compared to entire dogs
	Starling *et al.*, 2019	CS	Female	When barked, growled or lunged at by an unfamiliar dog: decreased with increasing PLGH (OR=0.997, 95% CI 0.996-0.999, P<0.001)	Detrimental for dogs with lower PLGH
	Spain *et al.*, 2004	RC	Both male and female	Urination when frightened: OR=0.74 for <5.5m (lower) than for dogs neutered at ≥5.5 months (95% CI=0.54-1.01, P=0.06). Noise phobia: OR=1.04 increase for each one month decrease in age at surgery (95%=CI 1.01-1.08, P<0.01).	Favourable for dogs neutered <5.5 months of age Detrimental for dogs neutered younger

				Separation anxiety: OR=0.72 for <5.5m (lower) than for dogs neutered at ≥5.5 months (95% CI=0.55-0.94, P=0.02).	Favourable for dogs neutered <5.5 months of age
Aggression	Starling *et al.*, 2019	CS	Female	Aggression when delivery workers approached the home = younger age at neuter (OR=0.999, 95% CI=0.998-0.999, P<0.001) and lower PLGH (OR=0.994, 95% CI=0.9992-0.9998, P<0.001) increased the likelihood. Aggression when approached by an unfamiliar male dog while on leash = lower PLGH increased the likelihood (OR=0.997, 95% CI=0.995–0.999, P<0.001). Aggression when being barked, growled or lunged at by an unfamiliar dog = lower PLGH (OR=0.996, 95% CI=0.995–0.998, P<0.001) increased the likelihood.	Detrimental for dogs neutered at a lower age and dogs with lower PLGH Detrimental for dogs with lower PLGH Detrimental for dogs with lower PLGH
	Farhoody *et al.*, 2018	CS	Both male and female	Simple unadjusted analysis – age at neutering had a significant relationship with aggression toward familiar people (P<0.001), aggression toward strangers (P<0.001) and aggressive behaviour toward other dogs (P=0.001). The direction of the relationships between age and aggression was not clear. A significant but low (22%) increase in moderate or severe aggression toward strangers for neutered compared to entire dogs (OR=1.219, P=0.014). This was based on data for dogs neutered between 7 and 12 months of age (OR=1.259, 95% CI=1.09-1.45, P=0.002).	Unclear Detrimental for dogs neutered between 7 and 12 months of age compared to entire dogs
	O'Farrell and	PC	Female	Increased risk of dominance aggression towards family members for neutered compared to entire bitches (P<0.05). This was greatest for bitches neutered under one year of age	Detrimental for bitches neutered under 12 months of

	Peachey 1990			that were already showing signs of aggression when compared to bitches neutered over one year of age and that were not showing signs of aggression (no statistics reported).	age that are already showing signs of aggression compared to those neutered over 12 months of age showing none
Excitability	Starling *et al.*, 2019	CS	Female	Increase in likelihood of excitable behaviour when the doorbell rings for age at neuter and PLGH (whether lower or higher was not stated for age nor PLGH). Only one OR (0.989, 95% CI=0.987-0.991) is reported therefore attributing this to age at neuter or PLGH was not possible. Excitable behaviour before being taken on a car trip: lower PLGH (OR=0.997, 95% CI 0.995-0.998, P<0.001) increased the likelihood.	Unclear Detrimental for dogs with lower PLGH
Training and obedience	Starling *et al.*, 2019	CS	Female	When off the leash returns immediately when called: higher PLGH (OR 0.997, 95% CI=0.995–0.999, P=0.001) decreased the likelihood. Will 'fetch' or attempt to fetch sticks, balls, or objects: higher age at spay (OR=0.997, 95% CI=0.997–0.998, P<0.001) and PLGH (OR=0.993, 95% CI=0.991–0.995, P<0.001) decreased the likelihood.	Favourable for dogs with lower PLGH Favourable for lower age at neutering and dogs with lower PLGH
Social behaviours	Starling *et al.*, 2019	CS	Female	Chews inappropriate objects: higher PLGH increased the likelihood (OR=1.004, 95% CI=1.003-1.006). Pulls excessively hard when on the leash: relationship with age at neuter (P<0.001) and PLGH (P=0.001) suggested but no OR or direction of relationship reported.	Favourable for dogs with lower PLGH Unclear Unclear

				Rolls in animal droppings or other smelly substances: relationship with age at neuter and PLGH suggested (both P<0.001) but no OR or direction of relationship reported. Howling: higher PLGH increased the likelihood (OR=1.004, 95% CI=1.002-1.006).	Favourable for dogs with lower PLGH
	Spain *et al.*, 2004	RC	Both male and female	Escaping from home: OR 0.93 decrease for each one month decrease in age at surgery (95% CI=0.87-0.98, P<0.01, when considered a serious problem).	More favourable as neutering age decreases
Sexual behaviours	Spain *et al.*, 2004	RC	Both male and female	OR 1.05 increase for each one month decrease in age at surgery (95% CI=1.01-1.09, P<0.01).	Less favourable as neutering age decreases
Withdrawal from assistance dog training for behavioural reasons	Zlotnick *et al.*, 2019	RC	Both male and female	Behavioural withdrawals about 40% less likely in dogs neutered between 7 and 11 months of age than dogs neutered at <7 months (RR=0.6, P<0.01) and 30% less likely than dogs neutered at >11 months of age (RR=0.7, P=0.05).	Favourable for dogs neutered between 7 and 11 months of age compared to younger and older age groups

Abbreviations: RC – retrospective cohort, PC – prospective cohort, CS – cross-sectional, CI – confidence interval, OR – odds ratio, RR – relative risk, PLGH - percentage lifetime gonadal hormone exposure.

2.3.4.2 Timing of neutering

Considering results by the timing of neutering rather than the behavioural factors investigated demonstrates that studies have classified neutering timing in different ways (Table 2.5). Categorical neuter age groups were used in 10 studies, one of which also considered age as a continuous variable for some analyses, and three of which did not make comparisons between age groups, only with a group of entire dogs. One study classified bitches as neutered before or after puberty and one classified bitches as pre or post-pubertal at the time of deslorelin acetate implant. The final study used age as a continuous variable along with percentage lifetime gonadal hormone exposure.

The only study to categorise dogs as being surgically neutered before or after puberty reported no impact on behaviour. The second study to categorise bitches as pre or post-pubertal, at the time of the first deslorelin acetate implant, reported numbers too low to perform statistical analysis for behavioural changes. Three of the four studies that compared dogs neutered at different ages with entire dogs reported detrimental impacts for the neutered dogs, but the fourth reported no impact on behaviour. Three of the six studies that compared behaviour between dogs neutered in different age groups did not identify any differences. For the three studies that reported differences, they included: differences in separation related behaviours and 'urination when frightened' for dogs neutered at less than 5.5 months of age, behavioural withdrawals from assistance dog training reported as less likely for dogs neutered between 7 and 11 months of age than those neutered at <7 months and or >11 months of age, and greater risk of dominance aggression towards family members for bitches neutered under one year of age that were already showing signs of aggression compared to those neutered over one year of age that showed no signs of aggression. Although for the latter, no statistical results were reported. One study examined the relationship between percentage lifetime gonadal hormone exposure and owner-reported behaviours and

reported four behaviours that were more favourable and five that were less favourable for lower percentage lifetime gonadal hormone exposure. All less favourable behaviours were related to fear or aggression. For the two studies that examined the relationship between behaviours and age as a continuous variable, again both favourable and detrimental effects on behaviour were reported for lower age at neutering.

Table 2.5. The impact on behaviours related to the different classification of neutering timing that were identified in a scoping review undertaken to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on the behaviour of female domesticated dogs.

Timing of neutering comparison	**Author**	**Study design**	**Sex**	**Impacts on behavioural outcomes**	**Favourable or detrimental impact of neutering summary**
Dogs neutered before compared to after puberty					
Before or after puberty	Balogh *et al.*, 2018	CS	Female	None	No impact
	Brandli *et al.*, 2021	RC	Female	None reported due to small numbers	None reported
Age at neutering compared to an entire population					
At or under 6 months of age compared to entire	Zink *et al.*, 2014	CS	Both male and female	Increased risk of developing a behavioural disorder compared to entire dogs (1.8x, P<0.001). Increased risk of fear of storms compared to entire dogs (OR=3.9, P<0.001).	Detrimental for dogs neutered at or under 6 months of age
Between 7 and 12 months compared to entire	Zink *et al.*, 2014	CS	Both male and female	Increased risk of fear of storms compared to entire dogs (OR=4.7, P<0.001).	Detrimental for dogs neutered between 7 and 12 months of age
	Farhoody *et al.*, 2018	CS	Both male and female	Increase in moderate or severe aggression for dogs neutered between 7 and 12 months of age compared to entire dogs (OR=1.259, 95% CI=1.09-1.45, P=0.002).	Detrimental for dogs neutered between 7 and 12 months of age

At or under 1 year compared to entire	Stellato *et al.*, 2021	CS	Both male and female	Higher fear score at veterinary clinics for dogs neutered at ≤1 year of age than older age groups when compared to entire dogs (<6 months P=0.015, 6-11 months P<0.001, 1 year P=0.008).	Detrimental for dogs neutered at or under 1 year of age
Over 12 months compared to entire	Zink *et al.*, 2014	CS	Both male and female	Increased risk of fear of storms compared to entire dogs (OR=4.0, P<0.001).	Detrimental for dogs neutered over 12 months of age
At 7 weeks, or 7 months compared to entire	Salmeri *et al.*, 1991	PC	Both male and female	None	No impact
Comparisons between dogs neutered at different ages					
<5.5m compared to ≥5.5 months	Spain *et al.*, 2004	RC	Both male and female	Urination when frightened: OR=0.74 for <5.5m (lower) than for dogs neutered at ≥5.5 months (95% CI=0.54-1.01, P=0.06). Separation anxiety: OR 0.72 for <5.5m (lower) than for dogs neutered at ≥5.5 months (95% CI=0.55-0.94, P=0.02).	Favourable for dogs neutered younger than 5.5 months of age
7 weeks compared to 7 months	Salmeri *et al.*, 1991	PC	Both male and female	None	No impact
<24 compared to ≥24 weeks of age	Howe *et al.*, 2001	PC	Both male and female	None	No impact
<7 months, 7 to 11 months, >11 months	Zlotnick *et al.*, 2019	RC	Both male and female	Behavioural withdrawals from assistance dog training about 40% less likely in dogs neutered between 7 and 11 months of age than dogs neutered at <7 months (RR=0.6, P<0.01) and 30% less likely than dogs neutered at >11 months of age (RR=0.7, P=0.05).	Favourable for dogs neutered between 7 and 11 months of age

<12 months compared to >12 months	O'Farrell and Peachey 1990	PC	Female	Greatest increased risk of dominance aggression towards family members for bitches neutered <12 months of age that were already showing signs of aggression compared to bitches neutered >12 months of age that were not showing signs of aggression (no statistics reported).	Detrimental for bitches neutered under 12 months of age that are already showing signs of aggression compared to those neutered over 12 months of age showing none
<6 months, 6 to 12 months, 12 to 18 months, >18 months	Edwards *et al.*, 2019	CS	Both male and female	None	No impact
<6 months, 7 to 12 months, 1 to 5 years, 6 to 10 years, >10 years	Palestrini *et al.*, 2021	PC	Both male and female	None reported due to small numbers	None reported
Relationships with percentage lifetime gonadal hormone exposure					
Higher percentage lifetime gonadal hormone exposure	Starling *et al.*, 2019	CS	Female	Decreased fear related behaviour when barked, growled or lunged at by an unfamiliar dog (OR=0.997, 95% CI=0.996-0.999, P<0.001). Decreased 'will 'fetch' or attempt to fetch sticks, balls, or objects' (OR=0.993, 95% CI=0.991–0.995. P<0.001). Decreased 'when off the leash returns immediately when called' (OR=0.997, 95% CI=0.995–0.999, P=0.001).	Detrimental for lower PLGH Favourable for lower PLGH Favourable for lower PLGH

				Increased likelihood of howling (OR=1.004, 95% CI=1.002-1.006). Increased chews inappropriate objects (OR=1.004, 95% CI=1.003-1.006).	Favourable for lower PLGH Favourable for lower PLGH
Lower percentage lifetime gonadal hormone exposure	Starling *et al.*, 2019	CS	Female	Increased aggression when delivery workers approached the home (OR=0.994, 95% CI=0.9992-0.9998, P<0.001). Increased aggression when approached by an unfamiliar male dog while on leash (OR=0.997, 95% CI=0.995–0.999, P<0.001). Increased aggression when being barked, growled or lunged at by an unfamiliar dog (OR=0.996, 95% CI=0.995–0.998, P<0.001). Increased excitable behaviour before being taken on a car trip (OR=0.997, 95% CI=0.995-0.998, P<0.001).	Detrimental for lower PLGH Detrimental for lower PLGH Detrimental for lower PLGH Detrimental for lower PLGH
Relationships with age at neutering					
Increasing or older age at neutering	Starling *et al.*, 2019	CS	Female	Decreased 'Will 'fetch' or attempt to fetch sticks, balls, or objects' (OR=0.997, 95% CI=0.997–0.998, P<0.001).	Favourable for lower age at neutering
Decreasing or younger age at neutering	Starling *et al.*, 2019	CS	Female	Increased aggression when delivery workers approached the home (OR=0.999, 95% CI=0.998-0.999, P<0.001)	Detrimental for lower age at neutering

	Spain *et al.*, 2004	RC	Both male and female	Increase in noise phobia (OR=1.04) for each one month decrease in age at surgery (95% CI=1.01-1.08, P<0.01). Increase in sexual behaviours (OR=1.05) for each one month decrease in age at neutering surgery (95% CI=1.01-1.09, P<0.01). OR=0.93 decrease in escaping from home for each one month decrease in age at surgery (95% CI=0.87-0.98, P<0.01, when considered a serious problem).	Detrimental for lower age at neutering Detrimental for lower age at neutering Favourable for lower age at neutering
Relationships suggested but are unclear					
Age at neutering and PLGH	Starling *et al.*, 2019	CS	Female	Rolls in animal droppings or other smelly substances and pulls excessively hard when on the leash: relationships with age at neuter and PLGH suggested (P</=0.001) but no OR or direction of relationships reported.	Unclear
				Increase in excitable behaviour when the doorbell rings for age at neuter and PLGH. However, only one OR (0.989, 95% CI=0.987-0.991) is reported therefore attributing to age at neuter or PLGH was not possible.	Unclear

Abbreviations: RC – retrospective cohort, PC – prospective cohort, CS – cross-sectional, CI – confidence interval, OR – odds ratio, RR – relative risk, PLGH - percentage lifetime gonadal hormone exposure.

2.4. DISCUSSION

2.4.1 Summary

This is the first scoping review that collates and describes the evidence relating to the effects of neutering bitches before or after puberty on behaviour. The review clearly identifies a lack of evidence, with 13 studies eligible for inclusion and only one of these examining behavioural data for bitches that were categorised as being surgically neutered before or after puberty. For that study, no definition of puberty was provided however, and classification was based on owner recollection. One other study considered bitches pre or post-pubertal at the time of the first treatment with deslorelin acetate for oestrus suppression. However, only four bitches were treated before puberty and numbers were too small for analysis of behavioural data. The remaining 11 studies examined the effects on behaviour related to age at neutering, even though one of these suggested that any age >24 weeks could be considered post-pubertal. This scoping review intended to collate and describe the available evidence to support veterinarians, dog behaviour specialists, those working with dogs and pet dog owners when discussing the timing of neutering relative to puberty. Instead, it has identified that robust evidence in which to base decisions does not yet exist. However, this provides information to vets to support conversations around the behavioural impacts of neutering and provides direction for future studies; the impact of neutering before or after puberty on the behaviour of female domesticated dogs remains unknown.

2.4.2 Study and participant characteristics

Two studies were identified that investigated the timing of neutering related to pubertal status. The first study involved 58 bitches from one breed that were categorised as being surgically neutered before or after puberty based on their owners' recollection. For this study there were limited numbers of bitches in each neuter group. However, behaviour was measured using a validated behaviour questionnaire and direct observations, which improves the reliability

of the results. The second study included four bitches that had their first deslorelin acetate treatment to suppress oestrus before puberty and 28 bitches that had their first treatment after puberty. In the latter study a definition of puberty was not provided, and behaviour was measured using owner questionnaires, however the questionnaires were not made available. The remaining 11 studies included in the review reported data for dogs based on age at neutering. This demonstrates the lack of available literature related to the impacts of neutering bitches before or after puberty on behaviour. However, the scoping review has enabled the evidence that is available, relating to age and puberty, to be summarised.

The majority of studies that were included in the scoping review retrospectively allocated dogs to groups based on when they were neutered. Where decisions about when to neuter were made by pet owners, some may have chosen to neuter their dogs earlier or later due to the animal's current behaviour and a belief about positive effects on future behaviours. The lack of random allocation to study groups presents a risk of bias that is difficult to overcome in studies that involve neutering bitches. Even if studies had been prospective and involved random allocation, it is not possible to blind owners to which group bitches are allocated to. The only study to randomly allocate dogs to groups was a prospective cohort study of 32 male and female dogs in a research facility. However, as well as a small study population, this study used subjective assessments of behaviour made by the primary care giver, which negatively impacts the reliability of the data.

Validated canine behaviour questionnaires are available and five of the studies used the C-BARQ, questions from the C-BARQ or data from the C-BARQ database, to examine behaviour. Owner questionnaires are commonly used to gather data on dog behaviour and although they can be affected by owner subjectivity, many studies have reported good reliability and validity (Tiira and Lohi, 2014; Harvey *et al.*, 2016a; Salonen *et al.*, 2021). Using data from

the C-BARQ database does allow for large study populations; three studies in this review that used data from C-BARQ included over 9,000, 13,000 and 25,000 dogs in each. Including such large numbers in prospective cohort studies would not be feasible. However, for large study populations, consideration should be given to statistical power and using the appropriate values of alpha. The remaining study populations ranged from 32 to 2,505 dogs, with the four prospective cohort studies including 32, 96, 269 and 300 dogs. Power analysis would also be important for these studies to determine the size of the effect that could reliably be detected. However, none of the nine studies reported using power analysis to determine appropriate values of alpha, and all studies used an alpha value of 5%.

The 11 studies that examined the impact of age at the time of neutering all considered or grouped age in different ways, such as before and after 5.5 months of age, neutering at seven weeks, seven months of age or entire, neutering at less than six months, between seven and 12 months or over 12 months. One study analysed age as a continuous variable and also considered the percentage lifetime gonadal hormone exposure. This does not allow for between study comparisons to be made easily when collating evidence from multiple studies, nor does it allow conclusions to be drawn about the impact of neutering in relation to puberty. One of the studies that grouped dogs as neutered at either less than 24 weeks of age, or 24 weeks of age or more, reported in the abstract and concluding paragraph of the discussion that the results indicate that neutering before puberty is not associated with increased behaviour problems. However, this is not a valid conclusion based on the age grouping of dogs at the time of neutering. The study that examined percentage lifetime gonadal hormone exposure appeared not to take age at neutering into account in that analysis, which may affect the results. For instance, a dog that was 12 months of age and was neutered at nine months of age would have the same percentage lifetime gonadal hormone exposure as a dog of 12 years

of age that was neutered at nine years of age. Thus, drawing conclusions about the impact of percentage lifetime gonadal hormone exposure is complicated for readers if they assume that greater percentage lifetime gonadal hormone exposure is associated with dogs that were older when they were neutered.

Most studies included male and female dogs and did not report results by sex separately; this prevents a clear understanding of the impacts specifically on bitch behaviour. Most studies included various or multiple breeds and did not state the breeds involved, or present results by breed, however some studies dealt with this by controlling for breed in the analyses or by matching control group bitches by breed. Breed can impact behaviour (Turcsán *et al.*, 2011; Asp *et al.*, 2015; MacLean *et al.*, 2019) therefore understanding the breeds, and controlling for breed in analysis is important so that results are not influenced by the inclusion of high or low numbers of certain breeds. However, this does pose difficulties for future study design. Undertaking individual studies across different geographical locations – to account for variations in behaviour that may be present in different subpopulations or cultural impacts on behaviour interpretation - for many breeds, or recruiting sufficient numbers of bitches of each breed to one prospective study would not be easy. Three studies did not state the age of bitches when behavioural data were collected. Therefore, there were differences between studies, and in some cases between dogs within studies, in the time elapsed since neutering, which was not always described or controlled for in analyses.

The type of neutering surgery was not reported in eight of the 12 studies that were presumed to involve surgical neutering. It is worth noting that in large studies with data extracted from the C-BARQ database, there is also the potential for medical suppression of fertility to be reported as 'neutering' for some dogs. While ovariohysterectomy, and to a lesser extent ovariectomy, are the most common surgical neutering procedures for bitches (Diesel *et*

al., 2010; Bushby and White, 2020), neutering by hysterectomy may also be undertaken (Belfield, 1972; De Cramer and May, 2015; Mattravers, 2017). Additionally, neutering can be performed either laparoscopically or by celiotomy in bitches. While there may be little direct impact of type of surgery on later behaviour, there are potentially different risks for post-operative complications resulting from different surgical techniques (Van Goethem *et al.*, 2006). Although the strength of the existing evidence for post-operative outcomes for bitches neutered by midline celiotomy and ovariohysterectomy compared to ovariectomy has been questioned (Pennington, 2020), differences in activity in the two-day period following surgery have been demonstrated between bitches neutered laparoscopically or by celiotomy (Culp *et al.*, 2009). Should post-operative complications differ between surgical techniques, more severe complications, or necessary treatment, could impact future behaviour, particularly fear and anxiety at future vet visits, or aggressive or fearful responses to certain people or stimuli (Edwards *et al.*, 2019; Wallis *et al.*, 2020). Should any of the bitches have undergone neutering via hysterectomy, gonadal hormones would still be present and influencing behaviour which could have impacted study outcomes.

Nine of the studies used pet dogs, two of which used dogs that had previously been in an animal shelter and were neutered during their time in the shelter. The lack of consistency in history and life experience prior to neutering, lack of knowledge of behavioural history and differences between studies make comparisons difficult. Some relationships between neutering and behaviour could have been influenced by age that dogs entered the animal shelters, age at adoption, duration of stay in a shelter, or by experiences before or during shelter accommodation. These unknown factors make determining cause and effect impossible for the relationship between neutering and future behaviour.

Nine of the 13 studies did not exclude dogs that were neutered for behavioural reasons, although one included reasons for neutering in the statistical analysis. When measuring the impact of an intervention on an outcome, it is important to either understand the status of the outcome measure prior to the intervention or to exclude those affected from the study. For instance, if a study was examining the impact of a treatment or intervention on the occurrence of a disease, prior to administration, study participants would all need to have been free from the disease. If they were not, or their disease status was unknown, understanding of the impact could be compromised by the fact that some participants may have been affected throughout the study, both before and after. Similarly here, if the dogs were already exhibiting signs of a behaviour prior to being neutered, neutering itself could not be deemed a risk factor for that behaviour being present in the follow up period after neutering. The lack of consideration for dogs' behaviour status prior to neutering, or lack of exclusion of dogs already exhibiting the behaviour being studied prior to neutering, impacts the confidence in the results. Where effects of neutering at younger ages were identified, it is not possible to determine whether these behaviours were present prior to neutering and therefore whether it was actually neutering that impacted the behaviours.

2.4.3 Summary of the evidence

Despite the lack of evidence relating specifically to puberty, this scoping review provided an overview of the available literature on the effects of neutering timing on behaviour in female dogs. Only two studies met the inclusion criteria, categorising bitches as pre or post-pubertal at surgical neutering or first treatment for oestrus suppression, highlighting a need for further research in this area and the importance of reporting this critical factor in studies. One of these studies did not identify any differences in behaviour reported by owners using the C-BARQ, or in observed behaviour (Balogh *et al.*, 2018). However, there were only a small number of bitches in each study group. Additionally, there was the potential for error in

classification of timing of neutering due to owner recollection. In the other study, where deslorelin acetate was used for oestrus suppression, any behavioural impacts that might have been identified would be complicated by potential hormonal changes influencing the behaviour as the implant lost efficacy. Such effects would not occur in studies where the ovaries had been surgically removed.

The remaining 11 studies presented results for dogs neutered at different ages. However, four of the studies that grouped dogs by age at neutering did not make comparisons between age groups to determine differences in behaviour; three only made comparisons between each age group and a cohort of entire dogs (Zink *et al.*, 2014; Farhoody *et al.*, 2018; Stellato *et al.*, 2021) and one did not have a sufficient number of dogs to allow for between age group analysis (Palestrini *et al.*, 2021). These studies therefore contributed no evidence relating to the impact of neutering at different ages on behaviour in female dogs.

Seven studies did make behavioural comparisons between dogs neutered at different ages. Three of these reported no impact of age at neutering on behaviour (Salmeri *et al.*, 1991; Howe *et al.*, 2001; Edwards *et al.*, 2019). The behaviours measured by Howe *et al.* in the owner telephone questionnaire that was used to collect the data were not stated, and it was not possible to obtain a copy of the questionnaire. However, behaviours measured by Salmeri *et al.* were similar to those in other studies where impacts on behaviour were noted including excitability, aggression and outgoing nature. However, behavioural assessments in the study by Salmeri *et al.* were not made using validated methods for collecting behavioural data, but using subjective behavioural assessments undertaken by the dogs' primary caregivers, therefore the validity of these behavioural data could be compromised.

Four studies identified differences in behaviour between dogs neutered at different ages. The most common behaviour traits shown to be impacted by age at neutering were those relating to aggressive, fearful and anxious and social behaviours. There were 15 behaviours reported to be affected by age at neutering, not including general behaviour disorders (Zink *et al.*, 2014) or withdrawals from a service dog programme for behaviour (Zlotnick *et al.*, 2019), but many that were not. These behaviours differed between studies. One study considered age at neutering as a continuous variable as well as percentage lifetime gonadal hormone exposure (Starling *et al.*, 2019). The study examined data from 100 C-BARQ questions for almost 9,000 bitches and identified five questions with scores that were associated with neutering age, although for three of these (rolls in animal droppings or other smelly substances, pulls excessively hard when on the leash, excitable behaviour when the doorbell rings) the direction of the relationship was not clear. Fetching behaviour scored more favourably for bitches neutered at younger ages while aggression towards delivery workers was more favourable for bitches neutered at older ages. Bitches that were neutered to prevent or correct a behavioural problem were excluded from the study. Therefore behaviour prior to neutering may not have influenced the results as much as some studies where such exclusions were not included. The retrospective nature of behavioural data collection may have been subject to owner recall bias concerning the dogs' behaviours or reasons for neutering.

O'Farrell and Peachey (1990) reported that for bitches already showing signs of aggression at the time of neutering, aggression was more likely to increase for bitches neutered at less than one year of age than for those neutered over one year of age. However, that study utilised owner questionnaires administered six months after neutering and no statistical evaluation was reported. Interestingly, the study by Spain *et al.* (2004), measured aggression but found no impact of neutering. Instead, urination

when frightened and separation anxiety were decreased in dogs neutered at less than 5.5 months of age, escaping from home decreased with each one month decrease in neutering age, while noise phobias and sexual behaviours increased with each one month decrease in neutering age. That study included data for male and female dogs from owner questionnaires and veterinary records and did not exclude dogs that already had behaviour problems, which will impact the confidence in the reported findings. It is interesting that some fear or anxiety related behaviours were favourably impacted and some negatively impacted by neutering at lower ages.

The final study that identified behavioural differences for dogs neutered at different ages did not directly measure behaviour, but withdrawals from a service dog training programme for behavioural reasons. Therefore, no conclusions about specific behaviours could be drawn. Again, this study did not report results for male and female dogs separately. The study reported that withdrawals for behavioural reasons were less likely in dogs neutered between seven and 11 months of age than in dogs neutered at less than seven, or greater than 11 months of age. Dogs in the study were in a service dog training programme where decisions about the withdrawal of a dog for behavioural reasons were made by training staff (Zlotnick *et al.*, 2019). Training staff would have been aware of the neutering age of each dog, and it is possible that their preconceptions around the impacts of neutering at different ages may have affected their beliefs about whether a dog would be successful in its training. The lack of behaviour measures within the study mean that it is not possible to confirm whether the dogs neutered in different age groups were actually displaying different behaviours, which could be a result of neutering age or whether decisions about their success were influenced by the staff that were responsible for them.

2.4.3.1 Consideration of scoping review findings compared to existing published reviews

Reviewing the 13 studies related to pubertal status or age at neutering and impacts on behaviour did not provide evidence to support opinions from review papers. A published accredited CPD article by De Cramer and May (2015) defines prepubertal neutering as neutering between three and five months of age. The authors stated that "Dogs neutered pre-pubertally have a higher success rate in becoming guide dogs" and references a paper by Root-Kustritz (2014) which itself is a review paper on paediatric neutering. Upon examination of the latter, the reference within relates to a study of male guide dogs. However, Root-Kustritz states "In one study of male guide dogs, there were no differences in behavior in placement rate as a working dog when comparing dogs castrated at 7 to 8 weeks, 6 to 8 months, or 10 to 14 months of age." Evidence from this scoping review only identified one paper relating to service dogs published in 2019, in which behavioural withdrawals from the service dog programme were less likely for dogs neutered between seven and 11 months of age than dogs in the younger or older age groups, however pubertal status at neutering cannot be inferred from these age groups. No studies relating specifically to guide dogs were identified, therefore the source of the statement made by De Cramer and May (2015) could not be confirmed.

Kustritz published an earlier review in 2002 that included the statement "Long-term effects of prepuberal gonadectomy are the same as those seen after gonadectomy performed at the more traditional age of 6 to 7 months." This highlights the confusion in the literature surrounding classification of dogs by age instead of actual onset of puberty. At six to seven months of age, bitches may still be prepubertal therefore the two age groups mentioned are likely to contain mainly prepubertal dogs. Kustritz continues "Anecdotal reports suggest that dogs and cats gonadectomized prepuberally are more likely to maintain juvenile behaviors and be

more difficult to train. Scientific evidence suggests the contrary. Groups of dogs spayed or castrated at 7 weeks of age (n=65) or 7 months of age (n=64) and trained as service dogs for the handicapped were equally likely to succeed in training (personal communication, Ruth Daniels, Canine Companions for Independence, April 2002). Prepuberal neutered dogs had a higher success rate in becoming guide dogs as compared to littermates neutered at traditional times (personal communication, Dr. Patricia Olson, Guide Dogs for the Blind, Inc., April 2002)." No published scientific evidence has been identified within the current review to support these claims and again, it is likely that all dogs in the referenced works were neutered before puberty due to the age groups studied.

Houlihan (2017) reported in a literature review "In another study conducted to evaluate gonadectomy in dogs before or after 24 weeks of age, investigators concluded that there was no increase in the incidence of behavioral problems or return rate to shelters for prepubertally gonadectomized dogs." Here Houlihan refers to the study by Howe *et al.* (2001) included in the final nine papers in this scoping review. However, as previously discussed, the classification of neutering age used by Howe *et al.* (2001), and lack of consideration of puberty in the study design, does not allow interpretation of findings in relation to pubertal status at the time of neutering.

A published review paper by Romagnoli (2017) defined prepubertal neutering as neutering between six and 14 weeks of age. Within the review they state "Effects of prepuberal gonadectomy on behaviour vary depending on species and sex. In dogs increased aggressiveness and barking towards family members and strangers, increased fear of noise, increased sexual behaviour but decreased separation anxiety and decreased urination at home have been observed. In general, excitability and degree of activity are increased in males and females gonadectomised at 7 weeks or 7

months, and when surgery is done at 7 weeks animals are found to be more excitable if compared to those gonadectomised at 7 months. However, inappropriate behaviors as reasons for relinquishment have not been reported in dogs neutered at an early age when compared to those neutered as adults, and in Labrador/Golden Retriever crosses raised as guide dogs for the blind, neutering at 7 weeks or 7 months does not affect the degree of training success." However, there are no references included for these statements therefore it is not clear from which studies the evidence originates. From the present scoping review and the study characteristics and findings described, it is likely that the study referenced by Romagnoli, which included dogs neutered at seven weeks and seven months, was that of Salmeri *et al.* (1991), although this study was not in the reading list in the review. The present scoping review has identified no literature to support the claim that there is evidence that prepubertal neutering of female dogs has any impact on behaviour. Furthermore, the present scoping review only identified two papers in total, published in 2018 and 2021, that investigated the impacts of neutering (or treatment for oestrus suppression) before or after puberty on behaviour.

In a guest editorial in 2011, Thrusfield defined early neutering as neutering before the first oestrus at less than six months of age, and states that early neutering has advantages including small reductions in some undesirable behaviour traits in female dogs. The retrospective cohort study by Spain *et al.* (2004) was referenced, a study that classified dogs as neutered either before or after 5.5 months of age. While it is true that those neutered at less than 5.5 months of age were likely to be prepubertal, those neutered at more than 5.5 months of age were not confirmed as post-pubertal and no record of puberty was made by Spain *et al.* Additionally, there are also some less favourable impacts on certain behaviours reported by Spain *et al.* (2004) for dogs neutered at younger ages that were not mentioned in the editorial, including increased risk of noise phobia and sexual behaviour.

The review of the available literature undertaken as part of this scoping review did not identify any literature to support the suggestions made by other authors regarding the impact of neutering before or after puberty on behaviour in female dogs.

2.4.4 Limitations

The database searches were conducted in February 2022. Since that time, further studies investigating the impacts of neutering before or after puberty on behaviour in female dogs may have become available.

A scoping review does not allow for analysis of the literature relating to a specific question, nor the reporting of a finding as could be expected from a systematic review. However, a scoping review is a recognised method suitable for the synthesis of evidence required of this project (Munn *et al.*, 2018; Sucharew and Macaluso, 2019; Peters *et al.*, 2020). A scoping rather than systematic review method was chosen due to the broad question posed and the need to examine and describe the potentially large range of literature that had not previously been synthesised (Peters *et al.*, 2015; Lockwood *et al.*, 2019). The review was conducted following JBI guidelines and using JBI critical appraisal tools (Tricco *et al.*, 2018) and was the appropriate review method to provide an overview of the available and relevant literature and to identify gaps in the research.

In comparison to a systematic review, scoping reviews do not limit the types of studies included and will therefore include a larger number of studies with various designs and methods (Sucharew and Macaluso, 2019). While this prevents the analysis of evidence that would be required for a systematic review, this can also be a benefit of a scoping review due to the ability to include a broader range of literature to explore the topic (O'Brien *et al.*, 2016). This was actually a benefit in terms of the current review. Only two studies

were identified that examined neutering in relation to puberty and these would have been the only studies reported in a systematic review. However, in this scoping review, other studies that examined age at neutering were reviewed and summarised. This provided a broader overview of the available evidence and identified problems relating to categorisation of dogs by age and other features of the study methodologies. This allowed reporting of the factors relating to study design and characteristics, and recommendations to be made for future studies. Additionally, bodies of evidence relating to certain behaviours such as fear and anxiety and aggression, were highlighted that may inform future systematic reviews.

There was the potential for selection bias (Sucharew and Macaluso, 2019). The search strategy may not have identified all of the available literature which could have affected the description of eligible studies presented. However, the databases that were included have been reported to provide very high levels of coverage of veterinary literature (Grindlay *et al.*, 2012).

A scoping review is recommended to be conducted by a team, including a librarian, rather than an individual. The inclusion of a librarian can help develop the research question and optimise the search strategy due to their knowledge of article retrieval from searches (Morris *et al.*, 2016; Hanneke *et al.*, 2017; Lockwood *et al.*, 2019; Peters *et al.*, 2020). This review was completed by two individuals, which was deemed appropriate for the volume of literature generated for review. However, there was no librarian included in the development of the search strategy. This could have resulted in additional work due to lack of refinement of the search strategy resulting in more articles than necessary being retrieved. There is also the possibility that some articles may have been missed, however the search terms and strategy were reviewed by a group of five specialists, one with extensive experience of conducting scoping reviews. Therefore, it is more likely that the

search would have been too broad resulting in more articles being retrieved than failing to identify relevant articles. The search term 'spey' was not included which could have resulted in articles being missed if they did not also include other terms relating to neutering that were in the search terms.

2.4.5 Future recommendations

Future studies that aim to examine the effects of neutering bitches before or after puberty should be undertaken for a variety of dogs (pet, assistance, shelter etc.) from many geographical locations. Studies should consider breed, which can impact behaviour and should consider using validated methods to measure behaviour, including either direct behavioural observations or validated behaviour questionnaires. Such studies should randomly allocate dogs to neutering groups where all bitches are neutered using the same surgical method. Surgical methods should be reported and where different methods are used, controlled for in the analysis. Studies should follow the dogs over time, ensuring that behaviours are monitored before and after neutering and, if appropriate, that dogs displaying the behaviour being investigated prior to neutering are either controlled for or excluded. Consideration should be given to the amount of time between neutering and collecting behaviour measures, or the age when behaviour is measured to ensure sufficient time has elapsed since neutering for any effects on behaviour to become apparent. Study population sizes should be considered in order to ensure that the minimum number of bitches needed for valid statistical analyses are recruited to the studies, and power analysis used to determine appropriate values of alpha for the study populations. Additionally, for studies examining the effects of timing of neutering relating to age of the dog, consideration of other studies when selecting age groups could enable easier comparison between studies. Finally, when considering the effects of neutering before or after puberty, a definition of puberty should be stated, puberty should be correctly identified, and studies should consider the onset of puberty itself rather than age.

2.5 CONCLUSION

This scoping review identified and described the available literature relating to the effects of neutering female domesticated dogs before or after puberty on behaviour. Only one study was identified that categorised the timing of surgical neutering as before or after puberty, and this categorisation was based on owner recollection from many years prior to the study. A second study was identified that examined the impacts of bitches receiving the first in a series of implants to supress oestrus either before or after puberty, but that study did not contain a sufficient number of dogs to examine impacts on behaviour. The remaining 11 studies used age of the bitch rather than puberty. Most studies were retrospective and varied in their methodologies, quality and means of categorising bitches into neutering age groups. The review has identified a lack of evidence to support understanding of the impacts of neutering bitches before or after puberty on behaviour. Recommendations are made for future studies that are undertaken investigating neutering bitches before or after puberty including conducting prospective cohort studies with random allocation of bitches to study groups, which are lacking in currently available studies. Despite the lack of evidence identified, the scoping review highlighted the lack of literature along with weaknesses in the existing studies caused by age rather than puberty being examined. These are important findings and will be of use to veterinarians, pet dog owners and others when making neutering decisions. Whether or not neutering before or after puberty has an impact on the behaviour of female domesticated dogs is currently unknown.

Chapter 3. Effect of timing of neutering in relation to puberty on health in the female dog – a scoping review

3.1 INTRODUCTION

The impact of neutering on female dog health has been studied and summarised in many narrative and systematic reviews (Beauvais *et al.*, 2012a,b; Smith, 2014; Houlihan, 2017; Oberbauer *et al.*, 2019; Urfer and Kaeberlein, 2019; Yates and Leedham, 2019; Kutzler, 2020; Stavisky and White, 2022). Both positive and negative effects on bitch health have been reported, and these are suggested to be associated with age at neutering for some diseases (Salmeri *et al.*, 1991; Howe *et al.*, 2001; Spain *et al.*, 2004; Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014, 2016, 2020a,b; Simpson *et al.*, 2019). However, comparing and consolidating evidence from different studies is challenging due to variations in study populations and methodological approaches. The direction of the effect can also be inconsistent depending on the disease studied. For example, earlier neutering has been associated with an increased risk of cranial cruciate ligament (CCL) rupture (neutering at less than 12 months of age: Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014, 2016; Ekenstedt *et al.*, 2017), but a decreased risk of certain cancers such as haemangiosarcoma (HSA), mammary neoplasia and mast cell tumours (MCT) (neutering before 2.5 years of age: Schneider *et al.*, 1969; Sonnenschein *et al.*, 1991; neutering at less than 12 months of age: Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014). For some diseases, such as urinary incontinence (UI), the association with age at neuter is unclear: Lutz *et al.* (2019) report an increased risk for bitches neutered at earlier ages, while Reichler *et al.* (2005) report the opposite, and others failed to find a significant association (Thrusfield *et al.*, 1998; de Bleser *et al.*, 2011; Forsee *et al.*, 2013). Interpretation of these findings is further complicated by breed differences which are reported for different conditions (Hart *et al.*, 2020a,b).

Two systematic reviews conducted in 2012 (Beauvais *et al.*, 2012a,b) investigated the effect of neutering and age at neutering (including studies that considered pubertal status at neutering) on mammary tumours and UI. The studies identified only weak associations and highlighted the risk of bias in the published studies. In other narrative reviews considering neutering timing in relation to puberty, mammary tumours are the most frequently mentioned disease, with a suggested protective effect of earlier neutering (Le Bars, 2006; Root-Kustritz, 2007, 2012; Schafer-Somi *et al.*, 2014; Speck, 2014; Howe, 2015; Goh, 2016). Most reviews reference the same work by Schneider *et al.* (1969) that identified a reduced risk for bitches neutered before the first oestrus. However, this study was suggested to be at moderate risk of bias by Beauvais (2012a). Other narrative reviews have suggested impacts on UI which varied dependent on individual study findings (Schafer-Somi *et al.*, 2014) while some suggest that the evidence relating to the impacts on UI is inconclusive (Romagnoli, 2017). Neutering before puberty has been suggested to impact growth and vulva development (Stubbs and Bloomberg, 1995; Root-Kustritz, 2012, 2014; Romagnoli, 2017), leading to epiphyseal fractures and urogenital disease, however the work of Salmeri *et al.* (1991), which did not consider neutering before or after puberty, is commonly referenced to support these suggestions. Inferring pubertal status based on studies that have used age at neutering is potentially flawed due to the variation in age at onset of puberty between dogs (six to 18 months; Hewitt and England, 1999), the fact that actual puberty (first oestrus) is not recorded, and that the age groups in studies are not designed to adequately distinguish between these groups. Howe *et al.* (2001) suggested that dogs neutered when younger than 24 weeks of age were prepubertal, however it is possible that dogs neutered aged older than 24 weeks in the study were also prepubertal.

To the author's knowledge, no systematic review or research synthesis have yet been published on the impact of neutering

bitches before or after puberty on atopy, developmental orthopaedic disease (DOD), neoplasia (other than mammary tumours), or obesity. Additionally, since the two systematic reviews on the impact of neutering on mammary tumours and UI (Beauvais *et al.*, 2012a,b), further studies have reported results that would benefit from review. Due to the potential for the timing of neutering in relation to puberty to impact various aspects of long-term health in female dogs, a scoping review was designed. Scoping reviews can provide a broad overview of literature relating to a topic, are a useful first step in collating the evidence and can be used prior to a systematic review (Peters *et al.*, 2015; Munn *et al.*, 2018; Lockwood *et al.*, 2019; Sucharew and Macaluso, 2019).

The aim of this study was to systematically search the available literature to identify and chart the current evidence on the effect of the timing of neutering in relation to puberty on the health of female domesticated dogs, and to highlight areas where knowledge is lacking.

The objectives of the study were to:

- Conduct a systematic search of databases using published methodology to identify current literature on neutering in female dogs.
- Review and appraise the identified literature on the effects of neutering bitches pre or post-pubertally on bitch health, specifically atopy, developmental orthopaedic disease (DOD), neoplasia, obesity and urogenital disease.
- Chart and summarise evidence on associations between these health problems and bitches neutered before puberty compared to bitches neutered after puberty.
- Chart study methodology from existing publications to identify knowledge gaps and construct recommendations for future studies to improve the evidence base.

3.2 METHODOLOGY

3.2.1 Protocol and registration

A search was conducted for existing scoping and systematic reviews on the impact of neutering bitches before and after puberty on atopy, DOD, neoplasia, obesity and urogenital disease. JBI Evidence Synthesis, PROSPERO, PubMed and VetSRev were searched on 07/09/2023. Two systematic reviews were identified, however these examined the evidence related to neutering age and not pubertal status at neutering (Beauvais *et al.*, 2012a,b).

A Preferred Reporting Items for Systematic reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR; Tricco *et al.*, 2018) was used for this scoping review. The scoping review protocol was developed before data extraction and was registered with the Open Science Framework (Freeman and Moxon, 2023).

3.2.2 Search strategy

Five primary literature searches were conducted on 17/05/2023 in three scientific databases; CAB Abstracts (1910 to present), Medline (1910 to present) and Web of Science (1950 to present) (Grindlay *et al.*, 2012).

Search terms were developed and reviewed by a team of five people, including the authors. The search terms were:

Neuter OR neuters OR neutering OR neutered OR spay OR spays OR spayed OR spaying OR ovariohysterectomy OR ovariohysterectomies OR sterilize OR sterilizes OR sterilization OR sterilise OR sterilises OR sterilisation OR gonadectomy OR gonadectomise OR gonadectomize OR ovariectomy OR ovariectomies OR de-sexing OR desex OR desexing OR de-sex OR castration OR castrate OR castrating OR sterilized OR sterilised

AND

Age OR pubert*

AND

Bitch OR bitches OR dog OR dogs OR canine OR canines OR canid OR canids OR canis

AND

Search 1

Obesity:

obese OR obesity OR "body fat" OR fatness OR adiposity OR overweight OR "over weight" OR grossness OR heaviness

Search 2

Neoplasia:

Neoplas* OR cancer* OR tumor* OR tumour* OR malignan* OR sarcoma* OR carcinoma* OR carcinosarcoma* OR haemangiosarcoma* OR hemangiosarcoma* OR osteosarcoma* OR lymphoma* OR lymphosarcoma* OR adenosarcoma* OR adenocarcinoma* OR lymphangiosarcoma* hemangiopericytoma* OR mastocytoma* OR mastocytosis* OR apudoma* OR mesothelioma OR lymphangiosarcoma OR myxosarcoma OR myosarcoma OR leiomyosarcoma OR fibrosarcoma OR chondrosarcoma OR liposarcoma OR hepatoblastoma OR mesenchymoma OR thymoma

Search 3

Urogenital:

Urinary tract infection* OR urinary incontinence OR urinary tract OR urologic* OR UTI* OR bladder OR cystitis OR vulva* OR vagina* OR vaginitis

Search 4

DOD:

"hip dysplasia" OR "elbow dysplasia" OR dysplastic OR coronoid OR "fragmented medial coronoid process" OR osteochondrosis OR osteochondritis OR "hypertrophic osteodystrophy" OR panosteitis OR "ununited anconeal process" OR "medial compartment disease" OR

"short ulna syndrome" OR "trochlear notch incongruity" OR "short radius syndrome" OR "cruciate ligament" OR "cruciate rupture" OR cruciate OR "cruciate injury" OR "cruciate tear"

Search 5
Atopy:
exp Dermatitis, Atopic/
OR atopy OR "atopic dermatitis" OR dermatitis OR atopic OR eczema OR "allergic skin" OR pruriti* OR allerg* OR "allergic inhalant dermatitis"

Additionally, BestBETs, RCVS Knowledge Summaries and VetSRev were searched to identify references. Papers not identified elsewhere were also included that were found in a previous literature search by the primary author.

3.2.3 Study selection

The search results were exported into EndnoteX8 (Clarivate Analytics[2]). Duplicate records were identified and deleted before publications were assessed against the inclusion criteria (Table 3.1). A three-stage systematic review and exclusion process was completed independently by two researchers involving 1) review of publication titles, 2) review of publication abstracts and 3) review of the full-text for the remaining publications. At title and abstract review, ambiguous publications were retained for the next review stage. Publications where abstracts could not be identified were retained for full-text review. For publications where full-text was not available, individual authors and journals were contacted to attempt to obtain the full-text. A study was excluded if English language full-text was not available as described in Chapter 2.

[2] https://endnote.com/

Table 3.1. The inclusion and exclusion criteria for a scoping review of the literature to identify and chart the current evidence on the effect of the timing of neutering in relation to puberty on the health of female domesticated dogs.

CRITERIA	INCLUSION	EXCLUSION
POPULATION	Female domesticated dogs	Male dogs Other species
EXPOSURES	Entire female dogs presented for surgical neutering	Papers comparing entire bitches to neutered bitches Papers not relating neutering to age or pubertal status Papers relating to the treatment of dogs with behaviour problems
INTERVENTION	Neutering procedure including chemical techniques and open and laparoscopic techniques which involve removal of the ovaries	Papers relating to neutering by hysterectomy
OUTCOME	Papers including assessments or measures of dog health Owner observations or survey of dog health Health to include obesity, neoplasia, developmental orthopaedic disease, urinary disease, atopic dermatitis, and vulval/vaginal disorders	Behavioural outcomes Surgical outcomes in the 14-day perioperative period and behaviour relating to recovery following surgery Pseudopregnancy Health problems other than those specified Student or owner opinions, perceptions or attitudes without health observation or measure Papers only examining effect of neutering on control of reproduction
LANGUAGE	English or papers with translation available	Translation not available
STUDY DESIGN	Case series, cohort, case control, cross sectional	Individual case reports, qualitative studies
PUBLICATION TYPE	Peer reviewed journals Conference proceedings	Narrative, textbook chapters, reviews (reference lists from systematic reviews were used to crosscheck we had all relevant studies but were not used themselves) Unable to obtain full study details (abstract or full text) Non-peer reviewed journals

3.2.4 Charting process

The remaining final full-text publications were assessed independently by two researchers to ensure eligibility for inclusion. Each search was described separately and then characteristics and relevant information were extracted and presented.

3.2.4.1 Data extraction

Following full text review, key information was extracted from each of the 33 retained publications using a standardised form (Appendix 2) and tabulated. Contents included in the study characteristics table were author, publication year, data source or country, study design, sample size, study aims, details of the intervention, health outcomes, how outcomes were measured and whether dogs were randomly or retrospectively allocated to study groups. Additional details were extracted and presented for participant characteristics including: how the sample was sourced, dog breed, age and sex, whether dogs neutered for a related health problem were excluded, whether previous breeding history was considered and how puberty was defined. This was carried out independently by one researcher.

3.2.4.2 Summarising the available evidence

The 33 publications were assessed by two researchers. Significant effects of timing of neutering on each health outcome were reported for either bitches or dogs and bitches (where studies did not present results for sexes of dogs separately). Studies that found no significant effects, reported significant effects only in male dogs, or made no comparisons between different neuter age groups were not included in the charting tables. A summary column was included to show the reported impact of neutering at certain times more clearly, including direction of any effect, for each health outcome included from each study.

3.3 RESULTS

3.3.1 Study selection

3.3.1.1 Atopy

The primary literature search identified 50 publications following the removal of duplicates. Thirty-five were excluded on title review, seven were excluded following abstract review and seven were excluded on full-text review. Therefore, one publication remained for assessment (Figure 3.1a).

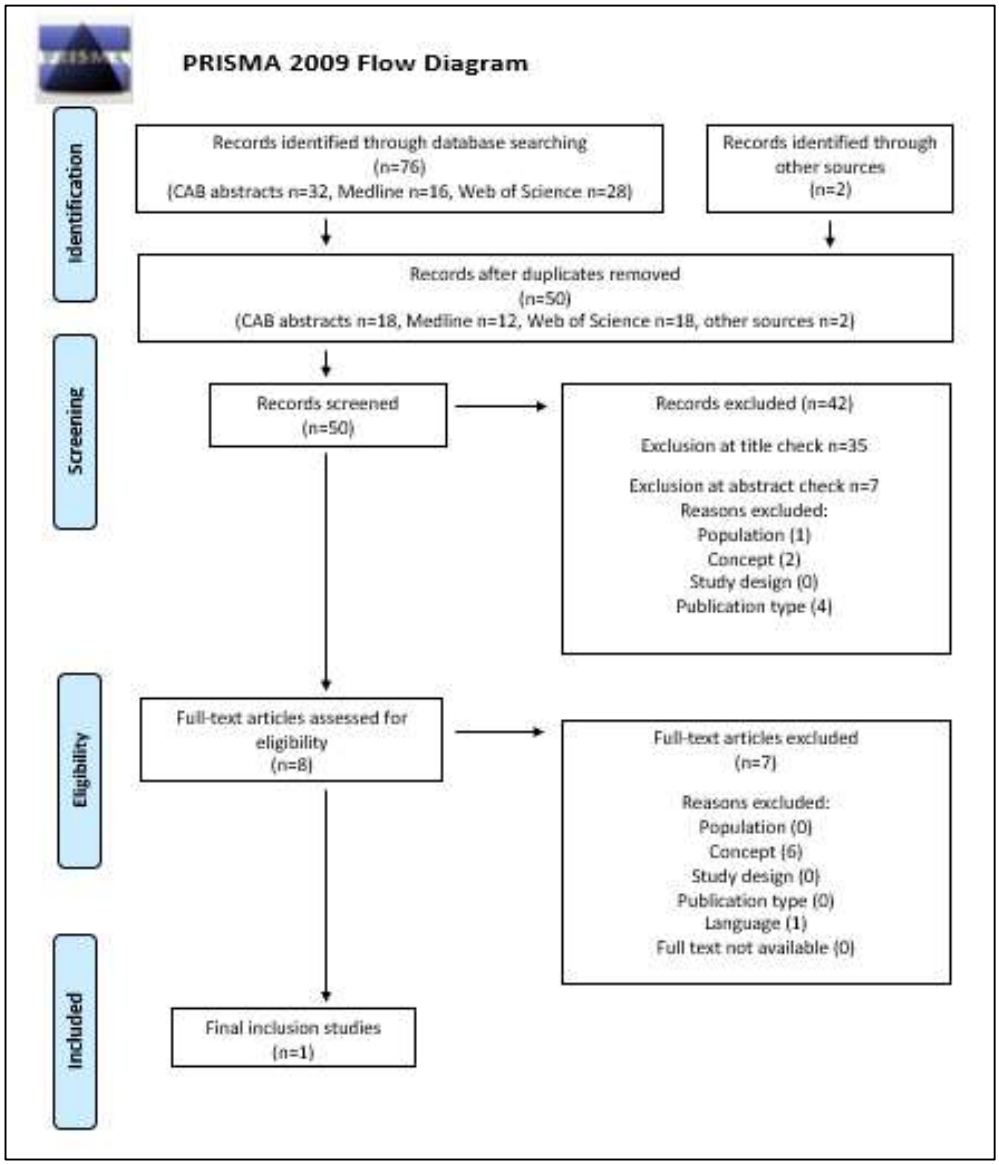


Figure 3.1a The PRISMA 2009 flow diagram showing the number of publications that were identified, reviewed, and excluded at title, abstract and full-text review in a scoping review to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on atopy in female domesticated dogs.

3.3.1.2 Developmental orthopaedic disease

The primary literature search identified 74 publications following the removal of duplicates. Twenty-eight were excluded on title review, 29 were excluded following abstract review and four were excluded on full-text review. Therefore, 13 publications remained for assessment (Figure 3.1b).

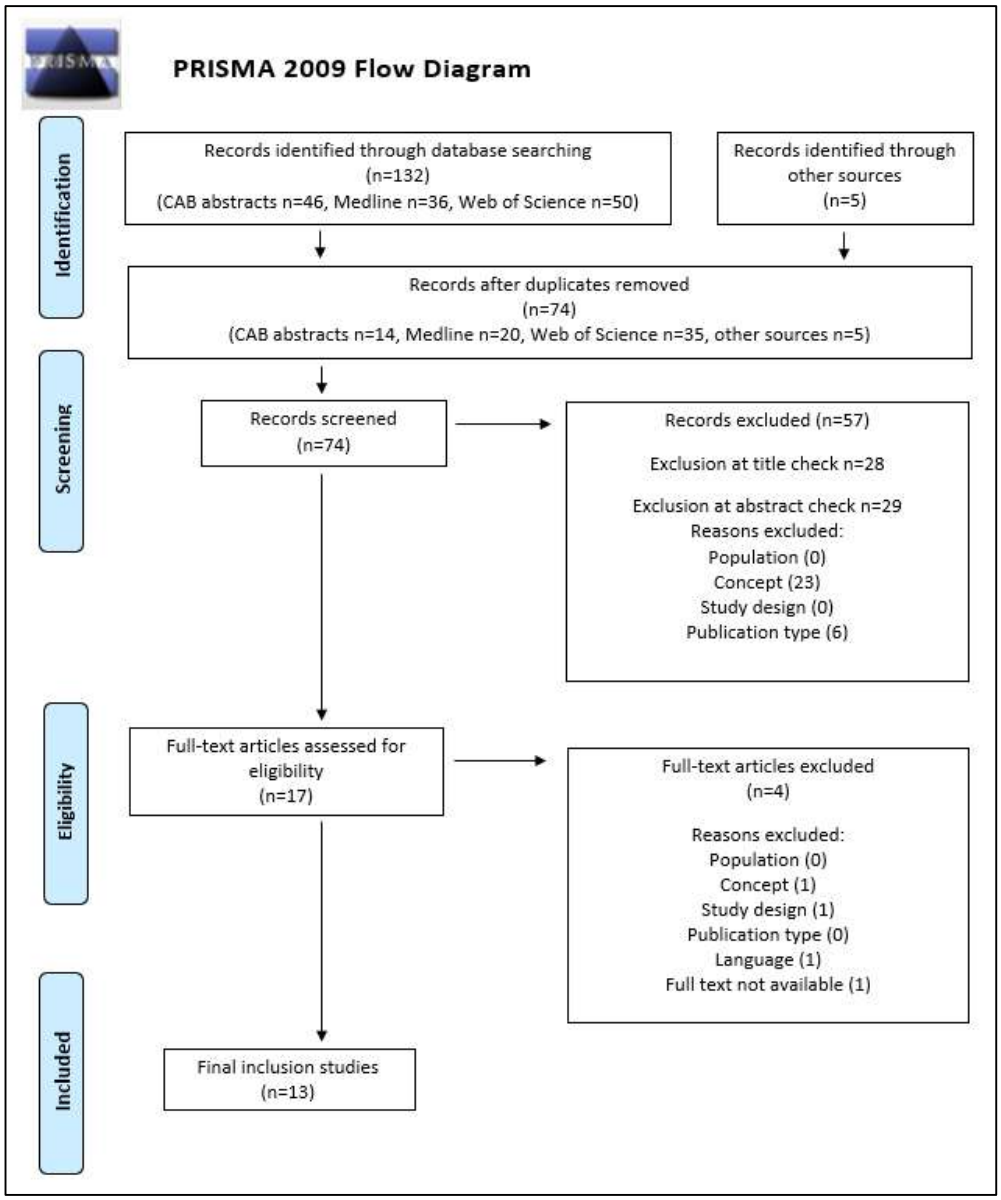


Figure 3.1b The PRISMA 2009 flow diagram showing the number of publications that were identified, reviewed, and excluded at title, abstract and full-text review in a scoping review to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on developmental orthopaedic disease in female domesticated dogs.

3.3.1.3 Neoplasia

The primary literature search identified 513 publications following the removal of duplicates. Three hundred and forty were excluded on title review, 137 were excluded following abstract review and 25 were excluded on full-text review. Therefore, 11 publications remained for assessment (Figure 3.1c).

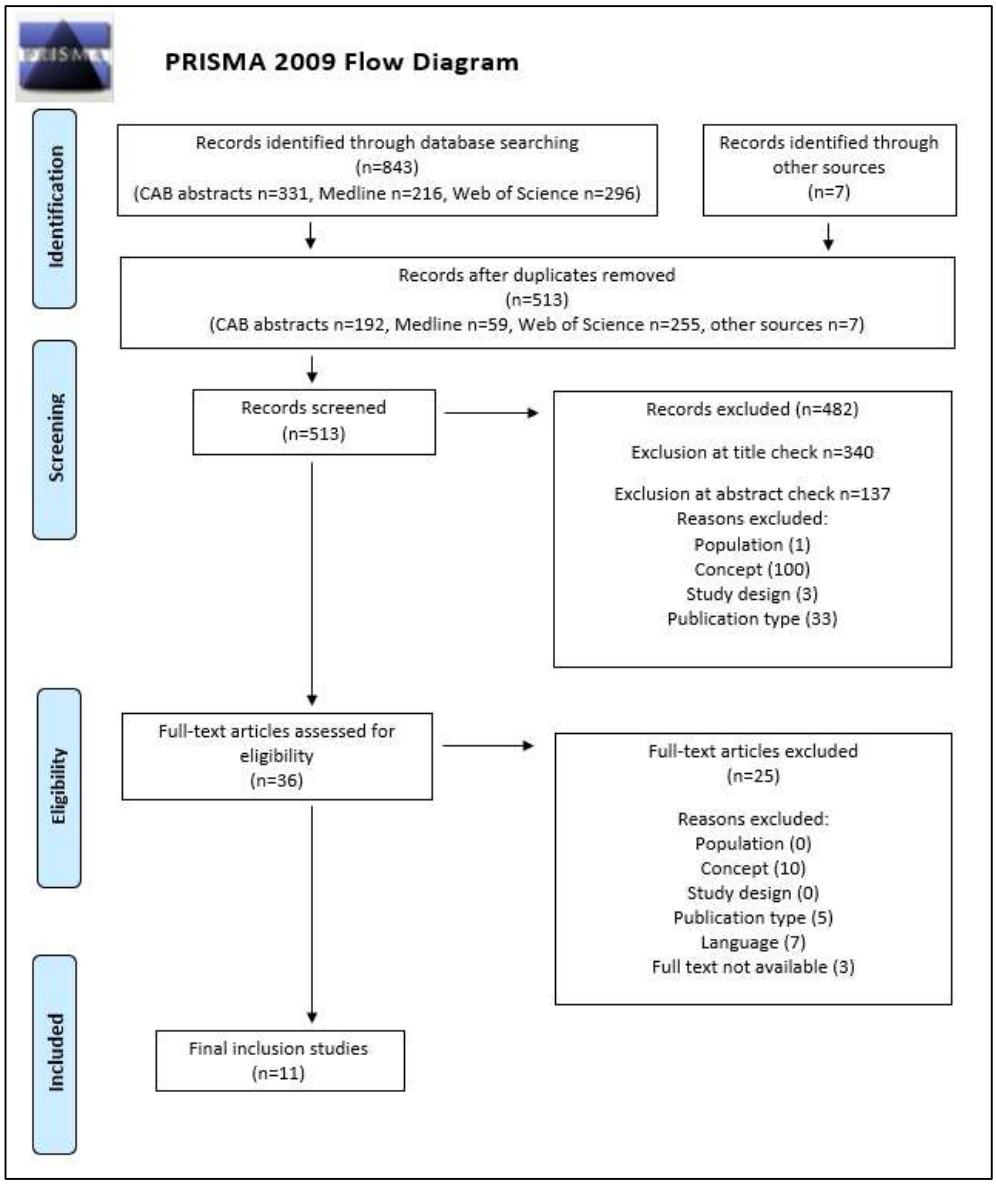


Figure 3.1c The PRISMA 2009 flow diagram showing the number of publications that were identified, reviewed, and excluded at title, abstract and full-text review in a scoping review to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on neoplasia in female domesticated dogs.

3.3.1.4 Obesity

The primary literature search identified 182 publications following the removal of duplicates. One hundred and seven were excluded on title review, 57 were excluded following abstract review and 12 were excluded on full-text review. Therefore, six publications remained for assessment (Figure 3.1d).

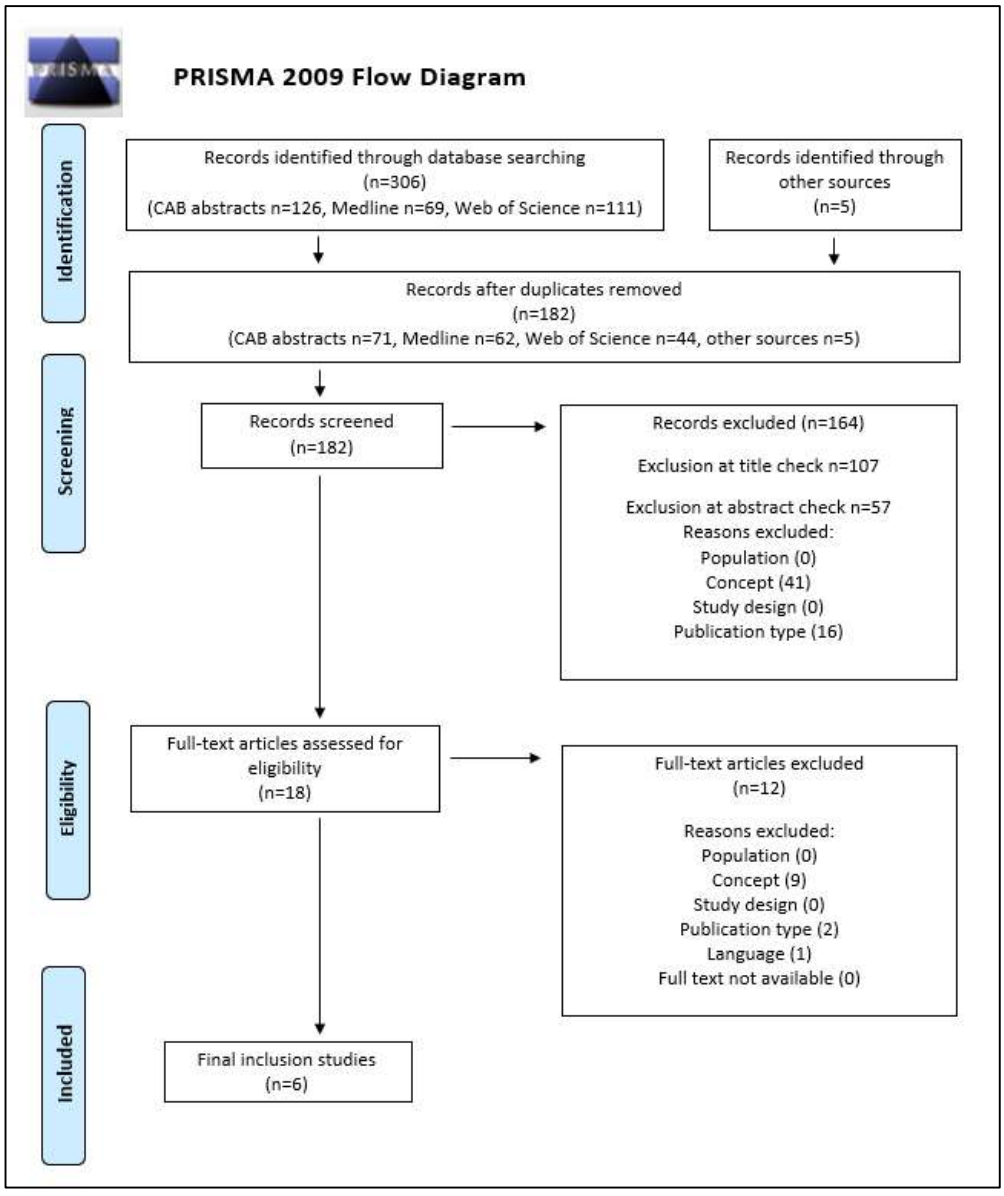


Figure 3.1d The PRISMA 2009 flow diagram showing the number of publications that were identified, reviewed, and excluded at title, abstract and full-text review in a scoping review to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on obesity in female domesticated dogs.

3.3.1.5 Urogenital

The primary literature search identified 326 publications following the removal of duplicates. Two hundred and twenty-five were excluded on title review, 63 were excluded following abstract review and 20 were excluded on full-text review. Therefore, 18 publications remained for assessment (Figure 3.1e).

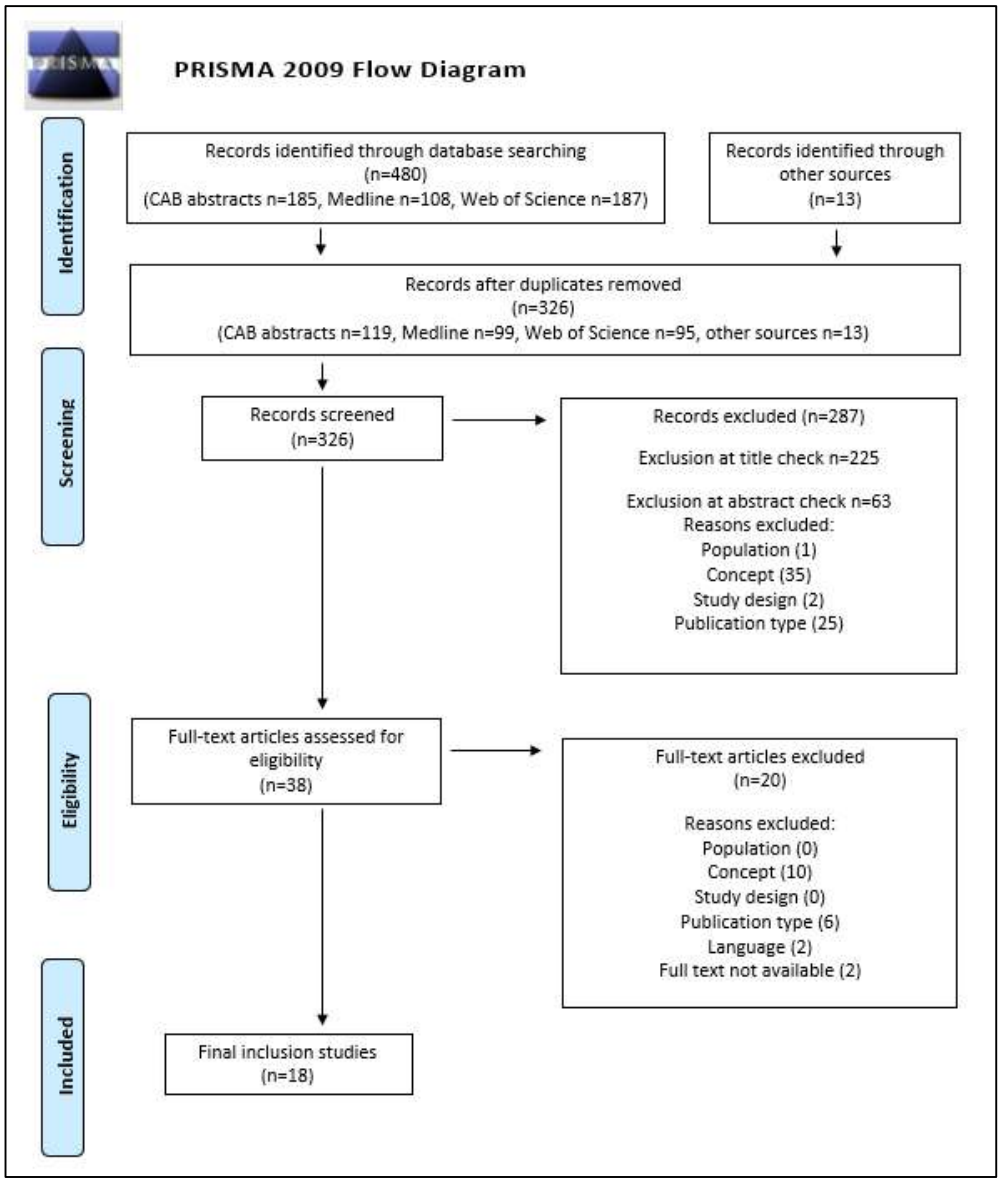


Figure 3.1e The PRISMA 2009 flow diagram showing the number of publications that were identified, reviewed, and excluded at title, abstract and full-text review in a scoping review to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on urogenital disease in female domesticated dogs.

3.3.2 Study characteristics

There were 33 individual papers identified in total across the five independent searches that were included in this review, some were identified in more than one of the searches (Table 3.2). Papers were published between 1969 and 2023 and were most commonly from the USA/North America (21/33 studies), including all of the papers identified for atopy, DOD and obesity, and 9/11 for neoplasia. Dogs were most commonly grouped retrospectively into neuter groups based on age (23/33 studies) or pubertal status (5/33 studies) at neutering. Two studies randomly allocated dogs to neutering age groups and two prospective studies grouped dogs based on age when neutered. Twenty-two studies did not report the type of neutering surgery performed. One study included bitches that had received deslorelin acetate implants for oestrus suppression. There were 27 different health outcomes examined and these were most frequently assessed by examining veterinary records (15/33) or by owner (8/33) or veterinarian questionnaires (4/33). Four studies used direct observations. Three owner questionnaire studies also used additional collection methods: data from veterinary records, assistance from the veterinarian or veterinarian questionnaires (Table 3.2).

Table 3.2. The study characteristics for the publications identified for inclusion in a scoping review of the literature undertaken to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on atopy, developmental orthopaedic disease (DOD), neoplasia, obesity and urogenital disease in female domesticated dogs.

Author and year	**Source / country**	**Study design**	**Sample size**	**Aims***	**Intervention**	**Relevant health outcomes***	**Outcome measures**	**Allocated randomly to neutering groups or retrospectively grouped**	**Comparisons reported between dogs neutered in different groups or just to the entire group?**
Beaudu-Lange *et al.*, 2021	France	RC	599 females	Determine the frequency of reproductive disorders over the lifetime of female dogs according to neuter status	Ovariectomy – early neuter (<2 years of age), late neuter (>2 years of age), entire	1) Neoplasia (Mammary tumour - including benign) 2) Urogenital disease (UI)	Veterinary records	Retrospectively grouped	Comparisons between early and late-neuter groups reported for mammary tumours only, not UI
Brandli *et al.*, 2021	Switzerland	RC	32 females	Evaluate the side effects of repeated deslorelin acetate application for sustained gonadal suppression	4 bitches first implant before puberty, 28 bitches after puberty, deslorelin acetate implant for oestrus suppression	Urogenital disease (UI)	Owner questionnaires	Retrospectively grouped	Descriptive comparisons reported between dogs first treated before or after puberty, no

									statistical analysis
Byron *et al.*, 2017	USA	CC	163 UI, 193 control – all female	Examine the impact of age at OVH and weight at presentation for incontinence on the hazard of USMI	OVH	Urogenital disease (USMI stated, however UI and incontinence also used interchangeably throughout so unclear)	Data supplied by veterinarians	Retrospectively grouped	Relationships with age at neuter reported
Cooley *et al.*, 2002	USA and Canada	RC	683 dogs (389 female)	Test the hypothesis that endogenous sex hormones significantly influence bone sarcomagenesis	Neutering, surgical method not stated	Neoplasia (appendicular bone sarcoma)	Owner questionnaires completed with veterinary assistance	Retrospectively grouped	Between age group comparisons reported
de Bleser *et al.*, 2011	UK	CC	202 cases, 168 controls	Estimate the strength of association between early spaying and acquired urinary sphincter mechanism incompetence	Neutering, surgical method not stated. 59 bitches neutered <6 months of age and 255 after 6 months of age. 100 bitches neutered before and 229 after puberty	Urogenital disease (acquired USMI)	Owner questionnaires	Retrospectively grouped	Between age group and pre/post-pubertal comparisons reported

Duerr *et al.*, 2007	North America	RC	For age at neuter analysis: 80 dogs, number of females not reported	Identify risk factors for excessive tibial plateau angle (TPA) in large-breed dogs with CCLD	Neutering age grouped: <6 (n=31) or >6 months (n=49), number of male and female in each neuter age group and surgical method not stated	DOD – TPA in dogs with CCL disease	Measurements of TPA from radiographs for cases and from dogs' medical records for controls	Retrospectively grouped	Between age group comparisons reported
Ekenstedt *et al.*, 2017	North America	RC	174 cases, 139 controls (108 neutered female dogs)	Examine the association between EIC and CCL rupture and examine aspects, such as sex and neutering	Neutering age grouped: ≤1 (83 female) or >1 year (25 female), surgical method not stated	DOD – CCL rupture	Most cases defined by surgical confirmation, others defined via physical exam, stifle palpation, and radiographs. Controls - no history of pelvic limb lameness or stifle surgery, no knee abnormalities via orthopaedic examination	Retrospectively grouped	Between age group comparisons reported

Forsee *et al.*, 2013	UK	RC	566 female	Identify the prevalence and determine associations of AUI with specific variables in OVH bitches	OVH. 109 bitches neutered <6 months of age, 355 neutered 6 to <18 months, 102 neutered ≥18 months	Urogenital disease (acquired UI)	Owner questionnaire	Retrospectively grouped	Between age group comparisons reported
Hart *et al.*, 2014	USA	RC	GR = 1,015 (543 males, 315 neutered; 472 females, 306 neutered). LR = 1,500 (808 males, 272 neutered; 692 females, 347 neutered)	Explore the effects of neutering on joint disorders and cancers in Labradors and Golden Retrievers	Neutering, surgical method not stated. Age grouped: <6 months, 6–11 months, 12-<24 months, and 2–8 years. Number of dogs neutered at each age not clear	DOD (HD, ED, CCL) Neoplasia (LSA, HSA, MCT, MC)	Veterinary records	Retrospectively grouped	No comparisons reported between dogs neutered at different ages

Hart *et al.*, 2016	USA	RC	705 male (245 neutered), 465 females (293 neutered)	Examine relationships between age at neutering and joint disorders and cancers	Neutering, surgical method not stated. Age grouped: <6 months, 6–11 months, 12-<24 months, and 2–8 years. Number of dogs neutered at each age not clear	DOD (HD, ED, CCL) Neoplasia (LSA, HSA, MCT, MC) Urogenital disease (UI)	Veterinary records	Retrospectively grouped	No comparisons reported between dogs neutered at different ages
Hart *et al.*, 2020a	USA	RC	Over 15,000 dogs, neutered and entire, male and female, exact numbers unclear	Provide information on breed-specific differences and guidelines for neutering ages considering long-term health risks of neutering. Document breed-specific differences in the increases in some cancers associated with neutering	Neutering, surgical method not stated. Age grouped: <6 months, 6–11 months, 12-<24 months, and 2–8 years. Number of dogs neutered at each age not clear	DOD (HD, ED, CCL) Neoplasia (LSA, HSA, MCT, MC, OSA) Urogenital disease (UI)	Veterinary records	Retrospectively grouped	No comparisons reported between dogs neutered at different ages

Hart *et al.*, 2020b	USA	RC	3139 total, 664 entire male, 875 neutered male, 441 entire female, 1159 neutered female.	Provide information for mixed breed dogs on the best age to neuter considering the risks of joint disorders and cancers	Neutering, surgical method not stated. Age grouped: <6 months, 6–11 months, 12-<24 months, and 2–8 years. Number of dogs neutered at each age not clear	DOD (HD, ED, CCL) Neoplasia (LSA, HSA, MCT, MC, OSA) Urogenital disease (UI)	Veterinary records	Retrospectively grouped	No comparisons reported between dogs neutered at different ages
Howe *et al.*, 2001	USA	PC	269 (153 female)	Determine long-term results and complications of neutering performed at an early age (prepubertal) or at the traditional age in dogs.	OVH or castration 94 dogs neutered ≥24 weeks of age and 175 neutered <24 weeks of age. Sex ratio in each neuter age group not reported	DOD (overall incidence of musculoskeletal disease and HD) Obesity (owner perception of dog's bodyweight) Urogenital disease (Urinary problems)	Owner telephone questionnaires 41-64 months after adoption. Contact with veterinarians for any unclear diagnoses.	Grouped based on age at surgery	Between age group comparisons reported
Lefebvre *et al.*, 2013	USA	RC	1,930 neutered and 1,669 entire dogs	Determine whether gonadectomy or age at gonadectomy was associated	Neutering, surgical method not stated. 782 neutered ≤6 months of age	Obesity (overweight or obese)	Body condition score 4 or 5 out of 5 in veterinary records	Retrospectively grouped	Between age group comparisons reported

				with the risk of becoming overweight	(454 female), 861 at >6 months to ≤1 year of age (469 female), 287 at >1 to ≤5 years of age (138 female)				
Lutz *et al.*, 2019	Switzerland	RC	131 matched-pair dogs	Investigate the risk factor "time of spaying relative to the onset of puberty" on AUI	Neutering, surgical method not stated	Urogenital (acquired UI)	Owner questionnaire	Retrospectively grouped	Before and after puberty compared
Moxon *et al.*, 2023b	UK	PC	306 female dogs	Compare vulval size and appearance at 17 months of age for bitches neutered before or after puberty	OVH before (n=155) or after (n=151) puberty	Urogenital (vulval size and appearance – recessed and juvenile vulva, vulval discharge, perivulval folds and skin changes, % dorsal fold coverage)	Veterinary assessment and assessment of digital vulval images	Randomly allocated	Before and after puberty compared
Palerme *et al.*, 2021	USA	CS	250 female dogs, 216 neutered	Assess the prevalence and severity of recessed vulvas	OVH. Number of bitches in each neuter	Urogenital (recessed vulva)	Direct observation, percentage of vulvar skin	Retrospectively grouped	Between age group comparisons reported

				and characterise differences between dogs with and without recessed vulvas	age group not reported		coverage scored out of 8 recessed vulva = score ≥7		
Pegram *et al.*, 2019a	UK	RC	23,499 neutered and 49,472 entire females	Explore association between neuter status and age at neuter with early-onset UI	Neutering, surgical method not stated. Neutering age grouped: <6 (n=3,418), 6 to <12 (n=10,543), 12 to <24 (n=5,426) and ≥24 months (n=4,112)	Urogenital (UI)	Veterinary records	Retrospectively grouped	Between age group comparisons reported
Pegram *et al.*, 2019b	UK	CC	106 case and 463 control bitches with age at neuter data	Explore the association between UI and neuter status, age at neuter and neuter relative to first oestrus	Neutering, surgical method not stated. Neutering age grouped: <6 (n=68), 6 to <12 (n=159), 12 to <24 (n=144) and ≥24 months (n=198)	Urogenital (UI)	Veterinary records	Retrospectively grouped	Between age group comparisons reported

Salmeri *et al.*, 1991	USA	PC	32 (17 female)	Determine how weight gain and body fat was affected by neutering at 7 weeks versus 7 months of age	Seven-week neuter: 7 male 7 female 7-month neuter: 4 male 4 female Entire: 4 male 6 female. Females neutered via OVH.	Obesity (weight gain and body fat)	Weight gain – monthly bodyweight measurements Body fat – back fat depth surgically at 14 months of age	Randomly allocated	Between age group comparisons reported
Schneider *et al.*, 1969	USA	CC	87 female case-control matches	To measure the effect on canine mammary cancer risk of various factors including neutering	Neutering, surgical method not stated. Neutered before first oestrus, after one or after two oestrous cycles	Neoplasia (mammary)	Neoplasia based on tumours submitted to neoplasm registry. Demographic data from owner interviews and veterinary records	Retrospectively grouped	Before and after puberty compared
Simpson, 2017	USA	PC	3,044 dogs (number of females not reported)	Examine whether age at gonadectomy is associated with overweight/ obesity and chronic non-traumatic	Neutering (gonadectomy) at <6 months, 6 to 12 months, >12 months or entire. Surgical method and number of	DOD (first occurrence of veterinarian-reported CCL rupture or osteoarthritis) Obesity (first occurrence of veterinarian-	Annual data collection from owners and veterinarians Overweight/ obesity – Purina Body Condition	Retrospectively grouped	No comparisons reported between dogs neutered at different ages

				orthopaedic injuries	dogs in each neuter group not reported	reported overweight or obese)	Score scale of >6/9		
Simpson *et al.*, 2019	USA	PC	2,764 or 2,754 dogs – unclear (number of females not reported)	Examine associations between gonadectomy and overweight/ obesity and orthopaedic injuries	Neutering (gonadectomy) at <6 months, 6 to 12 months, >12 months or entire. Surgical method and number of dogs in each neuter group not reported	DOD (first occurrence of veterinarian-reported CCL rupture or osteoarthritis) Obesity (veterinarian-reported overweight or obese)	Annual questionnaires from veterinarians and medical records Overweight/ obesity – Purina Body Condition Score scale of >6/9	Retrospectively grouped	Between age group comparisons reported
Sonnenschein *et al.*, 1991	USA	CC	428 female (150 cases, 147 cancer controls, 131 non-cancer controls)	Examine relationships between dietary intake and body conformation and the risk of breast cancer	Neutering, surgical method not stated	Neoplasia (mammary)	Veterinary records	Retrospectively grouped	No comparisons reported between dogs neutered at different ages
Spain *et al.*, 2004	USA	RC	1,659 (number of females not reported)	Evaluate the long-term risks and benefits of early-age (before 5.5 months) versus	Neutered <5.5 months or ≥5.5 months, number breakdown for each neuter age group not	DOD (CCL, HD, patella luxation) Neoplasia Obesity (overweight body condition)	HD, UI, neoplasia, vaginitis – owner questionnaire and veterinary records, CCL,	Retrospectively grouped	Between age group comparisons reported

				traditional-age gonadectomy	available. Surgical method not stated	Urogenital (perivulvar dermatitis, UI, urolithiasis, UTI-cystitis, vaginitis)	patella luxation, perivulvar dermatitis, UTI-cystitis, urolithiasis – veterinary records Overweight body condition –owner reported body shape determined by comparison to 5 images		
Stocklin-Gautschi *et al.*, 2001	Switzerland	RC	206 bitches	Determine the influence of early neuter on the incidence of UI in bitches	OVH or ovariectomy, all before puberty	Urogenital (UI)	Not stated	Retrospectively grouped	Before and after puberty compared
Sundberg *et al.*, 2016	USA	RC	6,281 dogs with age at neuter data (number of females not reported)	Examine associations between neuter status and diseases thought to be modulated by the immune system	Neutering, surgical method not stated	Atopy	Veterinary records	Retrospectively grouped	No comparisons reported between dogs neutered at different ages

Thrusfield *et al.*, 1998	UK	PC	310 females	Determine the incidence of acquired UI in neutered and entire bitches and assess the effects of time of neutering	Neutering, surgical method not stated; 92 neutered before first oestrus, 218 after first oestrus	Urogenital (acquired UI)	Veterinarian questionnaires	Based on owner decision	Before and after puberty compared
Torres de la Riva *et al.*, 2013	USA	RC	Males and females; 364 females (242 neutered)	Examine the effects of neutering on disease risk distinguishing between early or late neutering compared to remaining entire	Neutering, surgical method not stated; 172 females neutered <12m and 70 females neutered ≥12m	DOD (HD, ED, CCL) Neoplasia (LSA, HSA, MCT, MC, OSA)	Veterinary records	Retrospectively grouped	Between age group comparisons reported
Veronesi *et al.*, 2009	Italy	Unclear	750 females	Examine the influence of age at neutering on the occurrence of UI	OVH or ovariectomy	Urogenital (UI)	Veterinary records	Retrospectively grouped	Relationships with age at neuter reported
Whitehair *et al.*, 1993	North America	RC	10,769 dogs. Age at neuter known for 244.	Determine the prevalence of CCL rupture in dogs and whether CCL rupture was associated with	OVH, method of grouping by neuter age not described	DOD (CCL rupture)	Veterinary records	Unclear	Relationships with age at neuter reported

				age, breed, gender or bodyweight					
Zink *et al.*, 2014	25 different countries, majority USA, UK, Canada and Australia (USA 86.7%)	CS	2,505 (1,360 female)	Investigate associations between age at gonadectomy and sex of gonadectomised dogs and risk and age of onset of neoplasia	Owner selected age at gonadectomy: ≤6 months (209 female), 7-12 months (157 female) and >12 months (459 female), or entire. Surgical method not stated	Neoplasia (HSA, LSA, MCT, all other cancers, and all cancers combined)	Owner survey	Retrospectively grouped	No comparisons reported between dogs neutered at different ages
Zlotnick *et al.*, 2019	USA	RC	245 (77 female)	Investigate differences in the incidence of health or behavioural problems and completion of service dog training in dogs neutered at different ages	Neutered at <7 months (n=110) Neutered at 7-11 months (n=58) Neutered at >11 months (n=77). Surgical method not stated	DOD (dismissal from service dog programme for orthopaedic reasons)	From service dog records	Retrospectively grouped	Between age group comparisons reported

*For the purposes of this scoping review, only health aims and outcomes relevant to the subject of the review are included. Other aims or outcomes that may have been included in the studies, such as other health outcomes, or behaviour are not reported.

Abbreviations: RC – retrospective cohort, PC – prospective cohort, CS – cross-sectional, CC – case-control, AUI – acquired urinary incontinence, CCL – cranial cruciate ligament, DOD – developmental orthopaedic disease, ED – elbow dysplasia, EIC – exercise-induced collapse, OVH – ovariohysterectomy, HD - hip dysplasia, HSA – haemangiosarcoma, LSA – lymphosarcoma/lymphangiosarcoma, MC – mammary cancer, TPA – tibial plateau angle, UI - urinary incontinence, USMI – urinary sphincter mechanism incompetence, UTI – urinary tract infection.

3.3.3 Participant characteristics

Eighteen of the 33 studies included dogs of various breeds and usually did not report individual breeds. Fifteen studies included only bitches, 12 of these investigated urogenital outcomes, two neoplasia, and one urogenital and neoplasia. Eighteen studies included dogs and bitches, nine of these did not report results for each sex by neuter group independently, one reported some results by sex and eight did report results for male and female dogs separately. All papers identified for atopy, DOD and obesity were based on age not pubertal status at neutering. One paper for neoplasia and six for urogenital disease examined data by pubertal status at neutering. One further study aimed to examine data by pubertal status at the time of neutering however a lack of data prevented this analysis (Pegram *et al.*, 2019b). One study that reported results for bitches neutered before or after puberty had potential problems associated with grouping of bitches, with some older bitches reported to be classed as prepubertal (up to 1.4 years of age) and some younger bitches included in the post-pubertally neutered group (minimum 0.3 years of age; Lutz *et al.*, 2019). Only six studies examined data for bitches that were surgically neutered before or after puberty. One of these compared results for bitches neutered before puberty in the study to those for bitches neutered after puberty from another study (Stocklin-Gautschi *et al.*, 2001). One study examined data for bitches treated with deslorelin acetate to suppress oestrus, however data were not analysed statistically, and numbers were too small for comparative analysis. The remaining 26 studies either did not mention puberty (22/26), did not have the data available to examine puberty (1/26) or based conclusions regarding puberty on age at neutering (2/26) (Table 3.3).

Table 3.3. Participant characteristics for the publications identified for inclusion in a scoping review of the literature undertaken to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on atopy, developmental orthopaedic disease (DOD), neoplasia, obesity and urogenital disease in female domesticated dogs.

Author and year	**Sample source**	**Breed**	**Study duration**	**Sex**	**Consider health before neuter**	**Excluded dogs with a related health problem before neuter?**	**Previous breeding history considered?**	**How puberty was defined**
Beaudu-Lange *et al.*, 2021	Pet dogs identified from veterinary clinic records	Various breeds and crossbreeds	To at least 6 years of age, to death for 293 bitches	All female	Yes	No	No	N/A, puberty not considered within the study, only age
Brandli *et al.*, 2021	Pet dogs identified from clinical records from the Clinic for Small Animals, University of Zurich	31 purebred (14 different breeds) one mixed-breed	Varied from 0.7 to 11.3 years. Mean observation period 5.3 ± 3.4 years.	All female	No	No	Yes – all nulliparous	Not reported
Byron *et al.*, 2017	Not stated, data from veterinary practices	Various breeds, not stated	N/A – CC design	All female	No	No	No	N/A, puberty not considered within the study, only age

Cooley *et al.*, 2002	Pet dogs recruited via breed clubs and advertising	Rottweilers	Varied from 1.3 to 15.6 years. Mean observation period 8.8 and 8.6 years for cases and controls respectively.	Both male and female, reported separately	Yes	Unclear	Yes for entire and neutered >1 year	N/A, puberty not considered within the study, only age
de Bleser *et al.*, 2011	Not stated, data from veterinary practices	Various breeds and crossbreeds	N/A – CC design	All female	Yes	Yes	No	Before or after first oestrus
Duerr *et al.*, 2007	Not stated, data from veterinary surgeons and university veterinary medical centre records	Various breeds and mixed breeds	N/A – CC design	Both male and female, results not reported by sex	No	No	No	N/A, puberty not considered within the study, only age
Ekenstedt *et al.*, 2017	Mainly pet dogs from referral hospitals	Labradors	N/A – CC design	Both male and female, reported separately	No	Yes for controls	No	N/A, puberty not considered within the study, only age
Forsee *et al.*, 2013	Not stated, data from veterinary practices	Breeds not stated	4 to 7 years after neuter	All female	Yes	Yes	Yes	N/A, puberty not considered; whether oestrus cycles occurred

	and hospitals							before OVH was asked, but not reported on
Hart *et al.*, 2014	Not stated, data from veterinary hospital	Labradors and Golden Retrievers	To a maximum of 8 years of age for individual dogs	Both male and female, reported separately	Yes	No but dogs with health problem before neuter were classed as entire for that disease	No	N/A, puberty not considered within the study, only age
Hart *et al.*, 2016	Not stated, data from veterinary hospital	German Shepherd Dogs	To a maximum of 8 years of age for individual dogs	Both male and female, reported separately	Yes	No but dogs with health problem before neuter were classed as entire for that disease	No	N/A, puberty not considered within the study, only age
Hart *et al.*, 2020a	Not stated, data from veterinary hospital	35 different pure breeds	To a maximum of 11 years of age for individual dogs	Both male and female, reported separately	Yes	No but dogs with health problem before neuter	No	N/A, puberty not considered within the study, only age

			15 years of data for most breeds			were classed as entire for that disease		
Hart *et al.*, 2020b	Not stated, data from veterinary hospital	Crossbreeds	To a maximum of 11 years of age for individual dogs	Both male and female, reported separately	Yes	No but dogs with health problem before neuter were classed as entire for that disease	No	N/A, puberty not considered within the study, only age
Howe *et al.*, 2001	Pet dogs adopted from two shelters	Breeds not stated	41 to 64 months after neutering surgery	Both male and female, results not reported by sex	No	No	No	Based on age at neutering, ≥24 or <24 weeks of age
Lefebvre *et al.*, 2013	Pet dogs	Various breeds and crossbreeds	10 years or more of regular veterinary hospital visits	Both male and female, results not reported by sex	Yes	Yes	No	N/A, puberty not considered within the study, only age
Lutz *et al.*, 2019	Not stated, data from two veterinary hospitals	Various, not stated	4.9 to 15.6 years after neuter	All female	Not stated	Not stated	No	Not defined, however some neutered before puberty dogs were max 1.4

								years of age and some neutered after puberty dogs were 0.3 years of age so unreliable classification
Moxon *et al.*, 2023b	Guide Dogs UK	Labrador and Golden Retriever crossbreeds	To 17 months of age	All female	Yes	No	Yes – all nulliparous	Before or after first oestrus
Palerme *et al.*, 2021	Not stated, data from specialist referral centre	Various, not stated	N/A – CS design	All female	No (those already received vulvoplasty were excluded)	No	No	N/A, puberty not considered within the study, only age although suggest pre- or peri-pubertal neutering prior to 4 or 8 months of age
Pegram *et al.*, 2019a	Not stated, data from primary-care veterinary practices	Various breeds and crossbreeds	Up to 8 years	All female	Yes	Yes	No	N/A, puberty not considered within the study, only age
Pegram *et al.*, 2019b	Not stated, data from primary-care veterinary practices	Various breeds and crossbreeds	N/A – CC design	All female	Yes	Yes	No	Before or after first oestrus

Salmeri *et al.*, 1991	Dogs in a research kennel facility	Mixed-breed	Dogs followed from 0 to 15 months of age	Both male and female, reported separately	Yes	No	No	N/A, puberty was not considered within the study, only age
Schneider *et al.*, 1969	Pet dogs identified from neoplasm registry	Various breeds and crossbreeds	N/A – CC design	All female	Yes	No	Yes – not controlled for in oestrous cycle analysis	Based on number of oestrous cycles before neutering
Simpson, 2017	Pet, data from the Golden Retriever Lifetime Study	Golden Retrievers	Not stated	Both male and female, results not reported by sex	Not stated	Not stated	No	N/A, puberty was not considered within the study, only age
Simpson *et al.*, 2019	Pet, data from the Golden Retriever Lifetime Study	Golden Retrievers	Not stated	Both male and female, results not reported by sex for age at neutering	Yes	Yes	No	N/A, puberty was not considered, only age, although suggest <6 months – prepubertal; >6 to <12 months – dogs in puberty; >12 months post-pubertal
Sonnenschein *et al.*, 1991	Pet, data from School of Veterinary Medicine,	Various breeds and crossbreeds	N/A – CC design	All female	Yes	No, unclear whether dogs diagnosed	Yes	N/A, puberty was not considered

	University of Pennsylvania					before neutering were still included		within the study, only age
Spain *et al.*, 2004	Pet dogs adopted from a shelter	Various breeds, not stated	Median 4.5 years (range 0.3 to 11.3 years)	Male and female, sex ratio in each age at neuter group not reported for overweight analysis. Results reported separately for UI and cystitis	No	No	No (but maximum age at neuter was 12 months so breeding unlikely)	N/A, puberty was not considered within the study, only age
Stocklin-Gautschi *et al.*, 2001	Not stated	Not stated	A minimum of 3 years post-neuter	All female	Yes	Yes	No	Data for bitches neutered before puberty were compared to those for bitches neutered after puberty from a different study
Sundberg *et al.*, 2016	Pet dogs Data from a veterinary medical	Various breeds and crossbreeds	Not stated	Both male and female, results not reported by	Yes	No	No	N/A, puberty was not considered within the study, only age

	teaching hospital			sex for age at neutering				
Thrusfield *et al.*, 1998	Pet dogs, data from veterinary practices	Various breeds and crossbreeds	Five years	All female	Not stated, but all UI occurred after neutering	Yes	Yes	Before or after first oestrus
Torres de la Riva *et al.*, 2013	Not stated, data from veterinary hospital	Golden Retriever	To a maximum of 8 years of age for individual dogs	Both male and female, reported separately	Yes	No but dogs with health problem before neuter were classed as entire for that disease	No	N/A, puberty was not considered within the study, only age
Veronesi *et al.*, 2009	Pet dogs, source not stated	Various breeds and crossbreeds	Not clear	All female	Not stated, but all UI occurred after neutering	No	No	N/A, puberty was not considered within the study, only age
Whitehair *et al.*, 1993	Pet dogs, veterinary medical teaching hospital records	Various breeds and crossbreeds	Not clear	Both male and female, results not reported by sex for age at neutering	No	No	No	N/A, puberty was not considered within the study, only age
Zink *et al.*, 2014	Pet dog data gathered	Viszlas	Up to 16 years	Both male and female,	No	No	Yes for entire (53%), not	N/A, puberty was not

	from an anonymous online survey			results not reported by sex for age at neutering			mentioned for neutered	considered within the study, only age
Zlotnick *et al.*, 2019	Dogs in a service dog programme	Labradors, Golden Retrievers, various other breeds and crosses	Not stated	Both male and female. Sex breakdown by neuter group not reported, results not reported by sex	Yes	No	No	N/A, puberty was not considered within the study, only age

Abbreviations: CS – cross-sectional, CC – case-control, UI - urinary incontinence

3.3.4 Charting the studies against the aims of the scoping review

Thirteen different health outcomes were reported to be significantly affected by timing of neutering under the five health categories investigated (Table 3.4). The only study identified that examined atopy did not report results relating to pubertal status or age at neuter (Sundberg *et al.*, 2016). Two studies reported no impact on DOD (CCL rupture; Whitehair *et al.*, 1993; overall incidence and HD; Howe *et al.*, 2001). One study reported no impact on neoplasia (mammary neoplasia; Spain *et al.*, 2004). Three studies reported no impact on obesity (weight gain and back fat measurements; Salmeri *et al.*, 1991; owner perception of their dog's body weight; Howe *et al.*, 2001; overweight and obese based on body condition score; Lefebvre *et al.*, 2013). Five studies reported no impact on urogenital disease (acquired sphincter mechanism incompetence; de Bleser *et al.*, 2011; acquired UI; Thrusfield *et al.*, 1998; Forsee *et al.*, 2013; problems associated with the urinary system; Howe *et al.*, 2001, UI; Pegram *et al.*, 2019b). One study did not analyse data statistically due to small numbers of dogs (Brandli *et al.*, 2021). Seven studies did not compare between neuter groups; the only comparisons made were between each neuter group and entire dogs (Sonnenschein *et al.*, 1991; Hart *et al.*, 2014; Zink *et al.*, 2014; Hart *et al.*, 2016; Simpson, 2017; Hart *et al.*, 2020a; 2020b) and one study only compared between ages for one outcome measure, neoplasia and not the second, UI (Beaudu-Lange *et al.*, 2021). These studies where no effects were identified or that did not compare between different neuter groups were not included in the charting table.

One study (Pegram *et al.*, 2019b) aimed to investigate the impacts of neutering before or after the first oestrus, however data availability was too limited to enable analysis. Additionally, this study only reported comparisons for all neuter age groups to bitches neutered at 6 to <12 months; no comparisons were reported between other neuter age groups. However, the author has

confirmed that these comparisons were made and there were no differences identified (C. Pegram, *Pers. Comm.*).

The effects of neutering at younger and older ages were mixed, with earlier neutering commonly reported to be detrimental for DOD and urogenital disease, but favourable for neoplasia. For some diseases, findings were also mixed; four of the six studies that reported significant findings for UI found earlier neutering to be detrimental, whilst two suggested earlier neutering was favourable. However, one of the latter studies was primarily investigating onset of UI post-neuter, rather than focusing on comparisons of UI incidence in bitches neutered at different ages. The other study made comparisons between bitches in the study that were neutered before puberty and bitches from another study that were neutered post-pubertally. Four of the five studies that reported significant findings for neoplasia found earlier age at neuter to be favourable, however, one study examining the risk of bone sarcoma found an increased risk with fewer months entire (therefore earlier age at neuter). Spain *et al.* (2004) reported mixed effects of neutering age on HD, with increased hazard ratio in dogs neutered younger, but increased risk of being euthanised for HD for dogs with HD that were neutered at older ages.

The charting of study results highlights the inconsistencies in classification of timing of neutering between studies and the lack of information concerning the timing of neutering in relation to puberty.

Table 3.4. The health outcomes that were impacted by timing of neutering identified in a scoping review undertaken to identify and evaluate the current evidence on the effect of the timing of neutering in relation to puberty on atopy, developmental orthopaedic disease (DOD), neoplasia, obesity and urogenital disease in female domesticated dogs.

Health outcome	**Author**	**Study design**	**Sex**	**Relationship with timing of neutering**	**Age or puberty**	**Summary impact of neutering for each health outcome studied**
Developmental orthopaedic disease	Duerr *et al.*, 2007	CC	Both male and female	Dogs with TPA ≥35° were 3 times (95% CI=1.2–8.0) as likely to have been neutered <6 months of age than dogs with TPA <35° Dogs with TPA ≥35 ° in both limbs were 13.6 times (95% CI=2.72–68.1) as likely to have been neutered <6 months of age as were dogs with TPA ≤30 ° in both limbs	Age	Less favourable for dogs neutered at <6 months of age
	Ekenstedt *et al.*, 2017	CC	Female	Increased odds of CCL rupture for bitches neutered ≤1 year of age compared to those neutered >1 year of age (OR=4.30, 95% CI=1.55–12.72, P=0.0021)	Age	Less favourable for bitches neutered at ≤1 year of age
	Simpson *et al.*, 2019	PC	Both male and female	Increased risk of orthopaedic injury in dogs neutered ≤6 months compared to 6 to ≤12 months (HR=2.24, 95% CI=1.25-4.93, P=0.008) and to >12 months (HR=6.36, 95% CI=2.74–14.77, P<0.0001). Increased risk of orthopaedic injury in dogs neutered 6 to ≤12 months compared to >12 months (HR=2.56, 95% CI=1.08-2.25, P=0.03).	Age	Less favourable for dogs neutered at younger ages

				No influence of sex on the association between neutering and orthopaedic injuries (P=0.23). Results for each sex not presented separately.		
	Spain *et al.* (2004)	RC	Both male and female	Higher HR for HD in dogs neutered <5.5m than those neutered ≥5.5 months (HR=1.7, 95% CI=1.04–2.78, P=0.03) due to earlier age at diagnosis in dogs neutered <5.5m. Dogs with HD that were neutered ≥5.5 months of age were 3x as likely to be euthanised for HD as those with HD and neutered before 5.5 months of age (P=0.02).	Age	Less favourable for dogs neutered <5.5 months of age More favourable for dogs neutered <5.5 months of age
	Torres de la Riva *et al.*, 2013	RC	Female	Occurrence of CCL tear increased for bitches neutered <12 months (7.7%) compared to those neutered ≥12 months of age (0.0%) (P=0.001).	Age	Less favourable for bitches neutered <12 months compared to ≥12 months
	Zlotnick *et al.*, 2019	RC	Both male and female	Dogs neutered <7 months over 2x as likely to be dismissed from the service dog training programme for orthopaedic problems as dogs neutered at any older age (RR=2.2, 95% CI=1.1–4.3, P=0.03). Results for females not reported separately but stated that health-related dismissals did not differ by sex or breed. This is dismissals *not* diagnoses.	Age	Less favourable for dogs neutered <7 months

Neoplasia	Beaudu-Lange *et al.*, 2021	RC	Female	Early neutered (<2 years of age) females had a significantly lower risk of developing mammary tumours than late neutered or entire bitches (OR=0.10, 95% CI=0.01–0.41, P<0.001)	Age	More favourable for earlier neuter
	Cooley *et al.*, 2002	RC	Female	Females that developed bone sarcoma were entire for fewer months than those that did not (HR=0.98, 95% CI=0.97–0.99, P<0.0001). For each additional month of being sexually entire, there was a 1.4% reduction in bone sarcoma risk. Negative association between gonadal hormone exposure and risk of bone sarcoma (P=0.006 for females). In females neutered <1 year of age bone sarcoma incidence rate was >3x greater than for entire females (RR=3.1, 95% CI=1.1–8.3, P=0.02). There was no difference between entire bitches and those neutered >1 year of age.	Age	More favourable as age at neuter i.e. months remained entire increases
	Schneider *et al.*, 1969	CC	Female	Neutered bitches had lower risk of mammary neoplasia than entire multi-oestrus bitches, this was significant for those neutered before the first oestrus (RR=0.005).	Puberty	More favourable for bitches neutered before puberty
	Torres de la Riva *et al.*, 2013	RC	Female	Bitches neutered ≥12 months of age more likely to have HSA (RR=7.48, 95% CI=1.79–31.30) and MCT (RR=4.46, 95% CI=1.11–	Age	More favourable for bitches neutered earlier

				17.82) than those neutered <12months of age.		
Obesity	Simpson *et al.*, 2019	PC	Both male and female	Dogs neutered <6 months not different to dogs neutered 6–12 months (HR=0.82, 95% CI=0.66–1.01, P=0.06) or >12 months (HR=1.16, 95% CI=0.93–1.44, P=0.19). Dogs neutered 6–12 months had an increased risk for overweight/obesity compared to those neutered >12 months of age (HR=1.42, 95% CI=1.19–1.69, P=0.0001).	Age	Less favourable for dogs neutered at 6 to 12 months compared to those neutered at >12 months of age
	Spain *et al.*, 2004	RC	Both male and female	The prevalence of overweight body condition declined with decreasing age at neuter (OR for each 1-month decrease in age at neuter 0.94; P=0.04).	Age	More favourable for dogs neutered younger
Urogenital disease	Byron *et al.*, 2017	CC	Female	No significant difference in median age at OVH in isolation between UI and control dogs (both 8.5 months). An increased hazard of UI with higher adult body weight and earlier OVH was found that was statistically significant at 25 kg body weight (HR=0.89, 95% CI=0.82–0.97, P=0.006). For a 30 kg dog, a 1-month increase in OVH age decreased the hazard of UI by 24% (HR=0.86, 95% CI=0.78–0.94, P=0.001).	Age	More favourable as age at neuter increases– but not at every bodyweight
	Lutz *et al.*, 2019	RC	Female	UI reported in fewer dogs neutered after puberty (10.7%) than before puberty (22.9%; P=0.007). No OR or CI reported.	Puberty	Less favourable for bitches neutered before puberty
	Moxon *et al.*, 2023b	PC	Female	Significantly more bitches neutered prepubertally had vulvas that changed to be	Puberty	Less favourable for bitches

				smaller by 17 months of age (length: P<0.001; width: P<0.001). Bitches neutered prepubertally had smaller changes in vulval length (0.08 ± 0.06 cm vs 0.41 ± 0.05 cm, P<0.001) and width (-0.05 ± 0.04 cm vs 0.20 ± 0.04 cm, P<0.001) between six and 17 months of age. Significantly more prepubertally neutered bitches had vulvas that appeared juvenile (P<0.001) and recessed (P=0.005) in appearance on direct observation and more categorised as recessed/inverted on examination of digital images (P=0.002).		neutered before puberty
	Palerme *et al.*, 2021	CS	Female	Bitches neutered ≤12 months of age had significantly increased OR of recessed vulva than those neutered >12 months (OR=2.95, 95% CI=1.24–7.03, P=0.015. Bitches neutered ≤24 months of age had significantly increased OR of recessed vulva than those neutered >24 months (OR=3.08, 95% CI=1.48–8.26, P=0.026). No difference between ≤4m vs >4m or ≤8m vs >8m.	Age	Less favourable for bitches neutered earlier
	Pegram *et al.*, 2019a	RC	Female	Bitches neutered <6 months had significantly increased hazard of early-onset UI within the first year after neuter compared to those neutered 6-12 months (HR=1.82; 95% CI=1.15–2.88; P=0.011). The HR decreased 0.75-fold for every subsequent year.	Age	More favourable as neutering age increases

	Spain *et al.*, 2004	RC	Female	Incidence of cystitis higher for females neutered <5.5 months of age (HR=2.76, 95% CI=1.08–7.14, P=0.02). Decreasing age at neuter associated with increasing incidence of UI (HR for each 1-month decrease in age at neuter 1.2; 95% CI=1.06–1.35, P<0.01). Females neutered <3 months of age at highest risk, compared to those neutered ≥3 months (HR=3.46; P<0.001).	Age	More favourable as neutering age increases
	Stocklin-Gautschi *et al.*, 2001	RC	Female	Incidence of UI in bitches neutered prepubertally (9.7%) was significantly lower than the incidence for bitches neutered post-pubertally in a different study (20.1%; P<0.05).	Puberty	More favourable for bitches neutered before puberty
	Veronesi *et al.*, 2009	Unclear	Female	UI occurred earlier in bitches neutered at older ages. The authors suggest that this confirms a lower risk in bitches neutered at earlier ages.	Age	Favourable for bitches neutered earlier

Abbreviations: RC – retrospective cohort, PC – prospective cohort, CS – cross-sectional, CC – case-control, CI – confidence interval, HR – hazard ratio, OR – odds ratio, RR – relative risk, CCL – cranial cruciate ligament, OVH – ovariohysterectomy, HD - hip dysplasia, TPA - tibial plateau angle, UI – urinary incontinence.

3.4. DISCUSSION

3.4.1 Summary

This is the first scoping review that has attempted to collate and describe the evidence relating to the effects of neutering bitches before or after puberty on several health outcomes. The review identified a lack of evidence: 33 studies were eligible for inclusion, but only six categorised the bitches as being surgically neutered either before or after puberty. One study considered bitches that were pre or post-pubertal at the time of the first treatment with deslorelin acetate for oestrus suppression and 26 examined the effects on health related to age, rather than pubertal status, at neutering.

This scoping review suggests that robust evidence to support veterinarians, those working with dogs and pet dog owners when discussing the timing of neutering relative to puberty does not yet exist. The impact of neutering *before or after puberty* on atopy, DOD, neoplasia, obesity and urogenital disease in female domesticated dogs remains unknown. This information is important for veterinarians to support conversations around the health impacts of neutering in relation to puberty. This study summarises the current state of the evidence and provides direction for future studies.

3.4.2 Study and participant characteristics

Five of the six studies that considered neutering before or after puberty involved various breeds, included between 32 and 370 bitches and examined urogenital disease (five studies), or mammary neoplasia (one study). For one of these studies involving 101 matched pairs (Lutz *et al.*, 2019), method of determining pubertal status was not defined and potential problems with categorising bitches as pre/post-pubertal at neuter as evidenced by the ages at neutering of the bitches may reduce confidence in the results. Within the study that considered bitches that were pre or post-pubertal at the time of the first treatment with deslorelin

acetate for oestrus suppression, only four bitches were treated before puberty and numbers were too small for analysis (Brandli *et al.*, 2021). Stocklin-Gautschi *et al.* (2001) compared data to those from a group of bitches from another similar study to draw conclusions about the differences in incidence of UI for bitches neutered before or after the first oestrus. The study was excluded from a previous systematic literature review due to risk of bias. Additionally, the source of data regarding UI diagnosis was not stated. The studies by Schneider *et al.* (1969) and Thrusfield *et al.* (1998) have previously been reviewed and found to be compromised by a lack of controlling for confounders and problems with participant recruitment (Beauvais *et al.*, 2012a,b). For three of the four studies examining UI or USMI, there is a risk of bias due to unconfirmed diagnoses using owner-reported data.

The remaining 26 studies reported data for dogs based on age at neutering. This demonstrates the lack of available literature related to the impacts of neutering bitches before or after puberty on health outcomes. However, the scoping review has enabled the evidence that is available, relating to age and puberty, to be summarised.

The studies that examined the impact of age at the time of neutering considered or grouped age in different ways, such as neutering before or after 5.5 months of age; neutering at seven weeks or seven months of age; neutering at less than six months, between seven and 12 months or over 12 months. This does not allow for easy between-study comparisons to be made, nor does it allow conclusions to be drawn about the impact of neutering in relation to puberty. One of the studies that grouped dogs as neutered at either less than 24 weeks of age, or 24 weeks of age or more, reported in the abstract that the results indicate that neutering before puberty is not associated with increased incidence of physical problems. Additionally, the term ‘prepubertal’ was used throughout the discussion to demonstrate no differences in health problems between those that underwent ‘prepubertal’ neutering

compared to 'traditional age' neutering. However, this is misleading based on the age grouping of dogs at the time of neutering. Breeding history and previous pregnancies were commonly not included in studies, therefore may have impacted the findings.

Nine studies included male and female dogs and did not report results by sex separately including 7/13 studies on DOD and 5/6 studies on obesity. Differences in incidence of certain diseases between male and female dogs have previously been reported (Hart *et al.*, 2020a; O'Neill *et al.*, 2021) and this prevents a clear understanding of the impacts specifically on bitch health.

Most studies included various or multiple breeds and did not state the breeds involved, or present results by breed, however some studies dealt with this by controlling for breed in the analyses or by matching control group bitches by breed. Breed can impact disease incidence, with some breeds predisposed to certain diseases such as joint disorders, neoplasia and UI (Hart *et al.*, 2014; Reichler *et al.*, 2015; Hart *et al.*, 2020a,b). Additionally different breeds can reach puberty at different ages, with smaller breeds that reach adult bodyweight earlier experiencing puberty sooner (Hewitt and England, 1999). Therefore, understanding the breeds, and controlling for breed in analysis is important so that results are not influenced by the inclusion of high or low numbers of certain breeds. However, this does pose difficulties for future study design. Undertaking individual studies across different geographical locations – to account for variations that may be present in different subpopulations - for many breeds, or recruiting sufficient numbers of bitches of each breed to one prospective study would not be easy.

Studies also had different follow up times, with some not considering diseases that affected dogs in older age. While some authors stated a wish to reduce the impact of old age on disease occurrence to allow the focus to remain on the effect of neutering,

dogs with some diseases such as HD or mammary neoplasia may not be expected to present with clinical signs until later in life (Schafer *et al.*, 1998; Smith *et al.*, 2006; Sorenmo *et al.*, 2009; Pastor *et al.*, 2018; Burrai *et al.*, 2020). There were differences between studies, and in some cases between dogs within studies, in the time elapsed since neutering, and in the length of time dogs were followed for, which was not always described or controlled for in analyses. These are important considerations because such factors may not have allowed time for certain diseases to be observed.

Fifteen of the 33 studies did not report whether they excluded dogs that were already diagnosed with the disease prior to neutering, and for a further four studies this information was not clear in the paper. When measuring the impact of an intervention on an outcome, it is important to either understand the status of the outcome prior to the intervention or to exclude those already affected from the study. For instance, if a study was examining the impact of a treatment or intervention on the occurrence of a disease, prior to administration, study participants would all need to have been free from the disease. If they were not, or their disease status was unknown, understanding of the impact could be compromised by the fact that some participants may have been affected throughout the study, and to differing degrees. Similarly, if the dogs were already diagnosed prior to being neutered, neutering itself could not be deemed a risk factor for that disease being present in the follow up period after neutering. The lack of consideration for dogs' relevant disease status prior to neutering, or lack of exclusion of dogs already diagnosed prior to neutering, impacts the confidence in the results.

3.4.3 Summary of the evidence

Despite the lack of evidence relating specifically to puberty, this scoping review provided an overview of the available literature on the effects of neutering timing on health in female dogs. Only seven

studies were identified that categorised bitches as pre or post-pubertal at surgical neutering or first treatment for oestrus suppression, highlighting a need for further research and the importance of reporting this critical factor in studies. None of these studies examined atopy, DOD, or obesity, one considered mammary neoplasia and six investigated outcomes relating to urogenital disease.

3.4.3.1 Atopy

Only one study was identified that investigated the effect of neutering timing in relation to atopy (Sundberg *et al.*, 2016). The study considered age and not pubertal status at neutering, and although the authors suggested that age at neutering was to be examined, disease rates for age at neutering were not reported. The authors did report no significant differences in "age at gonadectomy as a function of disease (P>0.1)". However, which diseases were analysed and disease incidence data by age at neuter were not reported.

3.4.3.2 Developmental orthopaedic disease

All of the 13 studies that investigated DOD considered timing of neutering in relation to age rather than pubertal status. Five did not compare disease incidence between neuter age groups, only between each neuter age neuter group and a group of entire dogs (Hart *et al.*, 2014, 2016, 2020a,b; Simpson, 2017). Eight studies did make comparisons, two of those reported no impact of neutering age on DOD (Whitehair *et al.*, 1993; Howe *et al.*, 2001).

Six studies did report effects of neutering age on DOD, specifically on CCL rupture (Torres de la Riva *et al.*, 2013; Ekenstedt *et al.*, 2017), tibial plateau angle in dogs with CCL (Duerr *et al.*, 2007), HD (Spain *et al.*, 2004), "orthopaedic injury" (Simpson *et al.*, 2019) and dismissal from a service dog programme for orthopaedic problems (Zlotnick *et al.*, 2019). Despite differences between studies in how

age at neuter was grouped, in all six studies younger age at neuter groups were at increased risk of the particular outcome. In one study (Spain *et al.*, 2004), secondary to the main outcome of incidence of HD, dogs that had HD and were neutered at ≥5.5 months of age were more likely to be euthanised for the disease than those dogs with HD that were neutered before 5.5 months of age. Only two of the studies (Torres de la Riva *et al.*, 2013; Ekenstedt *et al.*, 2017) reported results for female dogs separately.

3.4.3.3 Neoplasia

Only one of the 11 studies that investigated neoplasia considered pubertal status at neutering (Schneider *et al.*, 1969). In that case-control study malignant mammary neoplasia was less likely in bitches neutered before experiencing any oestrus (relative risk 0.005) compared to those than had experienced one oestrus before neutering (relative risk 0.08) and two or more oestrous cycles before neutering (relative risk 0.26). However, as reported by Beauvais *et al.* (2012a), there is a risk of bias due to recruitment of cases and controls from different time periods and controlling for confounding variables in analysis.

The remaining 10 studies investigated the effects of timing of neutering related to age. Six of these made no comparisons of disease incidence between age at neuter groups, only between each age at neuter group and a group of entire dogs (Sonnenschein *et al.*, 1991; Hart *et al.*, 2014, 2016, 2020a,b; Zink *et al.*, 2014). Four studies did make comparisons and one of those reported no impact of neutering age on neoplasia (Spain *et al.*, 2004).

Three studies did report effects of neutering age on neoplasia, specifically on mammary tumours (Beaudu-Lange *et al.*, 2021); appendicular bone sarcoma (Cooley *et al.*, 2002) and HSA and MCT (Torres de la Riva *et al.*, 2013). Beaudu-Lange *et al.* (2021) reported a protective effect of neutering before two years of age on

mammary tumour development. While this is similar to the findings of Schneider *et al.* (1969), the study included a relatively small number of bitches (n=50) that were neutered <2 years of age compared to those neutered >2 years (n=243). Forty-seven of the bitches neutered >2 years of age were known to have a mammary tumour at the time of neutering and do not appear to have been excluded from the analysis. Torres de la Riva *et al.* (2013) also reported more favourable outcomes for earlier neutering for HSA and MCT for 242 female Golden Retrievers. In that study, bitches were grouped as those neutered before (n=172) or at/over 12 months of age (n=70). Data were gathered from veterinary hospital records which could affect how well they represent the general dog population however confirmation of diagnosis was reliable. Bitches were studied to nine years of age, which could impact disease occurrence reporting. Additionally, there was no difference between bitches neutered before or at/after 12 months of age in the incidence of LSA although, in contrast to other types of neoplasia, a higher percentage of bitches in the early (5.9%) rather than late neuter group (1.4%) were diagnosed. Bitches with disease prior to neutering were excluded, the study only included one breed and sex, addressing the potential for these confounding variables to impact the results.

In contrast to the first two studies, Cooley *et al.* (2002) found an increased risk with younger age at neuter for bone sarcoma in 683 Rottweilers, 389 of which were female. Data for neuter age were considered as months sexually entire on a continuous scale and were also grouped into neuter age groups and were reported for males and females separately. While data were gathered from an owner questionnaire, completed with veterinarian assistance, diagnoses were confirmed by reviewing radiographs which reduced the risk of incorrect diagnoses. Data on dog height and weight were included as confounding variables in the analysis. It is not clear whether there were any dogs with bone sarcoma diagnosed before

neutering and no mention of whether such cases were excluded from analysis.

3.4.3.4 Obesity

None of the six studies that investigated obesity considered timing of neutering by pubertal status; all investigated effects of neutering timing related to age. One of the studies did not compare disease incidence between neuter age groups, only between each neuter age group and a group of entire dogs (Simpson, 2017). Five studies did make comparisons and three of those reported no impact of neutering age on overweight/obesity (Salmeri *et al.*, 1991; Howe *et al.*, 2001; Lefebvre *et al.*, 2013).

Two studies did report effects of age at neutering on obesity with contrasting results (Spain *et al.*, 2004; Simpson *et al.*, 2019). Both studies considered being overweight and obese rather than obesity alone. Spain *et al.* (2004) reported a decreasing risk of overweight body condition with decreasing age at neuter for 1,659 dogs of various breeds, although results were not reported separately for male and female dogs. Data validity may have been compromised due to owners reporting overweight/obese status on a questionnaire by comparing their dog's body shape to five images, and because age was estimated in some cases for the shelter dogs. Confounding variables were reportedly controlled for, although it is unclear which variables specifically were included in the analyses. Simpson *et al.* (2019) examined data for over 2,700 Golden Retrievers, however the exact number of dogs, and the number neutered in each age group was not clear. The results suggested no difference in age at neuter between those neutered at <6 and those neutered at 6-12 or >12 months of age. However, dogs neutered at 6–12 months had an increased risk for overweight/obesity compared to those neutered at >12 months of age. Dogs that were overweight prior to neutering were excluded, data were reported by veterinarians on an annual questionnaire and were based on a body condition scoring

system, and confounding variables such as sex, physical activity level and age were controlled for.

3.4.3.5 Urogenital disease

Six of the 18 studies that investigated urogenital disease (four UI, one USMI, one vulval development) considered pubertal status at the time of neutering (Thrusfield *et al.*, 1998; Stocklin-Gautschi *et al.*, 2001; de Bleser *et al.*, 2011; Lutz *et al.*, 2019; Brandli *et al.*, 2021; Moxon *et al.*, 2023b). One of these (Brandli *et al.*, 2021) did not have a large enough sample size to enable statistical comparisons, and two reported no significant effects (Thrusfield *et al.*, 1998; de Bleser *et al.*, 2011). The strengths and risk of bias for these two studies have previously been reported by Beauvais *et al.* (2012b). Three studies did report effects of neutering before or after puberty on urogenital disease (Stocklin-Gautschi *et al.*, 2001; Lutz *et al.*, 2019; Moxon *et al.*, 2023b). Stocklin-Gautschi *et al.* (2001) suggested that the incidence of UI in bitches neutered prepubertally was lower than for bitches neutered post-pubertally, however there were issues with study methodology and a risk of bias that may compromise the results. The study only included bitches neutered before puberty and compared data to those for bitches that were neutered after puberty in a different study. The breeds and source of the bitches included and how outcomes were measured were not stated and the controlling for effects of confounding variables was not clear (Beauvais *et al.*, 2012b). Lutz *et al.* (2019) reported the opposite, with significantly more bitches neutered before puberty being reported with UI than those neutered after puberty. However, whether bitches with UI before neutering were excluded was not stated, UI was reported on an owner questionnaire and no OR or CI were reported. Importantly, results may be compromised by the grouping of bitches. There were some bitches aged up to 1.4 years in the group neutered before puberty and some aged as young as 0.3 years in the group neutered after puberty (presumably these are data errors).

The final study is based on published work from this thesis and reported effects on vulval size and appearance for 306 Labrador/Golden Retriever crossbreed bitches (Moxon *et al.*, 2023b). Significantly more bitches neutered prepubertally had vulvas that changed to be smaller by 17 months of age, had smaller changes in vulval length and width between six and 17 months of age and had vulvas that appeared juvenile or recessed. This study considered vulval size and appearance prior to neutering and accounted for confounding variables in the analysis of vulval size.

The remaining 12 studies investigated the effects of timing of neutering related to age. Four of these made no comparisons of disease incidence between neuter age groups, only between each neuter age group and a group of entire dogs (Hart *et al.*, 2016, 2020a,b; Beaudu-Lange *et al.*, 2021). Eight studies did make comparisons and three of those reported no impact of neutering age on urogenital disease (Howe *et al.*, 2001; Forsee *et al.*, 2013; Pegram *et al.*, 2019b).

Five studies did report effects of age at neutering on urogenital disease, specifically on recessed vulva (Palerme *et al.*, 2021); cystitis and UI (Spain *et al.*, 2004), and UI (Veronesi *et al.*, 2009; Byron *et al.*, 2017; Pegram *et al.*, 2019a). The only study (Veronesi *et al.*, 2009) to report more favourable results for bitches neutered earlier was a study examining UI onset post-neuter using data from veterinary records for 750 neutered bitches. While the authors reported that the age at neuter and UI onset were related, with bitches neutered at older ages having earlier onset UI, they suggest that this "seems to confirm the lower risk in early spayed bitches compared to bitches spayed after the first oestrus, as reported by Stocklin-Gautschi *et al.* (2001)". However, the statistical evidence to support this statement is weak, with supporting data not presented in the main section of the results and no description of confounding factors included in statistical analysis.

The four papers that reported less favourable findings for bitches neutered at younger ages examined UI, cystitis and recessed vulva. Spain *et al.* (2004) reported significantly higher incidence of cystitis in bitches neutered before 5.5 months of age and increasing incidence of UI with decreasing age at neuter, with the highest risk of UI for bitches neutered before three months of age. UI was only considered when bitches required medical treatment. The number of bitches in each neuter group was not stated. Dogs were of various breeds and crossbreeds, however pure/crossbreed status were included in the analysis as cofounding variables. It is not clear how reliable age at neuter determination was for the dogs that were in the shelter at the time of neutering. Byron *et al.* (2017) also investigated UI and found a decreased hazard for UI in bitches neutered at older ages that was significant for dogs weighing over 25 kg. This unmatched case-control study did not consider, or exclude bitches based on, health prior to neutering. Dogs were recruited via invitations to contribute data that were sent to 500 veterinary hospitals in the USA. The lack of matching of case-control dogs and the lack of controlling for confounding variables such as breed in the analysis affect the risk of bias in the study. Pegram *et al.* (2019a) also identified a relationship between age at neuter and early-onset UI when data for 23,499 neutered bitches were examined. In that study neutering before six months of age was associated with an increased hazard of early-onset UI. Data for a large number of bitches from UK primary care practices were examined with confounding variables including breed and bodyweight included in the analysis.

Finally, Palerme *et al.* (2021) reported that recessed vulva, determined by direct observation of lateral and dorsal vulvar skin fold coverage, was significantly more likely in bitches neutered at or less than 12 months or 24 months of age, compared to those neutered over these ages. However, no differences were identified when age at neuter was grouped as less or greater than four or

eight months of age. The study included various breeds and bitches were recruited from a university veterinary medical centre. Simple statistical analyses were performed with no controlling for confounding variables. The method of obtaining thorough history is unclear, therefore it is not obvious whether the approximate age at the time of OVH used for analysis was based on owner recollection or from veterinary records.

The effects on bitch health of the timing of neutering in relation to puberty have been investigated in only a small number of studies. Other studies have suggested that neutering before or after puberty has been examined, however on closer examination, age at neuter and not pubertal status has been used to categorise neuter timing (Howe *et al.*, 2001) or the reported ages at neuter for the prepubertal and post-pubertally neutered groups suggest a risk of bias (Lutz *et al.*, 2019). Seemingly the most commonly investigated health problem related to neutering before or after puberty in female dogs is UI.

In summary there are few studies that investigate the health implications of neutering bitches considering pubertal status at neutering. Most studies that do consider pubertal status investigate urogenital health, commonly UI, and are subject to a risk of bias. Studies generally propose that neutering bitches earlier appears to be protective for some (but not all) cancers but increases the risk of DOD and potentially UI; contrasting results which make applying the findings to benefit bitch health difficult.

3.4.4 Limitations

The database searches were conducted in May 2023. Since that time, further studies may have become available. Some papers that were referenced in other studies were not included due to not being available in English. This was not a systematic review; therefore, no specific questions were answered within this review and results from

papers were not subject to analysis. However, the methodology of a scoping review was suitable due to the broad question posed and the need to examine and describe the potentially large range of literature that had not previously been synthesised (Peters *et al.*, 2015; Lockwood *et al.*, 2019), at least for three of the five health outcomes included. The methodology was appropriate to provide an overview of the available and relevant literature and to identify gaps in the research (O'Brien *et al.*, 2016; Munn *et al.*, 2018; Sucharew and Macaluso, 2019; Peters *et al.*, 2020) and meant that the papers related to age at neuter could be included and summarised, rather than just the small number of papers that considered puberty, as would have been considered in a systematic review. This provided a broader overview of the available evidence and identified problems relating to features of the study methodologies. This allowed reporting of the factors relating to study design and characteristics, and recommendations to be made for future studies. The review also identified that there is insufficient literature for systematic reviews to be undertaken for most of these diseases.

There was the potential for selection bias (Sucharew and Macaluso, 2019). The search strategy may not have identified all of the available literature which could have affected the description of eligible studies presented. However, the databases that were included have been reported to provide very high levels of coverage of veterinary literature (Grindlay *et al.*, 2012).

This review was completed by two individuals, which was deemed appropriate for the volume of literature generated for review. There is the possibility that some articles may have been missed, however the search terms and strategy were reviewed by a group of five specialists, one with extensive experience of conducting scoping reviews. Additionally, the inclusion of a librarian helped to optimise the search strategy (Morris *et al.*, 2016; Hanneke *et al.*, 2017; Lockwood *et al.*, 2019; Peters *et al.*, 2020) and papers were included that were found in a previous literature search by the

primary author and did not appear in the search results. Therefore, it is more likely that the search would have been too broad resulting in more articles being retrieved than failing to identify relevant articles. The search term 'spey' was not included which could have resulted in articles being missed if they did not also include other terns relating to neutering that were in the search terms.

3.4.5 Future recommendations

Future studies that aim to examine the effects of neutering bitches before or after puberty should:

- be undertaken for a variety of dogs (pet, assistance, shelter etc.) from many geographical locations,
- consider breed and body weight, which can impact health outcomes,
- clearly define inclusion criteria including whether diseases are diagnosed before or after the neutering intervention, which should also be defined,
- randomly allocate dogs to neutering groups where all bitches are neutered using the same surgical method,
- consider study population size in order to ensure that the minimum number of bitches needed for valid statistical analyses are recruited, considering the likely disease incidence,
- use power analysis to determine appropriate values of alpha for the study population,
- identify and define puberty, considering the onset of puberty rather than age.

For studies examining the effects of timing of neutering relating to age, previous breeding history should be considered. Additionally, consideration of other studies when selecting age groups could enable easier comparisons to be made between studies.

3.5 CONCLUSION

This scoping review has identified a lack of evidence related to the impacts of neutering bitches before or after puberty on five aspects

of bitch health (atopy, DOD, neoplasia, obesity and urogenital disease). Only six studies were identified that categorised the timing of surgical neutering as prepubertal or post-pubertal, one investigating mammary neoplasia and the other five, urogenital disease, commonly UI. No studies were identified that examined the impacts on atopy, DOD or obesity. Twenty-six studies considered bitch age rather than pubertal status at the time of neutering. Most studies were retrospective and varied in their methodologies, quality and means of categorising bitches into neutering age groups. The review highlighted the lack of literature along with weaknesses in the existing studies caused by age rather than puberty being examined. Whether or not neutering before or after puberty has an impact on the health of female domesticated dogs is currently unknown.

Chapter 4. General methodology for prospective study

4.1 INTRODUCTION

In this prospective study extensive, detailed data collected at several time points were examined from a cohort of Guide Dogs' bitches from six months of age to approximately 11.5 years of age. Data were gathered on bitch behaviour and development through the Guide Dogs' production process to becoming a qualified guide dog or being withdrawn and rehomed and on long-term health outcomes. The data were examined independently and subsequently brought together in the discussion to report the findings of one large study into the effects of neutering bitches before or after puberty. Chapter 5 reports the findings from the physical assessments of bitches conducted at six- and 17 months of age. Chapter 6 investigates the behaviour of bitches reported using behavioural questionnaires completed at one and three years of age. Chapter 7 examines the training times, qualification and success rates for bitches as they progressed through the Guide Dogs' programme. Chapter 8 reports the findings from the examination of health data. Within this general methodology chapter, Guide Dogs' processes, the study population and the overall study design including the data collected throughout the study will be described. Detailed methods are explained in the relevant chapters.

4.2 STUDY DESIGN

A prospective study was undertaken using 306 Guide Dogs' bitches born between 22/02/2012 and 09/08/2015. The study gathered data for bitches from six months of age. The author was involved in the study from the study design stage.

4.3 STUDY SETTING

Guide Dogs' puppies are born to bitches cared for in volunteer family homes or at the Guide Dogs National Centre. Between six

and seven-weeks of age puppies enter the puppy block at the National Centre where they remain for seven days. During this time, they are vaccinated and microchipped. Between seven and eight-weeks of age, puppies enter the puppy raising stage, travelling nationally to their volunteer puppy raisers' homes where they remain until approximately 14 months of age. During puppy raising, dogs are socialised in a variety of environments and situations and learn basic obedience, supervised by a Puppy Development Advisor. During puppy raising, all dogs are neutered to ensure that surgery and recovery are undertaken while the dog is in a familiar home and formal training is not interrupted. Male dogs are castrated and female dogs are neutered by ovariohysterectomy. Dogs then enter the early training stage with a Guide Dog Trainer at a Guide Dog Training School where they develop a bond with their trainer and learn obedience and the basic skills required to become a guide dog. During early training, dogs are either housed in kennels at the Guide Dog Training School, or in homes with volunteer boarders. Following early training, dogs enter a period of advanced training at a Mobility Team, where a Guide Dog Mobility Specialist works with the dog to develop advanced guiding skills and matches the dog to a potential guide dog owner. During advanced training, dogs are housed with volunteer boarders. During the final 'on class' stage, the dog, potential guide dog owner and Guide Dog Mobility Specialist work together before qualifying as a guide dog partnership. Normal progression through the guide dog programme involves puppies moving sequentially through the puppy raising, early and then advanced training stages with one Puppy Development Advisor, one Guide Dog Trainer and one Guide Dog Mobility Specialist. The dates of movements between each of the stages are recorded on the dogs' individual records on Guide Dogs' electronic database, GDIR.

Detailed electronic health data for individual dogs are recorded on GDIR including the dates of any health investigations, diagnoses, health testing, surgeries, routine veterinary examinations, body

weights, body condition scores and routine treatments. Approximately 40% of the dogs that enter puppy raising are expected to be unsuitable to qualify as a guide dog due to health and/or behavioural reasons. GDIR contains details of the withdrawal reasons for any dogs that leave the programme.

4.4 STUDY PARTICIPANTS

4.4.1 Bitches allocated to the study

Three hundred and thirty crossbreed bitch puppies born between 22/02/2012 and 09/08/2015 were allocated to the study. Bitches were (sire breed listed first): Golden Retriever cross Labradors (n=219), Golden Retriever cross (Labrador cross Golden Retriever) (n=1), Labrador cross Golden Retrievers (n=47), Labrador cross (Golden Retriever cross Labrador) (n=55) or Labrador cross (Labrador cross Golden Retriever) (n=8). All puppies from a pure breed sire and a crossbreed dam were termed first-generation backcrosses. Bitches were allocated to the group to be neutered prepubertally (at six months of age, PPN, n=181) or the group to be neutered post-pubertally (after the first oestrus, Control, n=149)[3]. Whilst it is acknowledged that western convention is to refer to the Control group first, in this case the PPN group will appear first throughout the thesis for temporal consistency with the neutering intervention. Bitches were all neutered by ovariohysterectomy before entering early training. PPN bitches were neutered between 181 and 204 days of age (mean = 189.4 ± 0.4) and Control bitches between 278 and 528 days of age (mean = 387.8 ± 3.2). Surgeries were performed by experienced veterinarians at one of four veterinary practices in the United Kingdom (VP1, VP2, VP3 or VP4) following a standardised neutering protocol (Moxon *et al.*, 2023a).

[3] According to the Oxford English dictionary, 'prepubertal' is written without a hyphen, whereas 'post-pubertal' is hyphenated. www.oed.com

Bitches were selected to be included in the study by a Puppy Development Advisor if they were 1) the correct breed, 2) lived with puppy raisers within 25 miles of a Guide Dog Training School (either Forfar, Atherton, Leamington or Redbridge) to enable assessments and surgery to be performed by the centre veterinarians, and 3) were not marked as potential breeding stock. The Puppy Development Advisor contacted the author to advise they had a puppy that met the criteria once the puppy had entered puppy raising. The author then allocated the puppy to the PPN or Control group in alternating order.

4.4.2 Bitches removed from the study

Twenty-four bitches (eight PPN and 16 Control) were removed from the study. Data for the 24 bitches that were removed were excluded from all parts of the study and analysis. The removal reasons included being neutered at a veterinary practice other than one of the four study veterinary practices (n=10), being withdrawn from the Guide Dogs' programme before neutering (n=5) and having no six-month assessment completed (n=3; Figure 4.1).

4.4.3 Bitches changed from the PPN to the Control group

Following physical assessments at six months of age, 18 bitches were changed from the PPN to the Control group following veterinary advice. These included nine bitches for which decisions were made based on their clinical histories and a further eight bitches, for which decisions were made based on their current health and circumstances. There were eight different individual or combinations of reasons for bitches to be considered unsuitable for neutering at six months of age. Vaginitis, or a history of vaginitis and vulval discharge was the most common reason (n=10, Table 4.1). Therefore, the final study population included 155 PPN and 151 Control bitches.

Table 4.1. The reasons that 18 bitches were changed from the PPN group to the Control group following assessment by a veterinarian at six months of age.

Reason for changing from the PPN to Control group	Number of bitches
Vaginitis (or history of) and/or vulval discharge (or history of)	10
Surgery delayed due to kennel cough and neutering window missed	1
Vomiting and diarrhoea at the time of surgery	1
Recent history of diarrhoea	1
In season at the time of the assessment	1
Grade 2 heart murmur on examination which required further investigation	1
Juvenile, inverted and/or recessed vulva	2
Demodex diagnosed, bitch undergoing investigation and treatment	1

4.4.4 Final study population

The 306 bitches in the final study population were (sire breed listed first): Golden Retriever cross Labradors (n=204), Golden Retriever cross (Labrador cross Golden Retriever) (n=1), Labrador cross Golden Retrievers (n=43), Labrador cross (Golden Retriever cross Labradors) (n=50) or Labrador cross (Labrador cross Golden Retrievers) (n=8; Table 4.2). Bitches were from 64 unique sires and 173 unique dams, with the majority of sires (42/64) contributing puppies to both trial groups.

Table 4.2. The number of bitches in the final study population for the prospective study of the effect of neutering before or after puberty that were from each breed, showing the number in each trial group that were each breed and the total number in each trial group (PPN = neutered at six months of age, Control = neutered after the first oestrus).

Breed	Number of bitches in PPN group	Number of bitches in Control group
Golden Retriever cross Labrador	100	104
Golden Retriever cross (Labrador cross Golden Retriever)	0	1
Labrador cross Golden Retriever	24	19
Labrador cross (Golden Retriever cross Labrador)	28	22
Labrador cross (Labrador cross Golden Retriever)	3	5
Total	155	151

4.4.5 Progression and loss of bitches from the study and from the Guide Dogs programme

In addition to the 24 bitches that were removed from the study, a number remained in the study but were withdrawn from the Guide Dogs programme due to being unsuitable for progression to be a guide dog. Thirty-seven bitches were withdrawn during puppy raising and a further five bitches were transferred to alternative working homes during puppy raising. Fifty-six bitches were withdrawn during early training and 13 bitches were withdrawn from advanced training (Figure 4.1). Once withdrawn, these bitches were permanently rehomed and therefore no longer under the care and supervision of Guide Dogs. After this point health records were no longer available. For 17-month physical assessments and behaviour questionnaires, the persons that had rehomed the dogs were contacted where possible to allow data to be collected. Therefore, a number of bitches remained on the study with incomplete datasets, and the number of bitches included in each section of the study varied. Additionally, other bitches were missing one specific item from their data, for instance, the height measurement at six months of age. Within each section of the study, the number of bitches that were withdrawn prior to the data being collected and the number of bitches included in the analysis was specified.

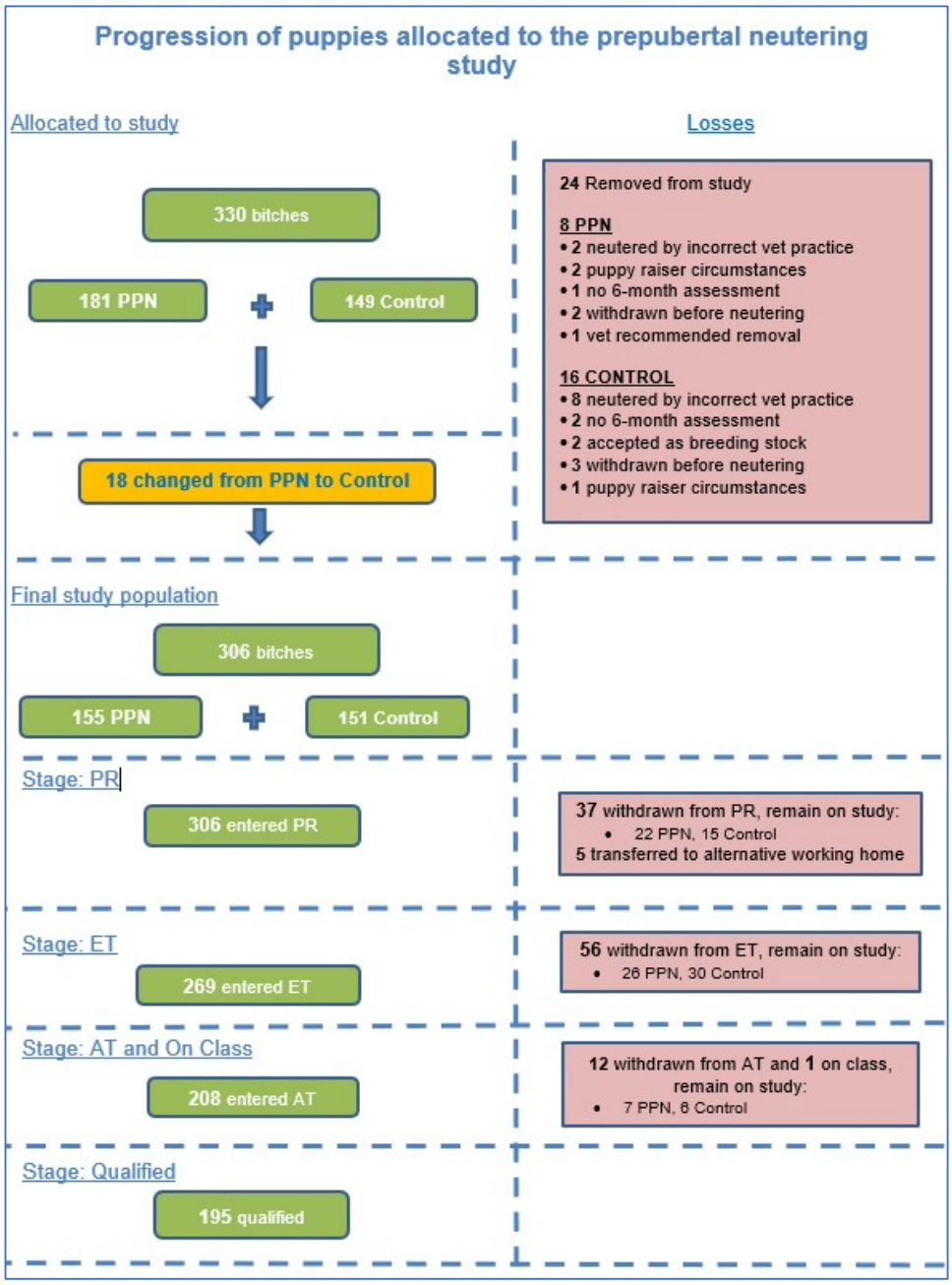


Figure 4.1. A flow chart to show the progression through the guide dog programme for 330 bitches allocated to the prospective study investigating the effect of neutering before (PPN) or after puberty (Control). The chart shows reasons for losses from study and stages at which study bitches were removed from the programme up to the point of qualification. PR-puppy raising, ET-early training, AT-advanced training.

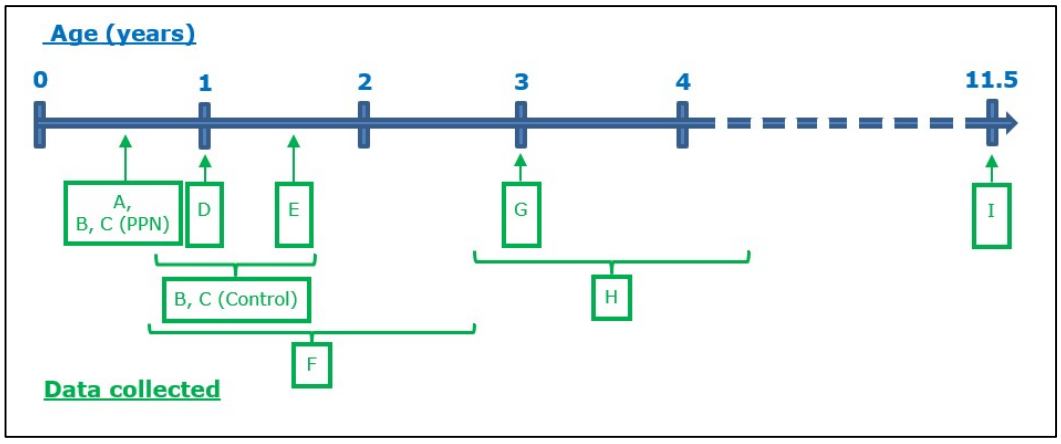
4.5 VARIABLES AND DATA SOURCES

Data were collected from puppy raisers, veterinarians, dog care staff, guide dog owners and rehomers using purpose designed data capture forms and behavioural questionnaires. Data were also

collected from Guide Dogs electronic database (GDIR), including details of the dogs' health, weight, BCS, and outcome (qualified or withdrawn, Table 4.3 (a) and (b)). Additionally, GDIR was used to search for any data missing from data collection forms, such as body weight at the time of physical assessments. PPN bitches were examined at the six-month assessment for suitability for neuter, and if suitable were neutered the same or the next day. Bitches that were unsuitable were transferred to the Control group. All physical assessments were performed at one of four veterinary practices (termed VP 1, VP 2, VP 3 and VP 4). Each of the veterinary practices are external to Guide Dogs but are associated with one of the four regional Guide Dog Training Schools. Detailed methodologies are described in each thesis chapter and are introduced below.

Table 4.3. For the 306 bitches in the prospective study examining the effect of neutering before or after puberty, the timeline (a) shows the ages at which data were collected for study bitches with reference to the table (b) which shows the data that were collected for bitches in each trial group. The ages and/or stages at which data were collected, the persons responsible for collecting the data and whether the data were collected using novel data collection methods designed specifically for the study or were collected retrospectively from pre-existing data, with reference to the timeline.

(a)



(b)

Reference for timeline	Age/stage	Group	Data collected	Data collected by	Novel or pre-existing data
A	6-months	PPN and Control	6-month physical assessment	Veterinarian	Novel
B*	6-months	PPN	Neutering questionnaire	Veterinarian	Novel
B*	Post-season spay window	Control	Neutering questionnaire	Veterinarian	Novel
C*	14 days post-neutering	PPN and Control	Recovery questionnaire	Puppy raiser or dog care staff	Novel
D	12-months	PPN and Control	Behaviour questionnaire	Puppy raiser	Novel
E	17-months	PPN and Control	17-month physical assessment	Veterinarian or DHWS	Novel
F	Qualification or withdrawal	PPN and Control	Qualification or withdrawal date Days in each stage of training	Researcher from GDIR	Pre-existing
G	3-years	PPN and Control	Behaviour questionnaire	GDO or rehomer	Novel
H	Qualification plus one and three years	PPN and Control	Whether bitch remained qualified with first GDO	Researcher from GDIR	Pre-existing
I	Up to 11.5 years	PPN and Control	Health data from electronic health records	Researcher from GDIR	Pre-existing

*data collected as part of the study but not reported within this thesis, see Moxon *et al.* (2023a). GDO–guide dog owner, DHWS-Dog Health and Wellbeing Specialist.

4.5.1 Physical assessments at six and 17 months of age

All bitches in the study had physical assessments performed by a veterinarian at one of the four veterinary practices at six and 17 months of age. Physical assessments for bitches that had been withdrawn and rehomed by 17 months of age were performed by Guide Dogs' Dog Health and Wellbeing Specialists.

4.5.2 Behaviour questionnaires at one and three years of age

Behavioural development was compared using an existing behavioural assessment tool, the C-BARQ (Canine Behaviour and Research Questionnaire, Duffy and Serpell, 2012). The version of the C-BARQ that was used in this study was adapted to measure six

factors of interest: 1) Training and obedience, 2) Aggression, 3) Fear and anxiety, 4) Excitability, 5) Attachment and attention seeking, and 6) Social behaviour.

The one-year behavioural questionnaires were completed by puppy raisers within a two-week window when the bitch was one-year to one-year two weeks of age. Three-year behavioural questionnaires were completed by either guide dog owners (for qualified dogs) or by rehomers (for dogs that had been withdrawn from the guide dog programme by three years of age) when the bitch was three years to three years and two weeks of age.

4.5.3 Training times, qualification and success

For each bitch, their outcome (qualified or withdrawn), the date that they entered each stage of training (puppy raising, early training, advanced training, on class) and the number of times in each stage of training were recorded. In addition, whether the bitch remained qualified with their original guide dog owner for at least a one-year and a three-year period (success) was noted. One and three-year periods following qualification are standard periods used by guide dog schools to determine successful partnerships. For dogs withdrawn from the Guide Dogs' training programme, the overall withdrawal reason (health or behaviour) and the detailed withdrawal reasons; the specific health and or behavioural reasons that the dogs were withdrawn, were recorded.

4.5.4 Health data

The detailed health records for each bitch were extracted from GDIR on 15/09/2023. All available health data to the point of data collation were included in the analysis. Health records and detailed veterinary notes were manually examined. Disease incidence and the number of episodes for each disease were noted. Disease data were grouped into one of five categories: musculoskeletal; neoplasia, urogenital, immune, and 'other' diseases. Health records relating to accidental injury, such as cuts or abrasions, dog attacks

and health records relating to behavioural observations, such as coprophagia, were not included in the analysis.

4.6 BIAS AND STUDY SIZE

Specific biases and confounding variables related to each analysis are described in detail in each chapter. Breed selection was limited to crosses of Labradors and Golden Retrievers as these crosses represent approximately 75% of the dog produced by Guide Dogs and would result in the greatest possible population to recruit from. Study size was limited by the available cohort of bitches produced by Guide Dogs during the recruitment period for the study that were placed in puppy raising within a suitable distance to travel to one of four national veterinary practices for neutering surgery. Power analysis was used to ensure appropriate values of alpha for the study size. Puppies were allocated to PPN or Control groups by the author, with no prior knowledge of the puppies, in alternating order at approximately eight weeks of age. This ensured that any confounding factors that may be influenced by time, such as staff capacity in the training programme, or demand for guide dogs, could be expected to act on both trial groups equally. Thirty-three puppy raisers had two bitches that were in the study (16 had one PPN and one Control, nine had two PPN bitches and eight had two Control bitches), and six puppy raisers had three bitches in the study (one had three PPN bitches, one had three Control bitches and four had a mixture of PPN and Control bitches). Most puppy raisers had one bitch in the study and due to the small number with more than one, it was not possible to include puppy raiser ID as a confounding variable in the analysis.

4.7 QUANTITATIVE VARIABLES AND STATISTICAL ANALYSIS

Detailed explanation of the statistical analyses and models used to examine the data for each element of the study are described in detail in the methodology section of the relevant study chapter. Throughout the thesis, age was referred to in months when used to broadly describe age at neuter, and years for diagnosis of disease in

later life. However, days were used in statistical analysis for age and associated descriptive statistics. All data were examined for normality and the appropriate parametric and non-parametric statistical tests and models were used. Loss to follow up in the study was caused by dogs being removed from the programme. Numbers of dogs removed were compared between trial groups to ensure that there were no differences, therefore no greater impact on any group. For longer term health data, survival analysis that accounts for censored data was used where the number of events was sufficient for analysis. Categorical covariates included in all statistical models were examined using descriptive statistics and any that had less than five bitches were identified and grouped.

Quality assurance of data entry was checked by duplicating 10 records from each section to check for errors (none identified). Results were described as mean ± SEM for continuous data, and median values were used for ordinal data such as BCS. Effect sizes (Glass's *delta*) were calculated using the formula $delta=(M_1-M_2)/S_c$. Where M_1 = PPN group mean, M_2 = Control group mean and S_c = standard deviation for the Control group. Data were analysed using appropriate statistical models in SPSS version 22-28 (IBM, USA[4]), XLStat (Addinsoft, USA[5]), and MLwiN v.3.04 (Rasbach *et al.*, 2019). Standardised residuals were saved and checked for normality (assumptions were met for all general linear models). Sequential Bonferroni correction was applied where multiple testing was conducted to minimise the risk of Type 1 error.

Individual values of alpha were calculated for each variable being tested, to minimise and balance the occurrence of Type I and II errors (Verrill and Durst, 2005; Payne, 2013) using G*Power version 3.1.9.2 (Faul *et al.*, 2007) for known population sizes and

[4] https://www.ibm.com/products/spss-statistics
[5] https://www.xlstat.com/en/

effect sizes that would be considered to represent an important change in the dependent variable for each analysis. In some cases, sensitivity analysis was used to determine the size of effect that could be detected with the available number of bitches.

Table 4.4. Values of alpha, beta, effect size and effective sample size for statistical tests conducted on each variable for each section of data. Effect size for each variable was determined using a percentage change in the Control population mean for a priori power analysis.

Chapter and variable	n (PPN, Control)	Statistical test	Change in Control population mean used for power analysis	Alpha, Beta, effect size and sample size
Chapter 5				
Six-month assessments				
Height (cm)	146,136	GLM	10% (5.2 cm)	8%, 5%, 2.04, 14
Body weight (kg)	145,134	GLM	10% (2.1 kg)	10%, 5%, 0.875, 58
Body condition score grouped as ideal and overweight	150,147	Binary logistic regression	10% decrease in the number that were optimum and 10% increase in the number that were overweight	4%, 5%, 0.225, 270
Vulval length (cm)	144,129	GLM	10% (0.3 cm)	6%, 3%, 0.601, 160
Vulval width (cm)	144,129	GLM	10% (0.18 cm)	6%, 3%, 0.554, 186
Anomalies in vulval appearance at the physical assessment	All = 152,151	Chi-square	10% increase in presence of anomalies	
Swollen				4%, 5%, 0.560, 66
Discharge				4%, 5%, 0.261, 304
Juvenile				4%, 5%, 0.325, 196
Recessed				4%, 5%, 0.265, 295
Perivulval skin folds				4%, 5%, 0.403, 128
Perivulval dermatitis				4%, 5%, 0.403, 128
Cumulative vulva score				7%, 9%, 0.230, 297
Anomalies in vulval appearance on digital image examination		Chi-square	Increase in presence of anomalies as indicated:	
Discharge	73,80		15%	9%, 10%, 0.302, 157

Discharge category	73,80		15%	7%, 9%, 0.323, 151
Dorsal skin folds	89,98		10%	4%, 5%, 0.904, 26
Estimated dorsal skin fold coverage	86,92		15%	4%, 5%, 0.354, 165
Recessed/ inverted	87,91		15%	4%, 5%, 0.362, 158
Perivulval skin changes	114,110		10%	4%, 5%, 1.01, 21
Nature of perivulval skin changes	114,110		10%	4%, 5%, 1.01, 21
17-month assessments				
Height (cm)	119,110	GLM	10% (5.7 cm)	7%, 5%, 2.14, 14
Body weight (kg)	119,110	GLM	10% (2.7 kg)	9%, 5%, 1.00, 48
Body condition score grouped as ideal and under/overweight	125,127	Binary logistic regression	10% decrease in the number that were optimum and 10% increase in the number that were under/overweight	4%, 5%, 0.280, 175
Vulval length (cm)	118,109	GLM	10% (0.3 cm)	8%, 4%, 0.733, 94
Vulval width (cm)	118,109	GLM	10% (0.2 cm)	8%, 4%, 0.639, 122
Anomalies in vulval appearance at the physical assessment	All = 125,128	Chi-square	10% increase in presence of anomalies	
Swollen				N/A none affected
Discharge				4%, 5%, 0.577, 42
Juvenile				4%, 5%, 0.797, 22
Recessed				4%, 5%, 0.577, 42
Perivulval skin folds				4%, 5%, 0.797, 22
Perivulval dermatitis				4%, 5%, 0.797, 22
Cumulative vulva score				4%, 5%, 0.406, 83
Anomalies in vulval appearance on digital image examination		Chi-square	Increase in presence of anomalies as indicated:	
Discharge	80,93		15%	4%, 5%, 0.359, 107
Discharge category	80,92		15%	4%, 5%, 0.373, 99
Dorsal skin folds	91,96		10%	4%, 5%, 0.959, 15

Estimated dorsal skin fold coverage	77,88		15%	4%, 5%, 0.385, 93
Recessed/ inverted	76,82		15%	4%, 5%, 0.301, 152
Perivulval skin changes	111,119		10%	4%, 5%, 1.01, 14
Nature of perivulval skin changes	111,119		15%	4%, 5%, 0.308, 145
Change in height (cm)**	115,110	GLM	N/A sensitivity analysis	9%, 5%, 0.447, 225
Change in weight (cm)**	114,108	GLM	N/A sensitivity analysis	9%, 5%, 0.450, 222
Change in vulval length and width (cm)**	113,107	GLM	N/A sensitivity analysis	9%, 5%, 0.452, 218
Chapter 6				
One-year behaviour questionnaires				
Mean Training and obedience score**	134,140	GLM	N/A sensitivity analysis	9%, 5%, 0.405, 274
Mean Aggression score*, **	134,140	GLZ with logit link	N/A sensitivity analysis	9%, 5%, 0.405, 274
Mean Fear and anxiety score**	134,140	GLZ	N/A sensitivity analysis	9%, 5%, 0.405, 274
Mean Excitability score**	134,140	GLZ	N/A sensitivity analysis	9%, 5%, 0.405, 274
Mean Attachment and attention seeking score**	134,140	GLM	N/A sensitivity analysis	9%, 5%, 0.405, 274
Mean Social behaviour score**	134,140	GLZ	N/A sensitivity analysis	9%, 5%, 0.405, 274
Three-year behaviour questionnaires				
Mean Training and obedience score**	102,91	GLM	N/A sensitivity analysis	10%, 5%, 0.476, 193
Mean Aggression score*, **	102,91	GLZ with logit link	N/A sensitivity analysis	10%, 5%, 0.476, 193
Mean Fear and anxiety score**	101,91	GLZ	N/A sensitivity analysis	10%, 5%, 0.477, 193
Mean Excitability score**	102,91	GLZ	N/A sensitivity analysis	10%, 5%, 0.476, 193
Mean Attachment and attention seeking score**	102,91	GLM	N/A sensitivity analysis	10%, 5%, 0.476, 193
Mean Social behaviour score**	102,91	GLZ	N/A sensitivity analysis	10%, 5%, 0.476, 193
Change in scores between one and three years				
Mean Training and obedience score**	89,82	GLM	N/A sensitivity analysis	10%, 5%, 0.506, 171
Mean Aggression score*, **	89,82	GLZ with logit link	N/A sensitivity analysis	10%, 5%, 0.506, 171
Mean Fear and anxiety score**	89,82	GLZ	N/A sensitivity analysis	10%, 5%, 0.506, 171
Mean Excitability score**	89,82	GLM	N/A sensitivity analysis	10%, 5%, 0.506, 171

Mean Attachment and attention seeking score**	89,82	GLM	N/A sensitivity analysis	10%, 5%, 0.506, 171
Mean Social behaviour score**	89,82	GLM	N/A sensitivity analysis	10%, 5%, 0.506, 171
Chapter 7				
Days in puppy raising	152,149	Multilevel model	10% (41 days)	10%, 5%, 0.837, 64
Days in early training**	130,134	Multilevel model	N/A sensitivity analysis	10%, 5%, 0.406, 264
Days in advanced training and on class**	105,105	Multilevel model	N/A sensitivity analysis	10%, 5%, 0.456, 210
Age at entry to early training	130,134	Multilevel model	10% (46 days)	10%, 5%, 1.095, 38
Qualified and withdrawn	152,149	Binary logistic regression	5% change in qualification	5%, 6%, 0.21, 281
Overall withdrawal reason (health or behaviour) (withdrawn dogs only)	54,51	Chi-square	10% change in withdrawn for each reason	10%, 10%, 0.3, 96
Behavioural withdrawal reasons	74,79	Chi-square	Equal distribution	5%, 5%, 0.4, 124
Stage at withdrawal	55,51	Chi-square	10% more bitches withdrawn earlier	10%, 10%, 0.3, 96
Success to one year	97,98	Chi-square	5% change in success	4%, 5%, 0.3, 152
Success to three years	95,97	Chi-square	5% change in success	4%, 5%, 0.3, 152
Chapter 8				
Years of health data	155,151	Chi-square	10% more bitches with >7 years of data and 15% less with <3	4%, 5%, 0.249, 315
At least one disease from the group: Musculoskeletal Neoplasia Urogenital Immune disease Immune disease (excluding otitis)	All=155,151	Chi-square	10% change in number diagnosed	 5%, 5%, 0.237, 232 4%, 5%, 0.316, 138 4%, 5%, 0.254, 213 4%, 5%, 0.204, 330 4%, 5%, 0.344, 116
0, 1 or 2 diseases from the group: Musculoskeletal Urogenital Immune	All=155,151	Chi-square	10% change in number diagnosed	 4%, 5%, 0.255, 250 4%, 5%, 0.265, 230

				5%, 5%, 0.218, 324
Disease incidence for:	All=155,151 unless stated	Chi-square	Increase in incidence as indicated:	
Forelimb lameness			10%	4%, 5%, 0.269, 190
Osteoarthritis			10%	4%, 5%, 0.384, 93
Cruciate ligament			5%	4%, 5%, 0.598, 39
Mast cell tumour			5%	4%, 5%, 0.239, 241
Other neoplasia			5%	4%, 5%, 0.314, 139
UTI/Cystitis			5%	4%, 5%, 0.211, 309
Perivulval dermatitis			5%	4%, 5%, 0.280, 175
Vulval/Abnormal discharge			5%	4%, 5%, 0.223, 275
Otitis externa			10%	4%, 5%, 0.208, 317
Atopy			5%	4%, 5%, 0.211, 309
Histiocytoma			5%	4%, 5%, 0.357, 108
Overweight/having obesity			10%	5%, 5%, 0.201, 323
Obese based on BCS	90,89		12%	5%. 5%, 0.270, 178
Age at first diagnosis for:				
Forelimb lameness**	29,25	Mann-Whitney U	N/A sensitivity analysis	10%, 5%, 0.910, 54
Osteoarthritis	18,11	t-test	20% increase in age of diagnosis	9%, 5%, 1.345, 28
Mast cell tumour**	4,7	Mann-Whitney U	N/A sensitivity analysis	13%, 5%, 2.124, 11
Other neoplasia**	12,4	Mann-Whitney U	N/A sensitivity analysis	12%, 5%, 1.934, 16
UTI/Cystitis**	12,9	Mann-Whitney U	N/A sensitivity analysis	11%, 5%, 1.481, 21
Perivulval dermatitis**	8,5	Mann-Whitney U	N/A sensitivity analysis	13%, 5%, 1.905, 13
Vulval/Abnormal discharge**	5,8	Mann-Whitney U	N/A sensitivity analysis	13%, 5%, 1.905, 13
Atopy**	12,9	Mann-Whitney U	N/A sensitivity analysis	11%, 5%, 1.481, 21
Otitis externa**	74,55	Mann-Whitney U	N/A sensitivity analysis	10%, 5%, 0.589, 129
Overweight/having obesity**	65,69	Mann-Whitney U	N/A sensitivity analysis	10%, 5%, 0.572, 134
Musculoskeletal disease and growth†		Mann-Whitney U	20% change in number affected	
Cruciate ligament	7,218			

Forelimb lameness	40,185			9%, 5%, 0.396, 286
Osteoarthritis	19,206			9%, 5%, 0.397, 286
At least one musculoskeletal disease	59,166			9%, 5%, 0.400, 282
				9%, 5%, 0.394, 290
Urogenital disease and vulval appearance at 17 months†		Chi-square	10% change in affected	
Perivulval dermatitis	13,265			4%, 5%, 0.494, 85
Urinary incontinence	7,271			4%, 5%, 0.580, 62
UTI/cystitis	20,258			4%, 5%, 0.438, 108
Vulval/abnormal discharge	13,265			4%, 5%, 0.494, 85
Urogenital disease and % dorsal fold coverage at 17 months†		Chi-square	10% change in affected	
Perivulval dermatitis	7,158			4%, 5%, 0.611, 56
UTI/cystitis	11,154			4%, 5%, 0.439, 107
Vulval/abnormal discharge	10,155			4%, 5%, 0.396, 132
Urogenital disease and overall vulval growth from six to 17 months†		Chi-square	10% change in affected	
Perivulval dermatitis	10,259			4%, 5%, 0.552, 68
Urinary incontinence	7,262			4%, 5%, 1.124, 17
UTI/cystitis	19,250			4%, 5%, 0.417, 119
Vulval/abnormal discharge	12,257			4%, 5%, 0.441, 107
Body condition score grouped as not obese/obese	90,89	Chi-square	12% decrease in % obese	5%, 5%, 0.270, 178
Overall health score	155,151	Chi-square	10% increase in number scored 0	5%, 5%, 0.246, 308
Withdrawn from working**	97,98	Chi-square	N/A sensitivity analysis	5%, 5%, 0.281, 195
Withdrawn for health	44,53	Chi-square	10% change in number withdrawn for health	4%, 5%, 0.380, 95
Number of bitches died	155,151	Chi-square	7% fewer bitches died	4%, 5%, 0.241, 236
Age at death**	18,14	Mann-Whitney U	N/A sensitivity analysis	10%, 5%, 1.200, 32

Abbreviations: GLM – generalised linear model, GLZ – generalized linear model
*Aggression scores converted to binary format for analysis.
**Computed using sensitivity analysis not a priori.
†For comparisons between growth and development and bitches affected with disease, the number of bitches shown is for affected and unaffected, rather than PPN and Control.

Chapter 5. A prospective study to investigate the impact of neutering bitches prepubertally or post-pubertally on physical development

5.1 INTRODUCTION

The effect of neutering on growth in dogs and cats, in terms of the length of the radius and ulna, the closure of the radial physes and the development of the external genitalia, has been investigated in a small number of studies (May *et al.*, 1991; Salmeri *et al.*, 1991; Stubbs *et al.*, 1996; Root-Kustritz *et al.*, 1997). In female cats, neutering, and earlier neutering has been associated with delayed closure of the proximal radial physes. Consequently, neutered female cats reached the growth plateau 18% later, and at 24 months of age had radial lengths that were 9% longer than cats that were left entire (Root-Kustritz *et al.*, 1997). In the most referenced work in the dog, Salmeri *et al.* (1991) compared neutered versus entire bitches and bitches neutered at different ages, and identified implications related to long bone growth and development of the external genitalia. However, the study included only small numbers and did not consider the timing of neutering in relation to puberty.

Previous studies have suggested that gonadectomy may impede vulval development (Salmeri *et al.*, 1991; Verstegen-Onclin and Verstegen, 2006; Nelson and Couto, 2009). Suboptimal vulva conformation is described clinically as a juvenile/infantile or recessed vulva. It is suggested by some authors to predispose to disease such as perivulval dermatitis, urinary tract infections (UTI) and chronic or juvenile vaginitis (Johnson, 1991; Feldman and Nelson, 2004; Nelson and Couto, 2009). Increased skin surrounding the vulva (perivulvar skin folds) is common in bitches with underdeveloped or recessed vulvas and the incidence is reported to be higher for neutered bitches and bitches that are obese (Johnston, 1991; Mathews, 2001). This morphological appearance can trap

urine and bacteria leading to bacterial overgrowth, dermatitis and an increased incidence of urogenital disease such as UTI, cystitis and chronic vaginitis (Lightner *et al.*, 2001, Mathews, 2001, Nelson and Couto, 2009, Miller *et al.*, 2013).

When considering effect of neutering on the development of external genitalia, Salmeri *et al.* (1991) reported that the vulvas of bitches neutered at seven weeks of age and seven months of age were immature in appearance, compared to entire bitches. While Salmeri *et al.* (1991) and Root-Kustritz *et al.* (1997) suggested that there may be no clinical problems associated with immature vulval development, Reichler (2009) proposed that obese bitches with infantile vulvas may be at higher risk of developing perivulvar dermatitis. Verstegen-Onclin (2006) suggested that early neutering of bitches can lead to chronic vaginal-vestibule infection due to the abnormal development of the external genitalia; of 27 dogs seen at a specialist small animal reproduction practice, 100% had vaginitis, 85% a recessed or hypoplastic vulva, 74% a recurrent urinary tract infection, 40% perivulvar dermatitis and 40% a redundant skinfold. Twenty-one of the 27 dogs were neutered prior to puberty. However, each of these cases was from a specialist referral hospital with the reason for referral unclear, and therefore the sample was potentially subject to bias. No quantitative data analysis has been conducted to examine the effect of neutering before or after puberty on the size and development of the vulva in female dogs.

Studies on bitch physical development and the effect of puberty and surgical neutering on characteristics such as height, weight and those relating to vulval development and abnormalities are not well documented. This study investigates the effects of timing of neutering in relation to puberty (rather than age at neutering) on these characteristics of physical development, in bitches neutered either before or after puberty. Such information would be useful to help with decision making on the optimum time for neutering bitches.

5.2 AIM AND OBJECTIVES

5.2.1 Hypothesis

Neutering bitches prepubertally does not impact their physical development compared to bitches neutered post-pubertally.

5.2.2 Aim

To use a prospective study design to compare measurements of height, weight, body condition score, vulval size and vulval appearance, in bitches neutered either before or after puberty.

5.2.3 Objectives

1) To determine whether there were any significant differences in measurements of height and weight at six and 17 months of age between bitches neutered before or after puberty.
2) To determine whether there were any significant differences in the change in height measurements between six and 17 months of age for bitches neutered before or after puberty.
3) To determine whether there were any significant differences in vulval length, width and appearance at six and 17 months of age between bitches neutered before or after puberty.

5.3 MATERIALS AND METHODS

5.3.1 Study design

A prospective study was undertaken using 306 Guide Dogs' bitches born between 22/02/2012 and 09/08/2015. Bitches were neutered either prepubertally or post-pubertally (see Section 4.4.4). Data were gathered from bitches at six and 17 months of age to examine physical development.

5.3.2 Study setting

Within Guide Dogs, whilst in the puppy raising stage, and before entering training at approximately 14 months age, all dogs are neutered. Study bitches had a physical assessment performed by experienced veterinarians at one of four veterinary practices in the United Kingdom (identified as VP1, VP2, VP3 or VP4) at six months

of age and again at 17 months of age. Physical assessments for bitches that had been withdrawn and rehomed by 17 months of age were performed by a Guide Dogs' Dog Health and Wellbeing Specialist. All assessments were requested to be completed within two weeks after the bitches reached (a) six months and (b) 17 months of age. For bitches neutered before puberty, the six-month physical assessment was conducted the day prior to neutering. At five and 16 months of age, data collection forms designed for the study were emailed to the Guide Dogs' staff member responsible for the dog. The forms were populated with the bitch's name, ID number, trial group and assessment window. Additionally, Guide Dogs' electronic health records were examined for bitches with missing assessment forms and missing data on completed assessment forms.

5.3.3 Study participants

The study population included 306 bitches from five different crossbreeds allocated to two groups; neutered prepubertally (at six months of age: PPN, n=155) or post-pubertally (after their first oestrus: Control, n=151) (see Section 4.4.4). All first-generation backcross bitches were grouped into one breed group for analysis. All 306 bitches were available for physical assessments at six months of age. By 17 months of age, 56 bitches had been withdrawn from the guide dog programme. Guide Dogs' Rehoming team were contacted to gather the data from these bitches, with the exception of those rehomed with alternative working homes. The number of bitches with data collected for each assessment was reported.

5.3.4 Variables and data sources

5.3.4.1 Physical assessments

Specially designed data capture forms were completed by the veterinarian or Dog Health and Wellbeing Specialist assessing the bitch (Appendix 3 and 4). Data collected at six and 17-month assessments included bitch height at the withers (cm) measured

using a wooden equine measuring stick, weight (kg) measured on veterinary practice scales, body condition score (BCS) measured using a 9-point scale (where a score of '4' or '5' represented optimum body condition at six and 17-months respectively), vulval measurements (length and width, cm; indicated on Figure 5.1) measured using a ruler, and descriptive appearance of the vulva. The presence of six features were noted using a '0' [not observed] or '1' [observed]; vulval swelling, perivulvar skin folds, perivulvar dermatitis, vulval discharge, recessed appearance, and juvenile appearance. These scores were later combined to give a cumulative vulva score out of six; bitches with a score of '0' were deemed to have normal vulval conformation.

At the six-month assessment the veterinarian also noted any relevant clinical history and signs, including evidence of previous conditions such as vaginitis and clinical signs associated with endocrine disease (specific diseases were not specified on the form; Appendix 3). Each bitch's suitability to be neutered at six months of age (PPN group only) was considered by the veterinarian based on current health and clinical history. The data related to clinical history and suitability to be neutered at six months of age were investigated in more detail for bitches moved from the PPN to the Control group and to establish the number of bitches in each trial group (reported in general methods, section 4.4.3).



Figure 5.1. The measurement points for the required length and width measurements recorded at the six-month and 17-month physical assessments and example of a digital image of a vulva.

For bitches with missing forms or missing data on completed forms for either the six or 17-month physical assessment, electronic health records were examined to determine whether the physical assessment was noted as completed. Any available height, weight or BCS data were extracted.

The outcome variables examined at six and 17 months of age were measurements of bitch height (cm), weight (kg), and vulval size (cm), BCS, responses to questions relating to vulval appearance and cumulative vulval score. Additionally, the change in measurements between six and 17 months of age were examined. Confounding variables were breed and the VP performing the assessment as fixed factors, the age at assessment and the number of days from neutering surgery (six and 17-month assessments) or the number of days between assessments (for the change in measurements between repeated assessments) as covariates. Confounding variable data were extracted from GDIR or physical assessment forms.

5.3.4.2 Digital images of the vulva

A digital photograph of the vulva was taken at the time of the six and 17-month assessments from a convenient (not fixed) distance (Figure 5.1). Following a period of training by a senior experienced veterinary reproduction specialist, all vulval images were examined by the same veterinary student who was blinded to bitch age and trial group. A vulval scoring system was devised for four aspects of vulval appearance; presence of vulval discharge, dorsal skin folds, recessed/inverted appearance and perivulval changes were denoted using '0' (absent) or '1' (present) for each bitch. Vulval discharge (if present) was categorised as: 1-mucoid, 2-mucoid purulent, 3-purulent, 4-haemorrhagic. Where present, the percentage cover of the dorsal skin fold over the vulva was estimated at 10% intervals (Figure 5.2). The nature of any perivulval changes were categorised as 1-skin discolouration, 2-hair discolouration, 3-hair and skin discolouration. If any image was of too poor quality to make a confident assessment of the above variables it was recorded as non-

diagnostic and was excluded from analysis for the corresponding variable. Vulval images were categorised as 'normal' or 'recessed/inverted' in appearance according to Figure 5.3. These categories were used to describe vulval appearance and examine vulval development for bitches with 'normal' and 'recessed/inverted' vulvas between six and 17-month assessments.

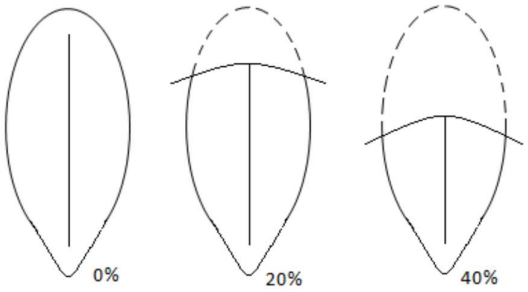


Figure 5.2 Schematic diagram illustrating 0%, 20% and 40% dorsal fold cover used during analysis of digital images of the vulva in a prospective study examining the differences in physical development for bitches neutered before or after puberty.

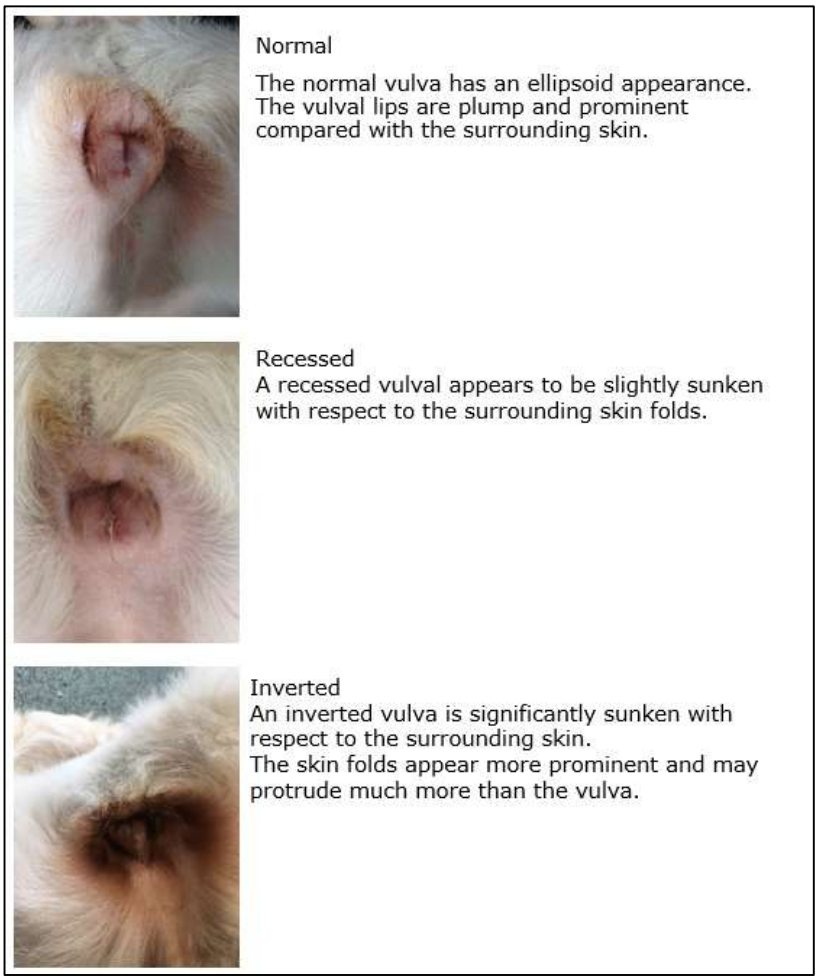


Figure 5.3. Images and descriptions (authors own) for normal, recessed and inverted vulval appearance used by a veterinary student to classify digital vulval images that were captured for bitches at six and 17-month physical assessments in a prospective study examining the differences in physical development for bitches neutered before or after puberty.

5.3.5 Bias and study size

Confounding factors were included in the statistical analysis for bitch height, weight, vulval length and width models to account for the effects of these factors when examining the main effect of trial group on the dependent variables. Height and weight can vary by breed. The effect of different crosses and back crosses on the adult size of female Labrador and Golden Retrievers crosses is unknown, therefore breed was included in the models. As up to a two-week variation in age was possible, that could in turn impact upon height and weight, age in days was included. The VP that completed the physical assessment, or whether the assessment was completed by

a Dog Health and Wellbeing Specialist was included due to potential bias in measurement or differences in calibration of weighing scales. The number of days between neutering surgery and the assessment was included in the models investigating differences in height, weight and vulval size at 17-month assessments.

Study size was limited by the available cohort of bitches produced by Guide Dogs during the recruitment period that were placed in puppy raising within a suitable distance to travel to one of four national veterinary practices for neutering surgery. Values of alpha were determined using G*Power for each analysis (see Section 4.7). The mean (±SEM) and confidence intervals (CI) were reported where appropriate.

5.3.6 Quantitative variables and statistical methods

Data were checked manually for obvious recording errors. Any incorrect dates or weights were checked in electronic health records and were corrected. Vulval measurements of length and width were checked manually, any cases where length<width were crosschecked with the bitches' digital images to determine whether the width appeared greater than the length. Where width in the image appeared less than the length, data were transposed (n=4).

Height at the wither (cm), weight (kg), vulval length (cm) and vulval width (cm) at the six and 17-month assessments were used to calculate the change in measurements between six and 17 months of age. For bitches with a calculated change in height that was zero or negative (representing a bitch not growing between six and 17 months of age, n=19), data were checked by examining the completed assessment forms to ensure recording errors were not present. These data were excluded from all height analyses and from analyses for other dependent variables where height was included as a covariate. The remaining data were used to examine differences between PPN and Control bitches at six and 17 months of age, and in the change in measurements between six and 17

months of age using univariate general linear models. The dependent factors were bitch height (cm), weight (kg), vulval length (cm) and vulval width (cm) for six and 17-month assessments, and were the calculated values for change in height (cm), change in weight (kg), change in vulval length (cm) and change in vulval width (cm). For all models, trial group, breed and VP were included as fixed factors. For models examining the change in measurements between six and 17 months of age, VP had five categories: VP1-4 for bitches with physical assessments completed by the same VP at each age, and 'Different' for bitches that had assessments completed by different VPs at each age. For six and 17-month models, age in days, and for 17-month models the number of days between neutering surgery and the assessment, were included as covariates. For models examining the change in measurements between six and 17 months of age, the number of days between measurements was included as a covariate. Height at the wither was included as a covariate in the general linear models for weight to account for any variation in weight attributable to bitches being taller. Height and weight were included as an interaction term in the general linear models used to examine vulval length and width measurements to control for any variation attributable to bitches being taller and heavier. Standardised residuals were saved and checked for normality. Pairwise comparisons within the models were used to describe significant differences for fixed effect variables. General linear models were performed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA).

For six-month assessments, for height measurements alpha and beta were 8% and 5% respectively with an effective sample size of 14 dogs, for weight measurements alpha and beta were 10% and 5% respectively with an effective sample size of 58 dogs. For both vulval length and width measurements, alpha and beta were 6% and 3% respectively with an effective sample size of 160 (length) and 186 (width) dogs.

For 17-month assessments, for height measurements alpha and beta were 7% and 5% respectively with an effective sample size of 14 dogs, for weight measurements alpha and beta were 9% and 5% respectively with an effective sample size of 48 dogs. For both vulval length and width measurements, alpha and beta were 8% and 4% respectively with effective sample sizes of 94 (length) and 122 (width) dogs.

For change in measurements between six and 17-month assessments, for height measurements alpha and beta were 9% and 5% respectively with an effective sample size of 225 dogs, for weight measurements alpha and beta were 9% and 5% respectively with an effective sample size of 222 dogs. For both vulval length and width measurements, alpha and beta were 9% and 5% respectively with effective sample sizes of 120 dogs.

A binary logistic regression model using stepwise backward elimination was used to examine the impact of trial group on BCS while controlling for confounding variables of breed, VP and body weight. For six-month assessments BCS were grouped as ideal (BCS = 4) and overweight (BCS greater than 4). For 17-month assessments BCS were grouped as ideal (BCS = 5) or under/overweight (BCS less or greater than 5). Alpha and beta were 4% and 5% respectively with effective sample sizes of 270 (6-month) and 175 (17-month) dogs.

Data for visual appearance of the vulva from physical assessment forms and digital vulval images, and cumulative vulval scores were examined using Chi-square tests. Chi-square with Yates' continuity correction was used for analysis of 2x2 contingency tables where frequencies were <5. Sequential Bonferroni correction was applied where multiple testing was conducted to minimise the risk of Type 1 error. Where frequencies were too small for Chi-square analysis, data were grouped and reported. Chi-square analyses and binary

logistic regression models were conducted using XLStat2016 (Addinsoft, USA).

For six-month assessments, for visual appearance of the vulva from physical assessment forms, alpha and beta were 4% and 5% respectively for all anomalies of appearance, with effective sample sizes between 66 and 304 dogs (see Section 4.7). For cumulative vulva score, alpha and beta were 7% and 9% respectively with an effective sample size of 297 dogs. For appearance of the vulva from examination of digital vulval images, for discharge alpha and beta were 9% and 10% respectively with an effective sample size of 157 dogs, for discharge category, alpha and beta were 7% and 9% respectively with an effective sample size of 151 dogs, and for all other anomalies, alpha and beta were 4% and 5% respectively with effective sample sizes between 21 and 165 dogs (see Section 4.7).

For 17-month assessments, for visual appearance of the vulva from physical assessment forms, alpha and beta were 4% and 5% respectively for all anomalies of appearance, with effective sample sizes between 22 and 42 dogs (see Section 4.7). For cumulative vulva score, alpha and beta were 4% and 5% respectively with an effective sample size of 83 dogs. For appearance of the vulva from examination of digital vulval images, alpha and beta were 4% and 5% respectively for all anomalies of appearance with effective sample sizes between 15 and 152 (see Section 4.7).

5.4 RESULTS

5.4.1 Participants

Three hundred and three bitches (152 PPN, 151 Control) had complete six-month physical assessment forms. Six-month assessment forms were not returned for three PPN bitches, however all three were neutered at six months of age at a VP and therefore had a physical assessment completed. All physical assessments at six months of age were completed by one of the four VPs. All bitches were entire at the time of the six-month physical

assessments. Two hundred and seventy-eight bitches (140 PPN, 138 Control) had complete 17-month assessment forms. Twenty-four of these were completed outside of the two-week window and one bitch was still entire at 17 months of age; these were excluded. A total of 253 bitches remained for analysis at the 17-month time point (125 PPN, 128 Control). Of these 253 bitches, 251 bitches (123 PPN, 128 Control) also had complete six-month physical assessment forms. At the time of the 17-month assessment, 36 of the 253 bitches were withdrawn from the guide dog programme and 19 bitches had assessments completed by either a Guide Dogs' Dog Health and Wellbeing Specialist or a veterinary practice other than one of the four VPs; 234 were completed by one of the four VPs. Nineteen bitches were excluded from all analysis that included height data due to potential errors represented by measurements suggesting a lack of growth between six and 17 months of age.

5.4.2 Height

5.4.2.1 Six-month

Two PPN bitches did not have a height recorded at the six-month physical assessment. The remaining 282 bitches (146 PPN, 136 Control) measured between 42.0 and 60.0 cm in height (mean 52.2 cm ± 0.2 cm). There was no significant difference in height between PPN and Control bitches at six months of age ($F=1.941$, $D.F.=1$, $P=0.165$; mean height of PPN bitches 52.0 ± 0.2 cm and Control bitches 52.3 ± 0.2 cm, Figure 5.4). Age at assessment ($F=7.396$, $D.F.=1$, $P=0.007$) and VP ($F=4.653$, $D.F.=3$, $P=0.003$) were significantly associated with bitch height. A one day increase in age was associated with a 0.09 unit increase in height ($\beta=0.086$, $S.E.=0.032$, 92% CI=0.030 to 0.142). VP3 had bitches with significantly lower measured heights than VP1 (mean difference =-0.806, $S.E.=0.402$, 92% CI=-1.512 to -0.100, $P=0.046$) and VP4 (mean difference =-1.501, $S.E.=0.409$, 92% CI=-2.220 to -0.782, $P<0.001$; Figure 5.5).

5.4.2.2 17-month

Two PPN and three Control bitches did not have height measurements recorded at the 17-month physical assessment. The remaining 229 bitches (119 PPN, 110 Control) measured between 51.0 and 66.0 cm in height (mean 57.6 ± 0.2 cm). There was no significant difference between PPN and Control bitches in height at the 17-month assessment (F=1.067, D.F.=1, P=0.303; mean height of PPN bitches 58.5 ± 0.2 cm and Control bitches 56.6 ± 0.3 cm; Figure 5.4). VP was the only factor significantly associated with bitch height measurement (F=7.872, D.F.=4, P<0.001). VP3 had bitches with significantly lower measured heights than all other VPs (Figure 5.5). Mean differences between VP3 and VP1 were -1.539 (S.E.=0.460, 93% CI=-2.376 to -0.702, P<0.001); VP2 were -1.666 (S.E=0.497, 93% CI=-2.571 to -0.762, P<0.001); VP4 were –2.311 (S.E.=0.475, 93% CI=-3.175 to -1.447, P<0.001); and 'Other' VP were -2.480 (S.E.=0.667, 93% CI=-3.694 to -1.266, P<0.001).

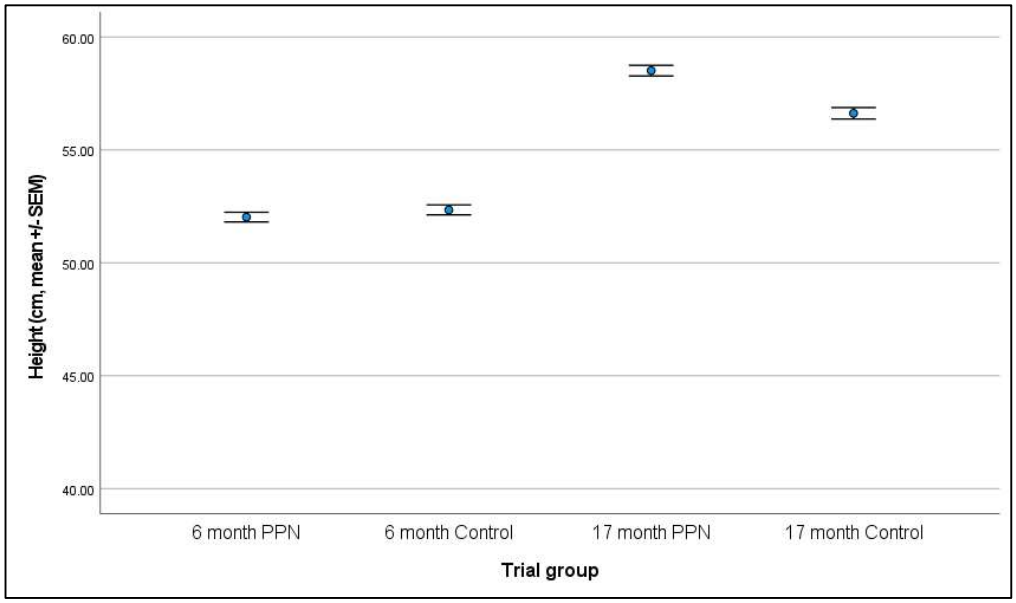


Figure 5.4. The mean (± SEM) height (cm) for bitches measured at six and 17 months of age that were neutered prepubertally (PPN) or post-pubertally (Control) in a prospective study.

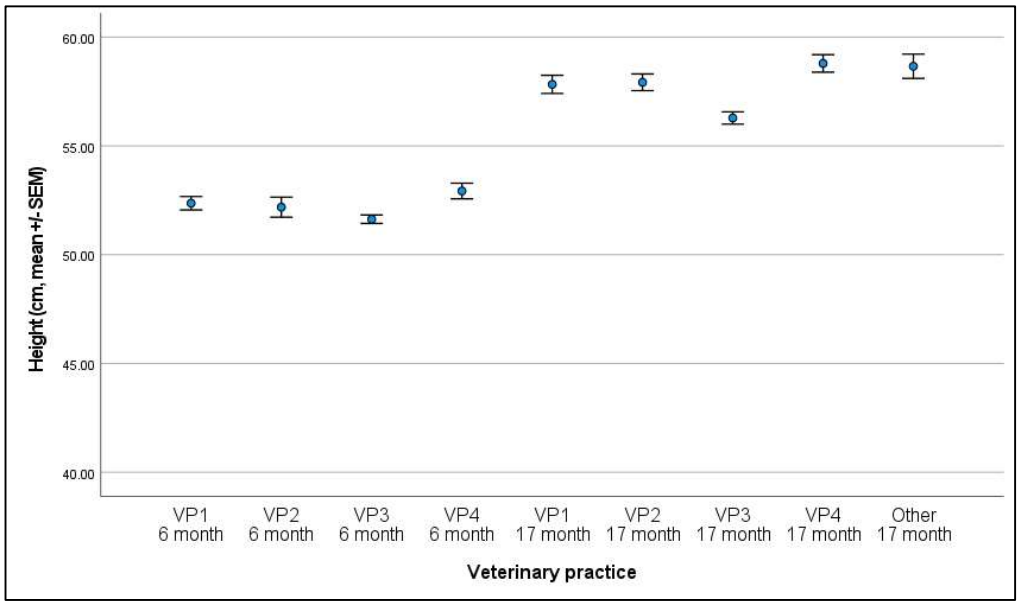


Figure 5.5. The mean (± SEM) height (cm) for bitches measured at six and 17 months of age that were measured at each veterinary practice (VP).

5.4.2.3 Change in height between six and 17-month physical assessments

Two hundred and twenty-five bitches (115 PPN, 110 Control) had height data available at six and 17-month assessments for calculation of the change in height between assessments. Mean change in height measurement for PPN bitches was 6.53 ± 0.22 cm and for Control bitches was 4.36 ± 0.25 cm ($F=37.329$, D.F.=1, $P<0.001$; Figure 5.6). PPN bitches had significantly greater change in height measurements than Controls ($\beta=2.039$, S.E.=0.334, 91% CI=1.471 to 2.608). Effect size was 0.82. The number of days between assessments ($F=5.816$, D.F.=1, $P=0.017$) and VP ($F=2.258$, D.F.=4, $P=0.064$) also significantly impacted the change in height measurement. A one day increase in days between assessments was associated with a 0.067 unit increase in change in height measurement ($\beta=0.067$, S.E.=0.028, 91% CI=0.020 to 0.115). VP3 had smaller change in height measurements than all other VPs (Figure 5.7) and these were significantly smaller than VP2 (mean difference =-1.002, S.E.=0.509, 91% CI=-1.869 to -0.135, P=0.050), VP4 (mean difference =-0.985, S.E.=0.484, 91% CI=-1.810 to -0.160, P=0.043) and for bitches that were measured

by 'different' VPs at six and 17 months of age (mean difference =-1.569, S.E.=0.604, 91% CI=-2.597 to -0.540, P=0.010).

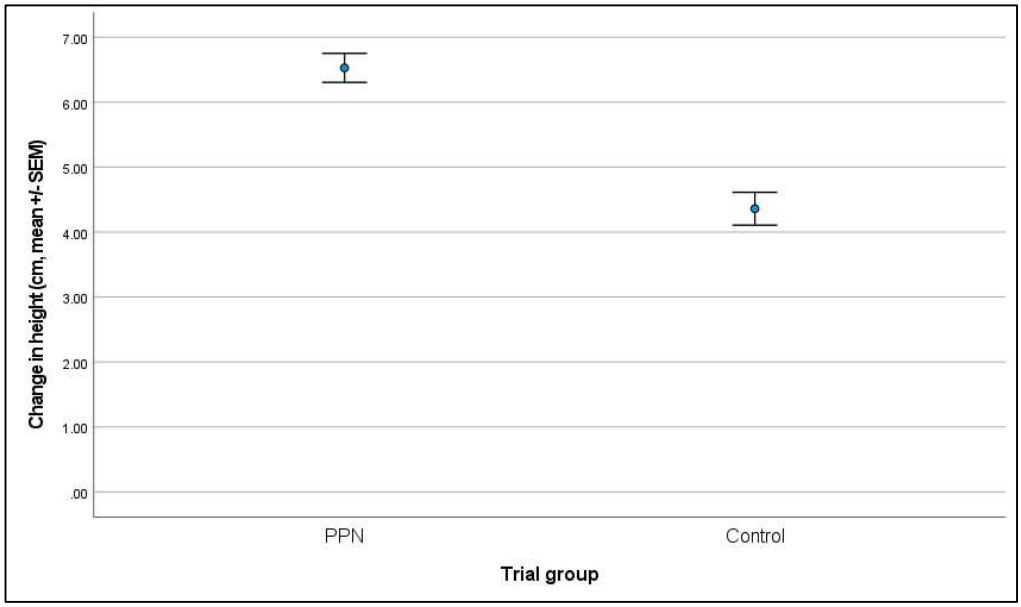


Figure 5.6. The mean (± SEM) change in height (cm) between measurements at six and 17 months of age for bitches that were neutered prepubertally or post-pubertally in a prospective study.

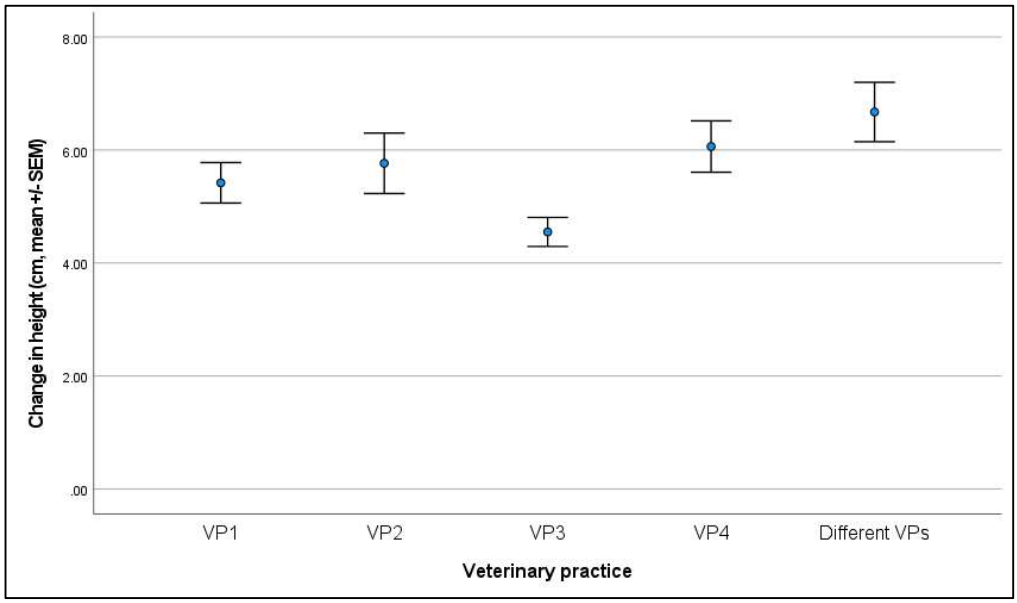


Figure 5.7. The mean (± SEM) change in height (cm) between measurements at six and 17 months of age for bitches that had physical assessments completed by each veterinary practice (VP).

5.4.3 Body weight

5.4.3.1 Six-month

Two Control bitches and one PPN bitch did not have a weight recorded at the six-month physical assessment. The remaining 300 bitches weighed between 15.0 and 27.2 kg (mean 21.0 ± 0.2 kg). Data were analysed for 279 bitches (145 PPN, 134 Control); two PPN bitches that did not have height data for inclusion as a covariate and 19 bitches with potential errors with height measurement were excluded. There was no significant difference in weight between PPN and Control bitches at six months of age ($F=0.952$, D.F.=1, $P=0.330$; mean weight of PPN bitches 21.0 ± 0.2 kg and Control bitches 21.0 ± 0.2 kg; Figure 5.8). Age at assessment ($F=17.221$, D.F.=1, $P<0.001$), height ($F=52.834$, D.F.=1, $P<0.001$) and VP ($F=2.688$, D.F.=3, $P=0.047$) were significantly associated with weight measurements. A one day increase in age was associated with a 0.11 unit increase in weight ($\beta=0.106$, S.E.=0.026, 90% CI=0.064 to 0.148). A one unit increase in height was associated with a 0.35 unit increase in weight ($\beta=0.349$, S.E.=0.048, 90% CI=0.270 to 0.429). VP2 had bitches that were significantly heavier at six months of age than all other VPs (compared to: VP1 mean difference =0.795, S.E.=0.387, 90% CI=0.155 to 1.434, $P=0.041$, VP3 mean difference =0.808, S.E. =0.352, 90% CI=0.227 to 1.388, $P=0.022$, VP4 mean difference =1.055, S.E.=0.392, 90% CI=0.407 to 1.702, $P=0.008$, Figure 5.9).

5.4.3.2 17-month

All 253 bitches with completed 17-month assessment forms had body weight data recorded. Bitches weighed between 21.7 and 33.5 kg (mean 27.80 ± 0.1 kg). Five bitches with no height measurement (required as a covariate) and 19 bitches with potentially incorrect height measurements were excluded from analysis. Data were analysed for 229 bitches (119 PPN, 110 Control). There was no significant difference in weight between PPN and Control bitches at 17 months of age ($F=1.289$, D.F.=1,

P=0.258; mean weight of PPN bitches 28.3 ± 0.2 kg and Control bitches 27.3 ± 0.2 kg, Figure 5.8). Height (F=39.050, D.F.=1, P<0.001) and VP (F=2.747, D.F.=4, P=0.029) were significantly associated with body weight at 17 months of age. A one unit increase in height was associated with a 0.35 unit increase in weight (β=0.345, S.E.=0.055, 91% CI=0.251 to 0.440). Body weight measurements by 'Other' VP were significantly lower than measurements by all four VPs. Mean differences between 'Other' and: VP1 were -1.758 (S.E.=0.566, 91% CI=-2.721 to -0.795, P=0.002); VP2 were -1.328 (S.E=0.579, 91% CI=-2.314 to -0.342, P=0.023); VP3 were –1.733 (S.E.=0.562, 91% CI=-2.691 to -0.775, P=0.002); and VP4 were -1.399 (S.E.=0.571, 91% CI=-2.370 to -0.427, P=0.015, Figure 5.9).

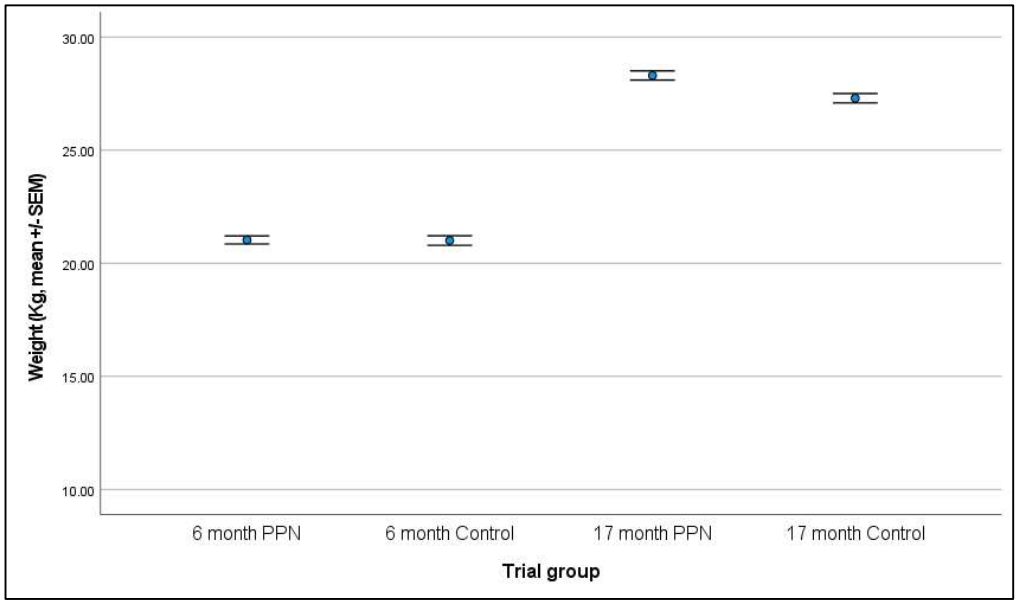


Figure 5.8. The mean (± SEM) body weight (kg) for bitches measured at six and 17 months of age that were neutered prepubertally (PPN) or post-pubertally (Control) in a prospective study.

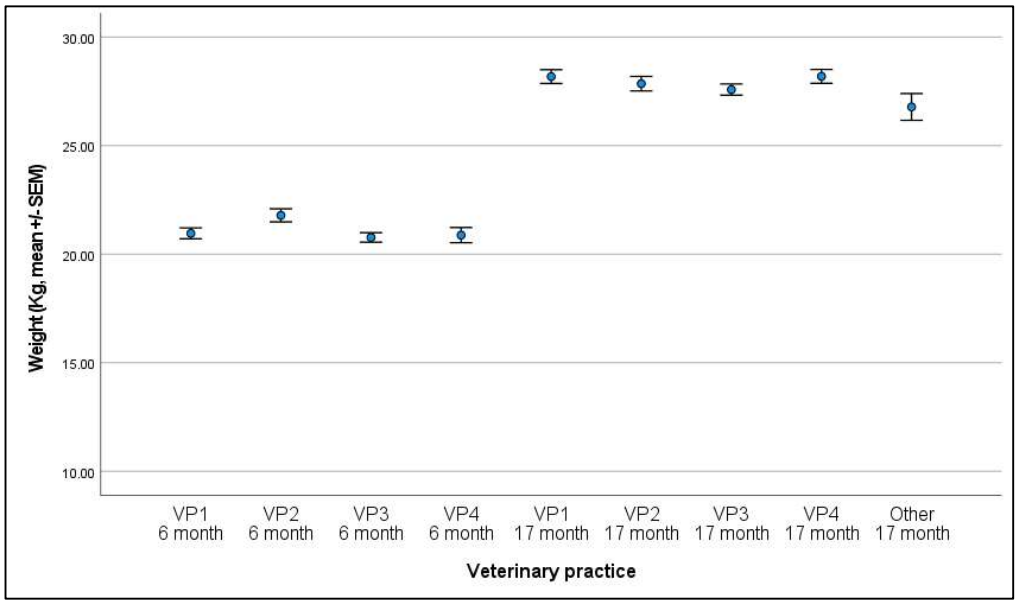


Figure 5.9. The mean (± SEM) body weight (kg) for bitches measured at six and 17 months of age that were neutered at each veterinary practice (VP).

5.4.3.3 Change in body weight between six and 17-month physical assessments

Two hundred and forty-eight bitches (122 PPN, 126 Control) had weight data available at six and 17 months of age for calculation of the change in weight between assessments. Mean change in weight for PPN bitches was 7.2 ± 0.2 kg and for Control bitches was 6.2 ± 0.2 kg. Bitches with potentially incorrect (n=19) or missing height measurements (n=7) were excluded from further analysis. Change in weight data were examined in a general linear model for 114 PPN (mean change = 7.2 ± 0.2 kg) and 108 Control bitches (mean change = 6.3 ± 0.2 kg). Trial group was not associated with change in weight measurements (F=2.450, D.F.=1, P=0.119). Breed was not included in the general linear model due to poor model fit.

The number of days between assessments (F=9.126, D.F.=1, P=0.003), change in height (F=8.262, D.F.=1, P=0.04) and VP (F=2.640, D.F.=4, P=0.035) were significantly associated with change in body weight. A one day increase in days between assessments was associated with a 0.077 unit increase in change in weight (β=0.077, S.E.=0.025, 91% CI=0.034 to 0.120). A one cm

increase in change in height was associated with a 0.177 unit increase in change in weight (β=0.177, S.E.=0.062, 91% CI=0.072 to 0.282). VP2 had smaller change in weight measurements than all other VPs (Figure 5.10) and these were significantly smaller than VP1 (mean difference =-1.350, S.E.=0.479, 91% CI=-2.166 to -0.534, P=0.005), VP3 (mean difference =-1.078, S.E.=0.454, 91% CI=-1.851 to -0.304, P=0.019) and VP4 (mean difference =-1.228, S.E.=0.492, 91% CI=-2.067 to -0.390, P=0.013).

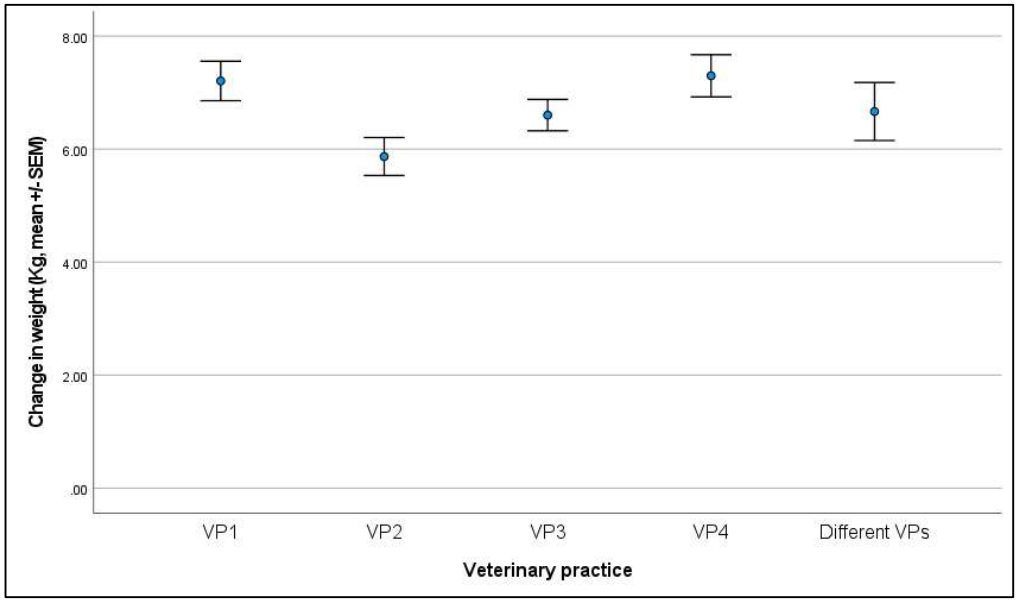


Figure 5.10. The mean (± SEM) change in body weight (kg) between measurements at six and 17 months of age for bitches that had physical assessments completed by each veterinary practice (VP).

5.4.4 Body condition score

5.4.4.1 Six-month

Two Control bitches did not have a BCS reported at their six-month physical assessment. For the remaining 301 bitches (152 PPN, 149 Control), BCS ranged from 3 to 6 (median = 4; Table 5.1).

Table 5.1. The number of bitches within a prospective study examining the differences in physical development for bitches neutered before (PPN) or after (Control) puberty that were each body condition score (BCS) at six months of age. The median BCS for each group was 4.

Variable	BCS 3	BCS 4	BCS 5	BCS 6
Breed				
Golden Retriever cross Labrador (n=200)	2	149	46	3
Backcrosses (n=58)	0	34	17	7
Labrador cross Golden Retriever (n=43)	0	31	12	0
Veterinary practice				
1 (n=65)	1	48	16	0
2 (n=52)	0	6	39	7
3 (n=118)	0	103	12	3
4 (n=66)	1	57	8	0
Trial group				
PPN (n=152)	1	106	44	1
Control (n=149)	1	108	31	9

n=number of bitches

Examination of BCS data using a binary logistic regression included data for 297 bitches; two bitches (1 PPN, 1 Control) that did not have weight data for inclusion as a covariate and the two bitches that were BCS 3 (1 PPN, 1 Control) were excluded. The model was significant (D.F.=4, Chi-square=134.135, P<0.001). There was no significant association between trial group and BCS. Body weight (OR=1.453, 96% CI=1.229 to 1.718, P<0.001) and VP influenced whether BCS were ideal (BCS 4) or overweight (BCS >4) and were retained in the final model. BCS reported by VP2 were significantly more likely to be reported as higher than ideal than for all other VPs. BCS were also significantly more likely to be reported as higher than ideal for VP1 than VP3 and VP4 (Table 5.2, Figure 5.11). The AUC was 0.873. The equation for the best fit model was:

$$Pred(BCS) = 1/(1 + exp(-(-9.045 + 0.374 \times Weight + 3.167 \times VP2 - 0.892 \times VP3 - 1.276 \times CVP4)))$$

Where BCS = body condition score, VP = veterinary practice (VP1 being the reference practice)

Table 5.2. The odds ratio (OR) and confidence intervals for each predictor variable category for the influence on body condition score at six months of age in comparison to the reference category veterinary practice (VP), showing comparisons with VP1 and VP2 as the reference categories (*).

Variable	Value	S.E.	Wald Chi-Square	Pr > Chi²	OR	OR Lower bound (96%)	OR Upper bound (96%)
Intercept	-9.045	1.800	25.258	<0.0001			
Body weight (kg)	0.374	0.082	20.955	<0.0001	1.453	1.229	1.718
Compared to VP1							
*VP1	0.000	0.000					
VP2	3.167	0.550	33.185	<0.0001	23.737	7.675	73.416
VP3	-0.892	0.420	4.499	0.034	0.410	0.173	0.972
VP4	-1.276	0.529	5.817	0.016	0.279	0.094	0.827
Compared to VP2							
VP1	-3.167	0.550	33.185	<0.0001	0.042	0.014	0.130
*VP2	0.000	0.000					
VP3	-4.059	0.550	54.529	<0.0001	0.017	0.006	0.053
VP4	-4.443	0.644	47.582	<0.0001	0.012	0.003	0.044

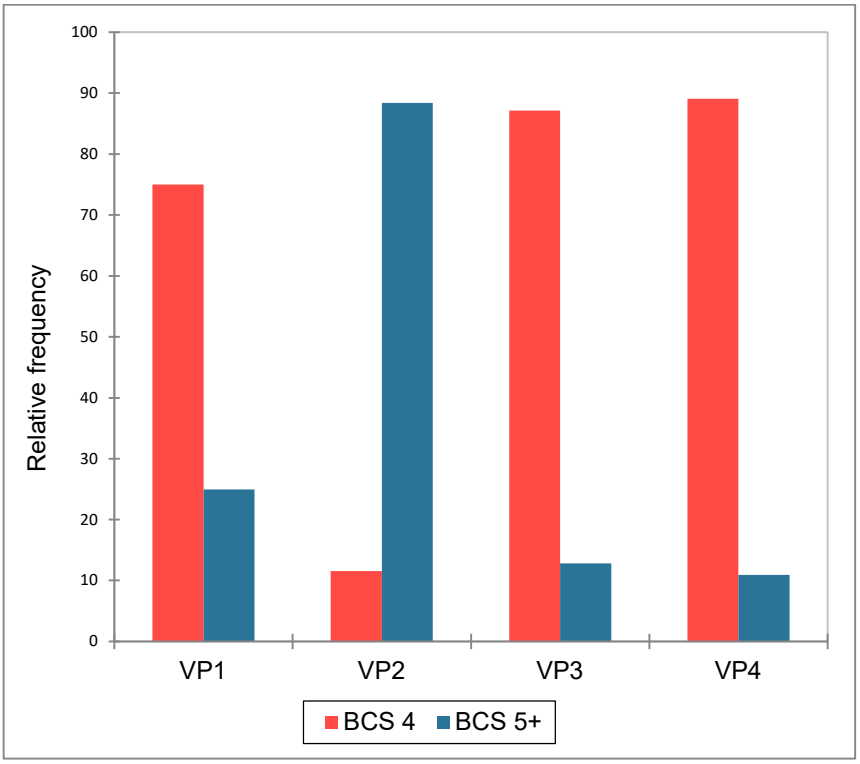


Figure 5.11. The relative frequency of BCS 4 and 5 or more (5+) reported by each veterinary practice (VP) for bitches at six months of age.

5.4.4.2 17-month

One Control bitch did not have a BCS reported at the 17-month physical assessment. For the remaining 252 bitches, BCS at 17 months of age ranged from 4 to 6 (median = 5; Table 5.3).

Table 5.3. The number bitches within a prospective study examining the differences in physical development for bitches neutered before (PPN) or after (Control) puberty that were each body condition score (BCS) at 17 months of age. The median BCS for each group was 5.

Variable	BCS 4	BCS 5	BCS 6
Breed			
Golden Retriever cross Labrador (n=171)	6	149	16
Backcrosses (n=49)	0	39	10
Labrador cross Golden Retriever (n=32)	2	25	5
Veterinary practice			
1 (n=52)	4	43	5
2 (n=44)	0	29	15
3 (n=87)	1	78	8
4 (n=50)	0	49	1
Other (n=19)	3	14	2
Trial group			
PPN (n=125)	4	105	16
Control (n=127)	4	108	15

n=number of bitches

Examination of BCS data used a binary logistic regression including data for 252 bitches. The model was significant (D.F.=5, Chi-square=30.113, P<0.001). There was no significant association between trial group and BCS. Body weight (OR=0.807, 96% CI=0.680 to 0.957) and VP influenced whether BCS were ideal (BCS 5) or under/overweight (BCS 4 or 6) and were retained in the final model. BCS reported by VP2 were significantly less likely to be ideal (BCS5) than those for VP1, VP3 and VP4. BCS reported by VP4 were significantly more likely to be ideal than those for VP1, VP2 and 'Other' (Table 5.4, Figure 5.12). The AUC was 0.771. The equation for the best fit model was:

$$Pred(BCS) = 1/(1 + exp\big(-(7.685 - 0.215 \times Weight - 1.030 \times VP2 + 0.475 \times VP3 + 2.346 \times VP4 - 0.773 - Other)\big))$$

Where BCS = body condition score, VP = veterinary practice (VP1 being the reference practice)

Table 5.4. The odds ratio (OR) and confidence intervals for each predictor variable category for the influence on body condition score at 17 months of age in comparison to the reference category veterinary practice (VP), showing comparisons with VP2 and VP4 as the reference categories (*).

Variable	Value	S.E.	Wald Chi-Square	Pr > Chi²	OR	OR Lower bound (96%)	OR Upper bound (96%)
Intercept	7.685	2.465	9.716	0.002			
Body weight (kg)	-0.215	0.083	6.671	0.010	0.807	0.680	0.957
Compared to VP2							
VP1	1.030	0.500	4.247	0.039	2.800	1.004	7.813
*VP2	0.000	0.000					
VP3	1.505	0.483	9.701	0.002	4.502	1.669	12.142
VP4	3.376	1.067	10.016	0.002	29.256	3.271	261.640
Other	0.257	0.630	0.166	0.684	1.293	0.355	4.711
Compared to V4							
VP1	-2.346	1.079	4.729	0.030	0.096	0.010	0.878
VP2	-3.376	1.067	10.016	0.002	0.034	0.004	0.306
VP3	-1.872	1.075	3.032	0.082	0.154	0.017	1.399
*VP4	0.000	0.000					
Other	-3.119	1.152	7.332	0.007	0.044	0.004	0.471

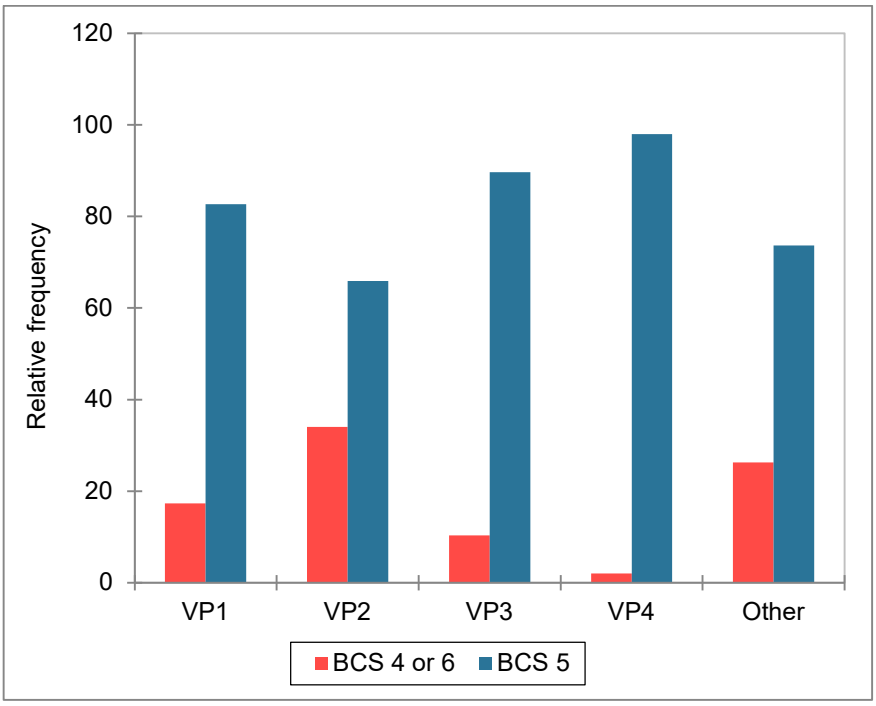


Figure 5.12. The relative frequency of BCS 4 or 6 and BCS 5 reported by each veterinary practice (VP) for bitches at 17 months of age.

5.4.5 Vulval size

5.4.5.1 Six-month

Vulval measurements were not reported for one PPN bitch. Six bitches (1 PPN, 5 Control) were reported to have a 'swollen' vulva appearance at their six-month physical assessment and were excluded from subsequent analysis for vulval size. For the remaining 296 bitches (150 PPN, 146 Control), vulval length ranged from 1.5 to 4.2 cm (mean 2.8 ± 0.03 cm, Figure 5.13A) and vulval width ranged from 0.8 to 3.0 cm (mean 1.8 ± 0.02 cm, Figure 5.13B). Bitches with no measurements for height (n=1) and weight (n=3), and bitches with potential errors in height measurements (n=19) were excluded from univariate models.

For the remaining 273 bitches (144 PPN, 129 Control), mean vulval lengths were 2.8 ± 0.04 cm for PPN and Control bitches and mean vulval widths were 1.8 ± 0.03 cm for both groups. In the model controlling for a height*weight interaction, there was no significant association between trial group and vulval length (F=0.394, D.F.=1, P=0.531) or width (F=2.769, D.F.=1, P=0.097) measurements.

Vulval length was affected by breed (F=3.808, D.F.=2, P=0.023), with backcross bitches having significantly smaller vulva lengths than Labrador cross Golden Retriever bitches (mean difference =-0.261, S.E.=0.097, 94% CI=-0.488 to -0.035, P=0.022). Vulval width was affected by age at assessment (F=7.794, D.F.=1, P=0.006). A one day increase in age was associated with a 0.01 unit increase in vulval width (β=0.012, S.E.=0.004, 94% CI=0.004 to 0.020). No other significant differences were identified.

5.4.5.2 17-month

Vulval measurements were not reported for six bitches (3 PPN, 3 Control) at their 17-month physical assessment. For the remaining 247 bitches (122 PPN, 125 Control), vulval length ranged from 1.0 to 4.4 cm (mean 3.0 ± 0.03 cm; Figure 5.13a) and vulval width ranged from 0.6 to 3.5 cm (mean 1.9 ± 0.03 cm Figure 5.13b). Bitches with no measurements for height (n=1) and with potential errors in height measurements (n=19) were excluded from univariate models.

For the remaining 227 bitches (118 PPN, 109 Control), mean vulval lengths were 2.8 ± 0.05 cm for PPN and 3.2 ± 0.04 cm for Control bitches and mean vulval widths were 1.7 ± 0.03 cm for PPN and 2.1 ± 0.03 cm for Control bitches.

In the model controlling for a height*weight interaction, there was no significant association between trial group and vulval length (F=0.011, D.F.=1, P=0.916) or width (F=0.175, D.F.=1, P=0.676) measurements. Vulval length (F=5.216, D.F.=4, P<0.001) and width (F=8.271, D.F.=4, P<0.001) were affected by VP. Measurements of vulval length were larger from VP1 (mean difference =0.291, S.E.=0.093, 92% CI=0.041 to 0.541, P=0.021) and VP2 (mean difference =0.398, S.E.=0.099, 92% CI=0.132 to 0.664, P<0.001) than VP 4. Measurements of vulval length were also larger from VP2 than VP3 (mean difference =0.255,

S.E.=0.092, 92% CI=0.010 to 0.500, P=0.058). Measurements of vulval width were larger from VP2 than VP1 (mean difference =0.338, S.E.=0.069, 92% CI=0.154 to 0.522, P<0.001), VP4 (mean difference =0.268, S.E.=0.070, 92% CI=0.081 to 0.456, P=0.002) and 'Other' VP (mean difference =0.289, S.E.=0.093, 92% CI=0.040 to 0.537, P=0.021). Measurements from VP3 were larger than from VP1 (mean difference =0.232, S.E.=0.060, 92% CI=0.071 to 0.393, P=0.001). No other significant differences were identified.

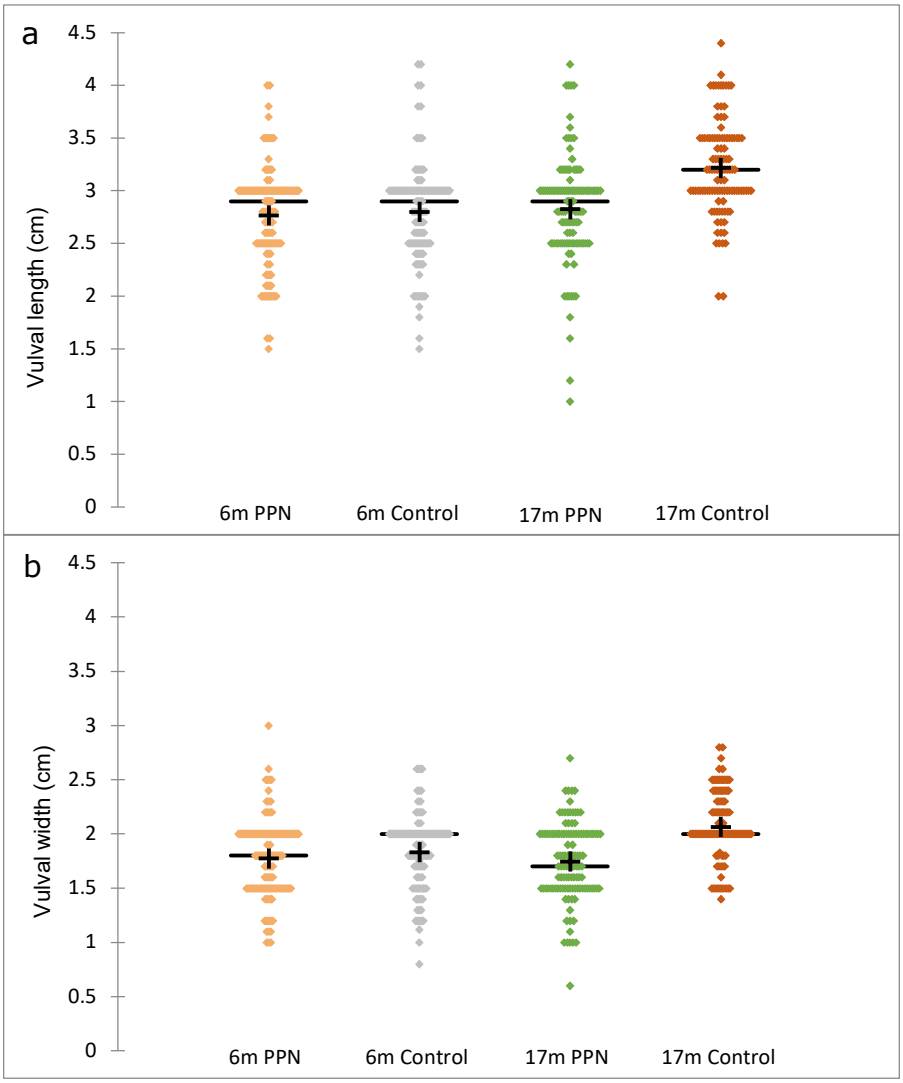


Figure 5.13. The distribution of vulval length (a) and vulval width (b) measurements at six and 17 months of age in a prospective study examining the effect of neutering bitches before (PPN, n=118) or after (Control, n=109) puberty. Graphs show the mean (+) and median (straight line).

5.4.5.3 Change in vulval size between six and 17-month physical assessments

Two hundred and forty-four bitches (119 PPN, 125 Control) had vulval length and width measurement data available at six and 17 months of age. Mean change in vulval length and width for PPN bitches was 0.08 ± 0.05 cm and -0.05 ± 0.04 cm respectively. Mean change in vulval length and width for Control bitches was 0.39 ± 0.05 cm and 0.19 ± 0.04 cm respectively (Table 5.5). Significantly more PPN than Control bitch vulvas changed to be smaller and fewer changed to be larger in length (Chi-square=22.334, D.F.=2, P<0.001) and width (Chi-square=20.131, D.F.=2, P<0.001) between six and 17 months of age (Figure 5.14).

Table 5.5. The minimum, maximum, mean (± SEM) change in vulval length and width measurements (cm) between six and 17 months of age for bitches in a prospective study examining differences in physical development for bitches neutered before (PPN) or after (Control) puberty.

	Change in vulval length (cm)		Change in vulval width (cm)	
Statistic	PPN n=119	Control n=125	PPN n=119	Control n=125
Mean ± SEM	0.08 ± 0.05	0.39 ± 0.05	-0.05 ± 0.04	0.19 ± 0.04
Range	-1.4 to 1.7	-1.0 to 1.9	-1.6 to 1.5	-1.0 to 1.3

n=number of bitches

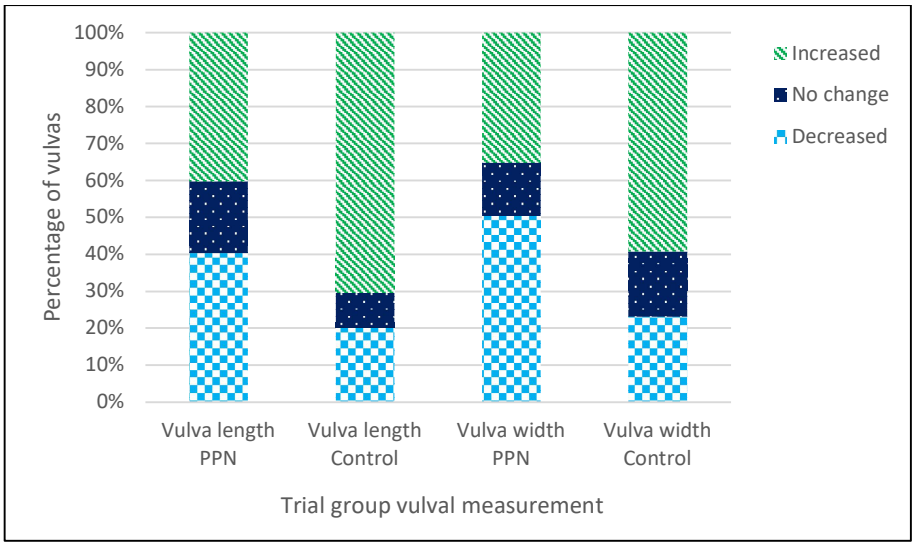


Figure 5.14. The percentage of bitches in a prospective study examining the effect of neutering before (PPN, n=119) or after (Control, n=125) puberty that had vulval length and width measurements that either increased, decreased, or did not change between the six- and 17-month physical assessments.

Bitches with potentially incorrect or missing height (n=19) or weight (n=5) measurements were excluded from univariate models. Changes in vulval length and width data were examined for 113 PPN (mean change vulval length = 0.08 ± 0.06 cm, mean change vulval width = -0.05 ± 0.04 cm) and 107 Control bitches (mean change vulval length = 0.41 ± 0.05 cm, mean change vulval width = 0.20 ± 0.04 cm). In the model controlling for a height*weight interaction, mean change in vulval length ($F=22.901$, D.F.=1, $P<0.001$) and width ($F=12.239$, D.F.=1, $P<0.001$) were significantly affected by trial group. Effect sizes were -0.53 for both measurements. Changes in both measurements were also affected by VP (vulval length $F=8.005$, D.F.=4, $P<0.001$; vulval width $F=6.932$, D.F.=4, $P<0.001$).

Changes in measurements of vulval length and width were smaller for PPN than for Control bitches (vulval length: mean difference =-0.377, S.E.=0.079, 91% CI=-0.511 to -0.243, $P<0.001$; vulval width: mean difference =-0.221, S.E.=0.063, 91% CI=-0.328 to -0.113, $P<0.001$). Changes in measurements of vulval length were smaller for VP4 than for VP1 (mean difference =-0.397, S.E.=0.112, 91% CI=-0.693 to -0.102, $P=0.005$), VP2 (mean difference =-0.676, S.E.=0.122, 91% CI=-0.997 to -0.354, $P<0.001$), and VP3 (mean difference =-0.303, S.E.=0.108, 91% CI=-0.588 to -0.019, $P=0.054$), and were smaller for VP3 than VP2 (mean difference =-0.373, S.E.=0.111, 91% CI=-0.666 to -0.079, $P=0.010$). Change in vulval width measurements were smaller for VP4 than for VP2 (mean difference =-0.468, S.E.=0.098, 91% CI=-0.726 to -0.211, $P<0.001$), and VP3 (mean difference =-0.304, S.E.=0.086, 91% CI=-0.531 to -0.076, $P=0.005$), and were smaller for VP1 than VP2 (mean difference =-0.353, S.E.=0.095, 91% CI=-0.603 to -0.103, $P=0.003$; Figure 5.15). No other significant differences were identified.

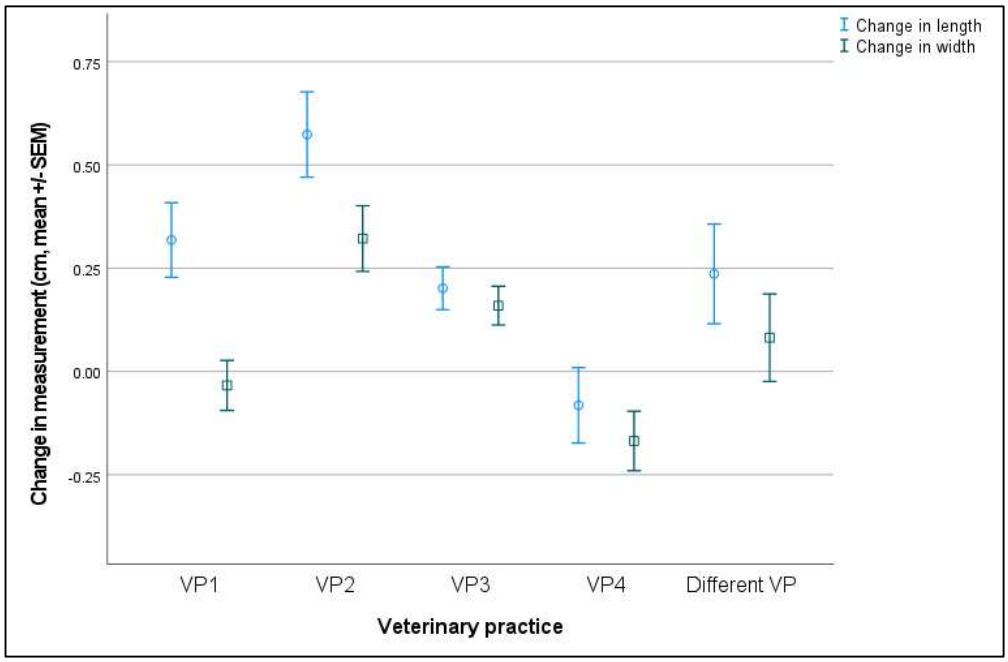


Figure 5.15. The mean (± SEM) change in vulval length and with measurements (cm) between six and 17 months of age for bitches that had physical assessments completed by each veterinary practice (VP).

5.4.6 Vulval appearance

5.4.6.1 Vulval appearance at the physical assessments

Data from six-month assessment forms were available for 303 bitches to examine the appearance of the vulva. The number of PPN and Control bitches that were reported to have each anomaly of vulval appearance at the six-month physical assessment were not significantly different following sequential Bonferroni correction (Table 5.6). Proportionally more Control bitches were reported to have a swollen vulva, vaginal discharge, a recessed or inverted vulva, prominent perivulval skin folds and perivulval dermatitis. There was no difference in the number of PPN and Control bitches with a cumulative vulva score of 0, 1, or 2 and 3 at the six-month assessment (Chi-square=3.933, D.F.=2, P=0.140). No bitch had more than three vulval anomalies noted (Figure 5.16a).

Data from 17-month assessment forms were available for 253 bitches (125 PPN, 128 Control) to examine the appearance of the vulva. Following sequential Bonferroni correction, significantly more PPN than Control bitches were reported to have vulvas that

appeared juvenile (Yates' Chi-square=14.834, D.F.=1, P<0.001) and recessed (Yates' Chi-square=7.792, D.F.=1, P=0.005) at the 17-month physical assessment (Table 5.6). No bitches from either trial group had vulvas that were reported to be swollen and there was no significant difference in the number of bitches reported to have discharge or perivulval folds. Analysis for perivulval dermatitis was not possible due to small numbers of bitches affected. Significantly more PPN than Control bitches had a cumulative vulva score of 1, or 2 and 3 at the 17-month assessment (Chi-square=13.773, D.F.=2, P=0.001). No bitch had more than three vulval anomalies noted (Figure 5.16b).

Table 5.6. The frequency of observations of anomalies in vulval appearance recorded by veterinarians at the six-month (n=303: 152 PPN, 151 Control) and 17-month (n=253: 125 PPN, 128 Control) physical assessments for bitches that were neutered before (PPN) or after (Control) puberty within a prospective study examining differences in physical development. The results for Chi-square tests are presented, significant results following sequential Bonferroni correction are highlighted in bold.

Reported anomaly in vulval appearance	Six-month assessment Observed % (n)	Chi-square (P value)	17-month assessment Observed % (n)	Chi-square (P value)
Swollen vulva				
PPN group	0.7 (1)	1.551* (0.213)	0.0 (0)	Analysis not possible
Control group	3.3 (5)		0.0 (0)	
Vaginal discharge				
PPN group	9.2 (14)	4.867 (0.027)	4.8 (6)	0.130* (0.718)
Control group	17.9 (27)		3.1 (4)	
Juvenile				
PPN group	15.1 (23)	1.389 (0.238)	16.0 (20)	**14.834* (<0.001)**
Control group	10.6 (16)		1.6 (2)	
Recessed/inverted				
PPN group	14.5 (22)	0.428 (0.513)	13.6 (17)	**7.792* (0.005)**
Control group	17.2 (26)		3.1 (4)	
Prominent perivulval skin folds				
PPN group	3.3 (5)	1.788 (0.181)	4.8 (6)	1.237 (0.266)
Control group	6.6 (10)		1.6 (2)	
Perivulval dermatitis				
PPN group	0.7 (1)	6.092* (0.014)	0.0 (0)	Analysis not possible
Control group	6.6 (10)		1.6 (2)	

*Chi-square with Yates' continuity correction. n=number of bitches

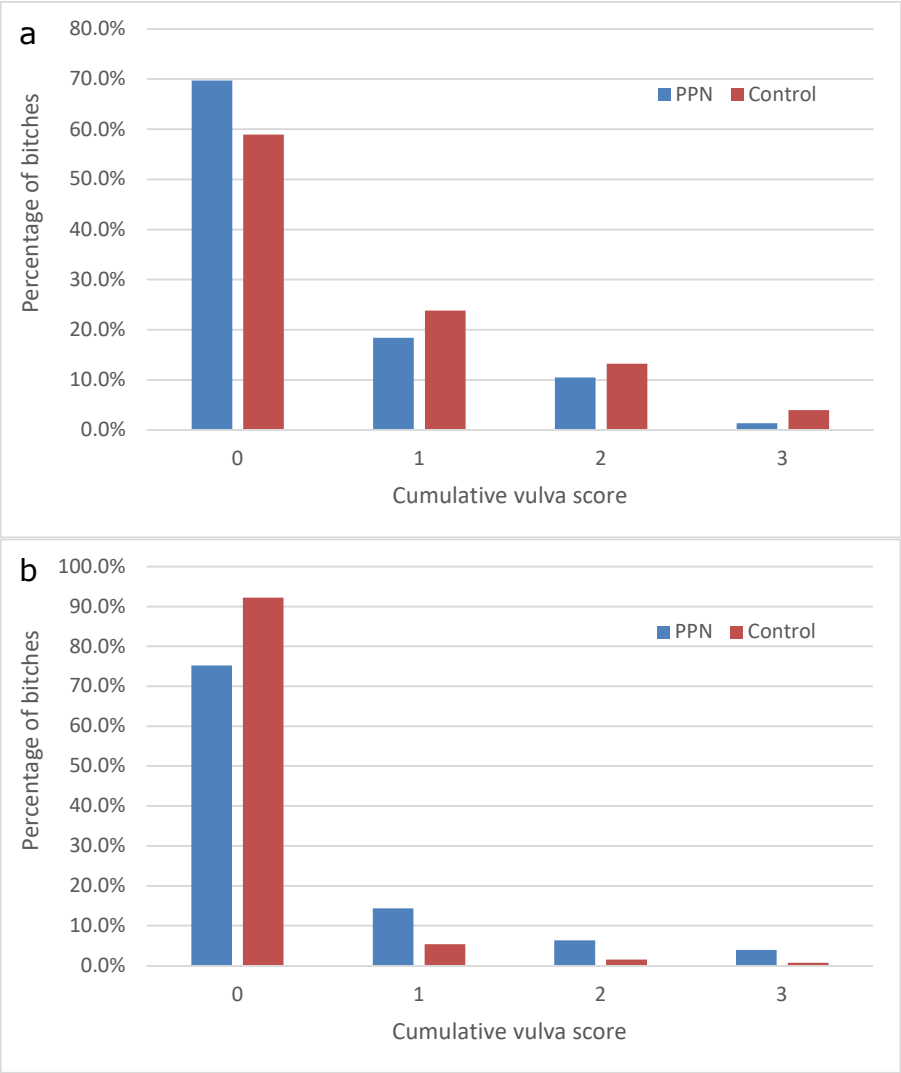


Figure 5.16. Cumulative vulva scores at six-month (a; 152 PPN, 151 Control) and 17-month assessments (b; 125 PPN, 128 Control) for bitches neutered before (PPN) or after (Control) puberty during a prospective study examining differences in physical development.

5.4.6.2 Vulval appearance from examination of digital images

Digital vulval images were captured for 274 bitches (134 PPN, 140 Control) at six-month assessments and for 270 bitches (137 PPN, 133 Control) at 17-month assessments. Some images were not of suitable quality to be considered diagnostic; the numbers examined for each anomaly in appearance are shown in Table 5.7. At six months of age, there were no significant differences between PPN and Control bitches in the number of images that had vulval discharge present, each category of vulva discharge present (excluding 'haemorrhagic' due to small n), estimated % dorsal fold

coverage grouped as less than 20%, 30%, 40% or greater than 50%, perivulval skin changes present, the nature of perivulval skin changes present (excluding 'hair changes only' due to small n) or 'recessed/ inverted' appearance (Table 5.7). Statistical analysis for the presence/absence of dorsal skin folds was not possible due to small numbers of bitches (1 PPN, 0 Control) with no dorsal fold present.

Seventy-two PPN (82.8%) and 71 Control (78.0%) bitches had vulval images that were classified as abnormal based on being recessed/inverted at six months of age. At 17-month assessments, significantly more PPN (71.1%, n=54) than Control bitches (46.3%, n=38) were reported to have vulvas that were 'recessed/inverted' in appearance (Chi-square=9.902, D.F.=1, P=0.002; Figure 5.17). Ninety-six bitches (46 PPN, 50 Control) had vulval images at both the six and 17-month assessments that were sufficient quality to classify bitches as 'recessed/inverted' or 'normal'. Ten PPN and 10 Control vulvas were 'normal' at six months. Of these, 3 PPN and 8 Control vulvas remained 'normal' at 17 months of age (Yates' Chi-square=3.232, D.F.=1, P=0.072). Thirty-six PPN and 40 Control bitch vulvas were 'recessed/inverted' at six months of age. Of these, 10 PPN and 17 Control changed to 'normal' by 17 months of age (Chi-square=1.793, D.F.=1, P=0.181). These differences were not statistically significant.

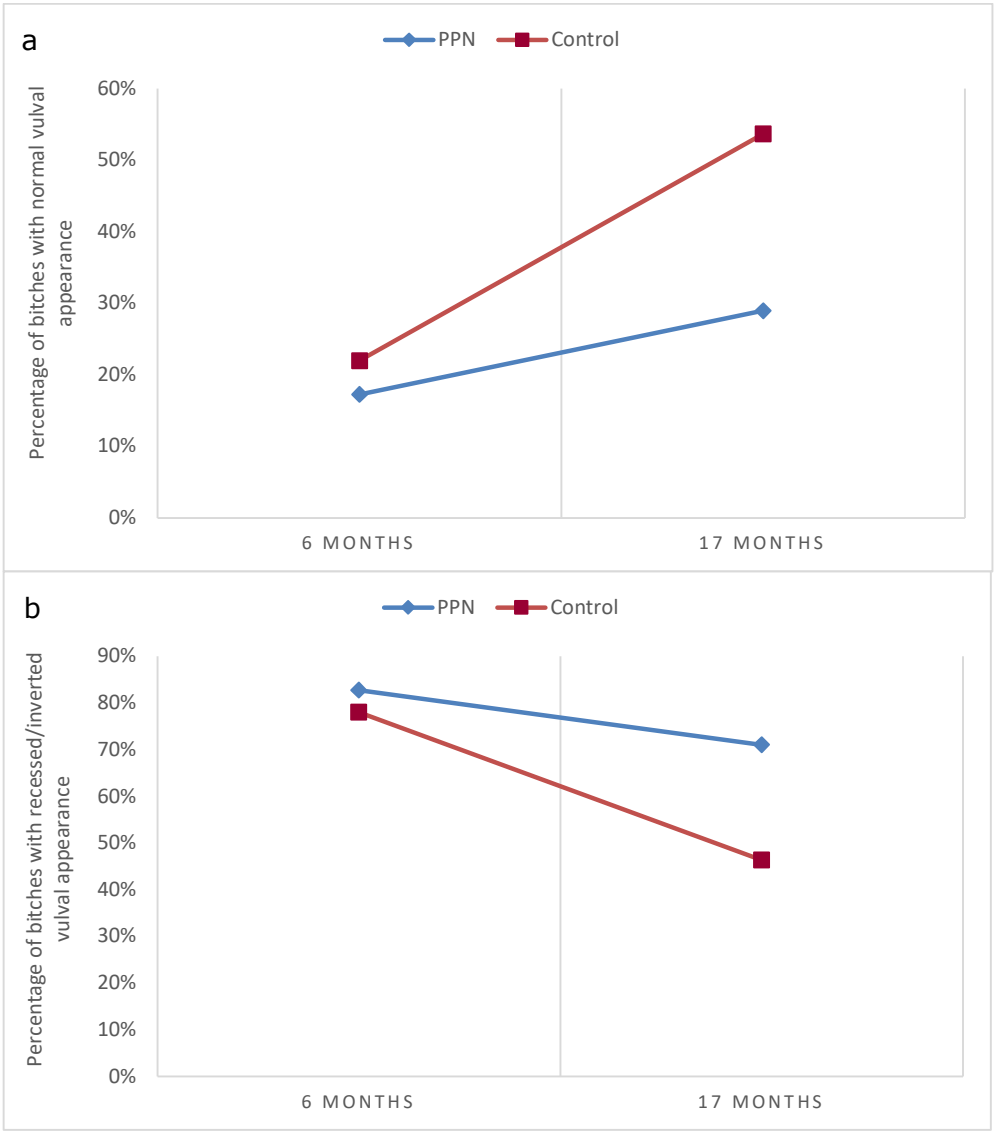


Figure 5.17. The percentage of bitches neutered before (PPN) or after (Control) puberty in a prospective study examining differences in physical development that had vulval images that were categorised as (a) normal or (b) recessed/inverted in appearance at six and at 17 months of age.

There was a significant difference between PPN and Control bitches in the number that had skin discolouration, and skin and hair discolouration noted on digital images at 17 months of age (Chi-square=5.563, D.F.=1, P=0.018). There were no significant differences between PPN and Control bitches in the number of images that had vulval discharge present, each category of vulva discharge present (excluding 'haemorrhagic' due to small n), estimated % dorsal fold coverage grouped as less than 20%, 30%,

40% or greater than 50%, or perivulval skin changes present (Table 5.7).

Table 5.7. The frequency of observations of anomalies in vulval appearance based on assessment of digital images of the vulva captured at the six- and 17-month physical assessment for bitches that were neutered before (PPN) or after (Control) within a prospective study examining differences in physical development. The results for Chi-square tests are presented, significant results following sequential Bonferroni correction are highlighted in bold.

Reported anomaly in vulval appearance	Six-month PPN % (n)	Six-month Control % (n)	Chi-square (P value)	17-month PPN % (n)	17-month Control % (n)	Chi-square (P value)
Vulval discharge						
Present on image	49.3 (36)	55.0 (44)	0.494 (0.482)	28.8 (23)	22.6 (21)	0.863 (0.353)
Not present	50.7 (37)	45.0 (36)		71.3 (57)	77.4 (72)	
Vulval discharge categorisation						
Mucoid	13.7 (10)	7.5 (6)	2.541 (0.281)*	8.8 (7)	6.5 (6)	4.398 (0.222)*
Mucoid purulent	12.3 (9)	18.8 (15)		6.3 (5)	9.8 (9)	
Purulent	23.3 (17)	27.5 (22)		13.8 (11)	5.4 (5)	
Haemorrhagic	0.0 (0)	1.3 (1)		0.0 (0)	0.0 (0)	
Dorsal skin folds						
Present on image	98.9 (88)	100.0 (98)	Analysis not possible	97.8 (89)	99.0 (95)	0.002 (0.963)†
Not present	1.1 (1)	0.0 (0)		2.2 (2)	1.0 (1)	
Estimated dorsal skin fold coverage						
0%	1.2 (1)	0.0 (0)	2.500 (0.475)**	2.6 (2)	0.0 (0)	4.089 (0.252)**
10%	1.2 (1)	3.3 (3)		7.8 (6)	10.2 (9)	
20%	26.7 (23)	21.7 (20)		32.5 (25)	35.2 (31)	
30%	24.4 (21)	34.8 (32)		28.6 (22)	37.5 (33)	
40%	29.1 (25)	27.2 (25)		19.5 (15)	13.6 (12)	
50%	16.3 (14)	10.9 (10)		9.1 (7)	3.4 (3)	
60%	1.2 (1)	1.1 (1)		0.0 (0)	0.0 (0)	
70%	0.0 (0)	1.1 (1)		0.0 (0)	0.0 (0)	
Recessed/ inverted appearance						
Present on image	82.8 (72)	78.0 (71)	0.632 (0.427)	71.1 (54)	46.3 (38)	**9.902 (0.002)**
Not present	17.2 (15)	22.0 (20)		28.9 (22)	53.7 (44)	
Perivulval skin changes						
Present on image	98.2 (112)	99.1 (109)		96.4 (107)	99.2 (118)	

Not present	1.8 (2)	0.9 (1)	0.001 (0.975)†	3.6 (4)	0.8 (1)	0.967 (0.325)†
Nature of perivulval changes						
Skin discolouration	28.8 (32)	31.8 (34)	0.187 (0.666)‡	23.4 (26)	38.7 (46)	**5.563 (0.018)**‡
Hair discolouration	2.7 (3)	2.8 (3)		0.9 (1)	0.8 (1)	
Skin and hair discolouration	69.4 (77)	67.3 (72)		72.1 (80)	59.7 (71)	

* Excluding haemorrhagic. **Dorsal fold coverage grouped as less than 20%, 30%, 40% or greater than 50%. †Chi-square with Yates' continuity correction. ‡ Excluding 'hair discolouration'.
n=number of bitches

5.5 DISCUSSION

The impact of neutering bitches before or after known puberty on physical development has not been well studied. As well as impacts on health and behaviour, physical development in bitches is an important consideration when making decisions regarding neutering timing due to potential consequences for future disease risk. This is the first prospective study investigating the associations between prepubertal or post-pubertal neutering and physical development in a large number of bitches.

The results showed that bitches neutered before puberty had significantly greater change in height measurements and smaller change in measurements of vulval size between six and 17 months of age than those neutered post-pubertally. Findings related to vulva appearance recorded on physical assessments and from examination of digital images of the vulva showed that significantly more bitches neutered before puberty had vulvas that appeared juvenile and recessed/inverted at 17 months of age. Additionally, a dorsal vulval skin fold was present in almost all vulval images from bitches at both ages and percentage cover of the skin fold was reported, which to the authors knowledge is the first time this feature has been documented in such a large study population. VP was one of the biggest and most consistent influencing factors for height, weight, BCS and vulva size measurements. The results from this part of the study in isolation could suggest that neutering bitches prepubertally could be suitable for large breed bitches. However, it is advised that any future consequences for health based on the differences presented here are considered prior to recommendations being made. These are considered in Chapter 8.

Many authors seem to suggest an impact of early neutering on bitch growth (Spain *et al.*, 2004; Root-Kustritz, 2007; Reichler, 2009; Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014; Balsa and Robinson, 2016; Oberbauer *et al.*, 2019), and the assertion that this is due to an absence of oestrogen makes sense. However, when

examined, most of these are review papers and they reference the one study in dogs by Salmeri *et al.* (1991) that included dogs neutered at seven weeks of age (seven male, seven female), seven months of age (four male, four female) and entire dogs (four male, six female), as well as literature relating to cats (Stubbs *et al.*, 1996; Root-Kustritz *et al.*, 1997). Of the two studies identified that examined long bone length in bitches that were surgically neutered, both reported increases in early neutered bitches, however the differences were only significant in one of the studies for bitches neutered at seven weeks of age (Salmeri *et al.*, 1991). For the other, no significant differences were identified, and the authors suggested that there are no skeletal developmental implications of prepubertal gonadectomy (Sontas and Ekici, 2007). Both of these studies included small numbers of bitches with no consideration of power analysis or adjustment of alpha for the sample size. Only Salmeri *et al.* (1991) considered bitches neutered at different ages; the study by Sontas and Ekici (2007) included bitches that were all neutered, or underwent sham surgeries, at 10 weeks of age and measured final radial length at six months of age. Therefore, there are issues with study design and methodology that should be considered when interpreting these findings.

The present study, which included a larger number of bitches than previous studies, showed no significant difference between bitches neutered pre or post-pubertally in height and weight at 17 months of age. However, the change in height between timepoints did differ significantly (PPN = 6.5 cm, Control = 4.4 cm). Repeated radiography and measurement of long bone length was not undertaken in the present study. This would have enabled more accurate assessment of growth and determination of age at growth plate closure; measures that were undertaken by Salmeri *et al.* (1991). However, by 17 months of age physeal growth plates, which close sequentially from distal to proximal, would be expected to be closed in all but giant dog breeds (Kilborn *et al.*, 2002; Von Pfeil *et al.*, 2009a; Hammond and McConnell, 2013). Therefore, our

measurements, in contrast to those of Sontas and Ekici (2007), are likely representative of adult height. Bitches neutered before puberty may have an extended growth period, reduced physeal closure and consequently greater growth, in agreement with Salmeri *et al.* (1991). However, Salmeri *et al.* (1991) failed to include whether the bitches had undergone puberty, making direct comparison to the present study difficult.

The lack of significant findings when comparing height measurements for bitches neutered before and after puberty in the present study may be explained by the timing of neutering. Major growth in dogs occurs between three and six months of age (Von Pfeil *et al.*, 2009a), therefore bitches in both groups were neutered after this period. It is possible that growth plate closure was delayed in bitches neutered before puberty and that this influenced the greater change in height measurements, but that neutering at six months of age, after the period of major growth had ended, prevented greater differences in height at 17 months of age being observed. Salmeri *et al.* (1991) neutered bitches at seven weeks and seven months of age, and therefore compared bitches neutered before and after the major growth phase, which could have caused greater differences in length of long bones. Indeed Salmeri *et al.* (1991) only reported significantly greater radial lengths in bitches neutered at seven weeks (18.5 cm) compared to bitches neutered at seven months of age and entire bitches (both 16.6 cm). However, direct comparisons between the studies are impossible due to the different methods of measurement. Age at assessment significantly impacted height and weight measurements at six but not 17 months of age in the present study, as would be expected due to more rapid growth around six months of age. When considering growth, time before puberty as well as pubertal status at neutering are important factors to consider.

While literature relating to the impacts of neutering on vulval conformation and development are rare, some authors have

suggested that normal vulva development may be impacted by neutering due to the removal of oestrogen, which is essential for development of the reproductive tract and external genitalia (Hewitt and England, 1999; Verstegen-Onclin and Verstegen, 2006; Nelson and Couto, 2009). In the present study, vulval size and appearance at 17 months of age differed for bitches neutered before or after puberty. While measurements of vulval length and width were not significantly different between groups at 17 months of age, vulval size was larger for bitches neutered after puberty and the change in vulval length and width between the two time points was significantly greater for post-pubertally neutered bitches. Vulval size at 17 months of age in prepubertally neutered bitches was similar to that reported for bitches at six months of age. In a previous preliminary analysis of the data from the present study presented as an abstract, a significant difference was identified; bitches neutered before puberty were found to have significantly smaller vulvas (length 2.9 cm, width 1.8 cm) than bitches neutered post-pubertally (length 3.2 cm, width 2.1 cm) at 17 months of age (Godfrey-Hunt *et al.*, 2020). In that study, data from entire bitches were also included and vulval sizes for bitches neutered after puberty were not significantly different to entire pure-bred Labradors and Golden Retrievers and their crosses. The reasons why differences were no longer significant in the present study, despite 0.1 cm smaller mean measurements in vulval length and width for prepubertally neutered bitches, are likely due to the more complex method of statistical analysis including confounding variables, and the removal of bitches with missing confounding variable data and incorrect height measurements for inclusion as covariates from the models.

Vulval size has been measured by other authors and compared for bitches neutered at different ages. Salmeri *et al.* (1991) reported that vulvas were smaller and appeared infantile for bitches neutered at seven weeks (17.8mm, n=7) and seven months of age (16.8mm, n=4) than in entire bitches (19.8mm, n=6), although numbers of

bitches were small and statistical analysis was not performed. Marino *et al.* (2014) reported vulval appearance as juvenile and "sunken" for eight bitches that received repeated GnRH implants to delay puberty starting at 4.5 months of age, although actual vulval measurements were not performed. In contrast, Kaya *et al.* (2015) reported no difference in vulval size for 13 bitches that received either a deslorelin acetate or a placebo implant between four and five months of age for 40 weeks after insertion. In a study considering the prevalence and severity of recessed vulvas for bitches neutered at different ages, Palerme *et al.* (2021) reported increased odds of bitches having recessed vulva appearance if they were neutered at or before 12 months of age compared to after 12 months of age, or at or before 24 months of age compared to after 24 months of age. Interestingly, the authors reported no significant differences when bitches neutered before or after four or eight months were compared. The findings of the present study support the suggestion that vulval development may be impeded by neutering before puberty.

Results relating to veterinarian assessment of vulval appearance and examination of digital images of the vulva in the present study confirmed that vulva size and development are impacted by neutering before puberty. By 17 months of age, there were significantly more pre than post-pubertally neutered bitches with vulvas that were reported to be juvenile (16% vs 2%) and recessed (14% vs 3%) based on veterinary examination, and more classified as recessed/inverted (71% vs 46%) based on digital image examination. These findings are to be expected due to the earlier removal of oestrogen for bitches neutered before puberty. Such anomalies in appearance are suggested to predispose to urogenital disease (Johnson, 1991; Feldman and Nelson, 2004; Nelson and Couto, 2009). However, seemingly in contrast to the findings related to vulval development and the potential for urogenital disease later in life, fewer bitches neutered before than after puberty had skin discolouration noted when digital images from 17

months of age were examined (23% vs 39%), and more had skin and hair discolouration (72% vs 60%). The reasons for this are not clear but could relate to the presence of disease, such as dermatitis or vaginitis; this could result in greater time spent licking or cleaning the perivulval area (Fontbonne, 2010). Further investigation of urogenital disease incidence for the bitches in this study later in life will be undertaken in Chapter 8.

Dorsal occlusion of the vulva is suggested to be an abnormality in vulval conformation which, if covering a large percentage of the vulva, can lead to mating difficulties and can predispose to disease such as vaginitis and cystitis (Fontbonne, 2010). In the present study, a dorsal vulva skin fold was observed in almost all bitches on digital images at six and 17 months of age. By 17 months of age, the percentage coverage reduced to be 40% or less for most bitches. It would seem that some percentage coverage of a dorsal fold is a normal anatomical variant in bitches of these breeds, as most of the study population had no other clinical disease. However, the anatomical purpose of such a fold is unknown.

As mentioned previously, some studies have begun to investigate the effect of GnRH agonists implanted in small numbers of prepubertal bitches with the aim of delaying puberty. Within these are conflicting results regarding the impacts on height and vulval development, which may relate to the age at first implantation, or the number of repeated implants administered (Rubion *et al.*, 2006; Marino *et al.*, 2014; Kaya *et al.*, 2015). Marino *et al.* (2014) recruited 24 prepubertal Sicilian hounds to a study and treated eight with GnRH implants at 4.5, 9 and 13.5 months of age and 16 bitches did not receive an implant; eight of these were ovariohysterectomised at 18 months of age and eight at 4.5 months of age. The authors reported that the vulvas of the eight bitches that received repeated GnRH agonist implants remained juvenile in appearance at 18 months of age. They also identified no significant difference in weight measurements between bitches in different

groups and they noted that all growth plates were closed on radiographs taken at 15 months of age. However, no radiographs were taken at time points throughout the study to identify age at growth plate closure, therefore differences could not be compared. Kaya *et al.* (2015) studied the impacts of a GnRH agonist implant in 13 medium-sized crossbreed bitches; five bitches that received a 9.4 mg deslorelin acetate implant, four bitches that received a 4.7 mg deslorelin acetate implant and four bitches that received a placebo implant. Results suggested that there were no significant differences between the groups in bodyweight, height at the withers, vulval size and humeral length. However, growth plate closure determined radiographically was delayed for longer in bitches that received the higher dose 9.4 mg implant (83.5 ± 8.5 weeks of age) compared to bitches with the 4.7 mg implant (73.4 ± 4.5 weeks of age) and those with the placebo implant (60.9 ± 9.9 weeks of age). The authors suggest that incomplete suppression of hormones due to a single treatment may have resulted in the lack of observable differences in most measures.

5.5.1 Study limitations and generalisation

As with previous chapters, the results of the study are limited by the characteristics of the study sample. These factors are discussed in more depth in the general discussion (see Chapter 9). When considering this study, it is important to note that no comparisons were made to measurements of height, weight, vulval length or width for entire bitches of the same crossbreeds. Therefore, no conclusions can be made regarding whether the physical development measurements for bitches neutered post-pubertally reflect those that would be seen in entire bitches, although the results reported previously (Godfrey-Hunt *et al.*, 2020) suggest that vulval size in bitches neutered after puberty is similar to that in entire bitches, indicating maturation of the external genitalia. Whether vulval size in these neutered bitches would decrease with age is unknown; no later life vulva measurements were obtained. Two methods of determining vulval appearance were used,

assessments made by veterinarians and from digital images of the vulva. While in-person assessments by an experienced veterinarian may be considered useful, the ability to use the digital images to allow one trained person that was blinded to trial group to examine all images significantly reduced the risk of bias.

The findings related to height are limited by the method of measurement. Previous studies used repeated radiographs to measure long bone length and identify age at growth plate closure. This was not possible within the present study. Height measurements were also impacted by potential inaccuracies. Nineteen bitches were excluded from height analysis that showed zero or 'negative' growth results between six and 17-month assessments. There were no similarities identified across these 19 physical assessments that could explain the reason for the apparent error in height measurement and all veterinary practices were represented. While exclusion of these noticeably incorrect measurements prevented these data influencing the findings, the existence of these errors does suggest that height may have been improperly measured for other bitches that were included, however such errors are likely to have occurred equally for both groups. Despite the potential errors, height data were not excluded due to the importance of considering growth differences between dogs neutered before or after puberty, and the results do indicate an impact on growth in a direction that was expected. Specific studies examining long bone length in a larger number of animals would be required to adequately conclude findings related to the impacts of neutering before or after puberty on growth, as have been performed by others in smaller numbers of dogs (Salmeri *et al.*, 1991; Marino *et al.*, 2014; Kaya *et al.*, 2015). Method of measurement for vulval size could have been improved if standardised callipers had been provided to the four veterinary practices. Such tools have been used by others (Kaya *et al.*, 2015) and are recommended for future studies.

There were effects of VP in all analyses for which it was included as a confounding variable. Effects of VP within height were relatively consistent, VP3 reported height measurements that were significantly lower at both time points, and lower change in height measurements. For BCS, VP2 was significantly less likely to score bitches as 'ideal' body condition at both time points. It is important when analysing weight data to also have an indication of weight status, and whether the animals are under, ideal or overweight. BCS was included in this study, however, the majority of bitches were a BCS '5' at six-month assessments completed by VP2. At six months of age, BCS of '4' is considered ideal. Despite VP2 reporting significantly higher body weights for bitches at six months of age than all other VPs, it is unlikely that most bitches were overweight and more likely that BCS was incorrectly scored. Effects of VP were less consistent for vulva measurements. The importance of this finding should not be underestimated and highlights the value of limiting the persons conducting measurements and controlling for this confounder in analysis. While this doesn't compromise the findings related to trial group, as VP was included as a covariate in the models, it is a limitation of findings related to veterinary practice. The reasons for the variation between VP are unclear, and could be caused by differences in equipment (although this was supplied and was consistent across VP for measurement of height) or method of measurement; this warrants further consideration and should be considered in future studies and when using multiple veterinary practices throughout the UK to manage a large population of dogs.

5.6 CONCLUSION

This is the first time that the effect of neutering bitches before or after known puberty on physical development has been investigated. The results suggest that for Labrador/Golden Retriever crossbreed bitches, prepubertal neutering is associated with growth and physical development. There were insignificant differences in height, weight and vulval size at 17 months of age with bitches

neutered before puberty being taller, heavier and with smaller vulvas at this age. Changes in height and vulval size were significantly associated with neutering before puberty and more bitches neutered pre-pubertally had vulvas that appeared juvenile, recessed or inverted at 17 months of age. While these findings suggest that prepubertal ovariohysterectomy could be a suitable option for neutering bitches, the longer-term health implications of the differences identified need to be investigated and better understood before recommendations could be made. These findings improve understanding of the impacts on physical development from neutering bitches in relation to known puberty.

Chapter 6. A prospective study to investigate the behavioural development of bitches neutered prepubertally or post-pubertally

6.1 INTRODUCTION

Behavioural problems can negatively impact dog wellbeing and are frequently reported as reasons for dogs being relinquished to animal shelters and for failed adoptions from shelters (Patronek *et al.*, 1996; Salman *et al.*, 2000; Wells and Hepper, 2000; Mondelli *et al.*, 2004; Shore, 2005; Sherman and Mills, 2008; Hawes *et al.*, 2020). Behavioural problems or subsequent training difficulty are the most commonly reported reasons for dogs being unsuccessful in assistance dog training programmes (Goddard and Beilharz, 1982, 1983; Tomkins *et al.*, 2011; Bray *et al.*, 2019; Zlotnick *et al.*, 2019). Understanding the factors that affect the likelihood of behaviour problems being observed is therefore important for maintaining the human-animal bond and for dog welfare.

Neutering has been suggested to impact behaviour in female dogs by many authors (O'Farrell and Peachey, 1990; Salmeri *et al.*, 1991; Guy *et al.*, 2001; Serpell and Hsu, 2005; Duffy and Serpell, 2006; Kim *et al.*, 2006; Maejima *et al.*, 2007; Kubinyi *et al.*, 2009; Duffy and Serpell, 2012; Starling *et al.*, 2013; Casey *et al.*, 2014; Balint *et al.*, 2017; Balogh *et al.*, 2018; Farhoody *et al.*, 2018). The effects of the timing of neutering, most commonly related to age at neutering, on behaviour have also been studied. Similar to findings related to comparisons of behaviour between entire and neutered dogs, the reported effect of age at neutering is not consistent across behaviours or between sexes (Salmeri *et al.*, 1991; Spain *et al.*, 2004; Kubinyi *et al.*, 2009). Some authors suggest no effect of neutering at different ages on female dog behaviour (Salmeri *et al.*, 1991 [seven weeks versus seven months of age]; Howe *et al.*, 2001 [less or greater than 24 weeks of age]; Flint *et al.*, 2017). While others have reported differences in the prevalence and onset of

behavioural problems, including withdrawal from service dog training for behavioural reasons, that are related to age at neutering (O'Farrell and Peachey, 1990; Spain *et al.*, 2004; Zink *et al.*, 2014; Zlotnick *et al.*, 2019).

As highlighted in the scoping review (see Chapter 2), there is a lack of evidence regarding the effects of timing of neutering in relation to puberty on female dog behaviour. Only one retrospective study was identified that considered whether bitches were neutered before or after puberty based on owners' recollections of when neutering surgery occurred. In that study, Balogh *et al.* (2018) identified no differences in owner-reported behaviour or in urination behaviour between bitches neutered before or after puberty. Despite the lack of evidence, many authors have suggested differences in behaviour related to neutering before or after puberty (Kustritz, 2002; Thrusfield, 2011; Borstad, 2015; De Cramer and May, 2015; Romagnoli, 2017; Warnes, 2018). Others claim there to be no effect on behaviour related to neutering bitches prepubertally (Stubbs and Bloomberg, 1995; Houlihan, 2017). These imply that studies have been conducted, however, the comments appear to be a misinterpretation of studies that investigate the impacts of neutering at different ages. Other authors have acknowledged the lack of evidence regarding the effects of neutering before or after puberty on behaviour (Le Bars, 2006; Warnes, 2013; McGreevy *et al.*, 2018; Warnes, 2018) and large-scale, longitudinal studies that measure specific behaviours over time, and examine behavioural development of dogs through their lifetimes have been recommended (Warnes, 2018; Starling *et al.*, 2019; Zlotnick *et al.*, 2019).

Due to the lack of evidence for the impacts of time of neutering in relation to puberty on dog behaviour, decision making for working or assistance dog organisations that routinely neuter their dogs is difficult. This study investigates the effects of timing of neutering bitches from an assistance dog programme in relation to puberty

(rather than age at neutering) on behaviour measured using a validated questionnaire in a prospective study design for the first time. This will provide important information on dog behaviour for assistance and working dog programmes to aid decision making regarding whether to neuter their bitches before or after puberty to minimise the occurrence of undesirable behaviours.

6.2 AIM AND OBJECTIVES

6.2.1 Hypothesis

Neutering bitches prepubertally or post-pubertally can affect their behavioural development measured using an adapted C-BARQ tool.

6.2.2 Aim

To examine data for bitches neutered before or after puberty using a prospective study design to compare six behavioural factor scores measured using an adapted version of the C-BARQ.

6.2.3 Objectives

1) To determine whether there were any significant differences in scores for six behavioural factors at one and three years of age between bitches neutered before or after puberty.
2) To determine whether there were any significant differences in the change in scores for six behavioural factors between one and three years of age for bitches neutered before or after puberty.

6.3 MATERIALS AND METHODS

6.3.1 Study design

A prospective study was undertaken using 306 Guide Dogs' bitches born between 22/02/2012 and 09/08/2015. Bitches were neutered either prepubertally or post-pubertally (see Section 4.4.4). Data were gathered to examine bitch behaviour reported at one and three years of age.

6.3.2 Study setting

Within Guide Dogs, dogs are placed into puppy raising from the nest between seven and eight weeks of age and remain there until they enter training at approximately 14 months age. During puppy raising, dogs live with puppy raisers and their families. During training stages dogs are housed in kennels or with boarders in their homes. By three years of age dogs have qualified as a guide dog and are living with their guide dog owner or have been withdrawn from the training programme and are rehomed with their new owner (rehomer). Some dogs are rehomed to their puppy raisers. All of these dog movements are recorded in Guide Dogs' electronic database (GDIR).

One month prior to bitches reaching 12 months of age, behaviour questionnaires were sent to the puppy raisers for completion within a two-week window between the bitches being 12 months and 12 months and two weeks old. Owners of working three-year-old guide dogs were contacted by telephone and invited to take part, and questionnaires were then completed by telephone or by email depending on preference. In the case of bitches that were withdrawn and rehomed by three years of age, behaviour questionnaires were sent by email to the bitches' new owners.

6.3.3 Study participants

6.3.3.1 Behaviour questionnaires

The study population included 306 bitches from five different crossbreeds allocated to two groups; prepubertally neutered (PPN, n=155) and Control (n=151) (see Section 4.4.4). All first-generation backcross bitches were grouped into one breed group for analysis. Before sending behaviour questionnaires, Guide Dogs' people data management system was checked to ensure that the person responsible for each bitch had not opted out of contact for research. For those that had not opted out, the person's main Guide Dogs' staff contact was then consulted to ensure that there were no known issues with contacting the person at that time. Bitches that

were qualified as buddy dogs with a child or young person and that were in alternative working homes were excluded (n=8).

One-year behaviour questionnaires were sent by email to puppy raisers (n=303) or rehomers (n=2) for 305 bitches. One bitch was in buddy dog training and a questionnaire was not sent. For three-year behaviour questionnaires the owners of 243 bitches were invited to participate. These were 151 guide dog owners (by telephone), 91 rehomers and one boarder (by email). Twenty-five of the 91 rehomers had also been the bitch's puppy raiser. Three-year behaviour questionnaires were not administered for 63 bitches; 32 guide dog owners had opted out of contact, Guide Dogs' staff requested that 10 guide dog owners were not contacted, nine bitches were in alternative working homes and nine were working as a buddy dog. Bitches with questionnaires that were completed outside of the two-week window were excluded from analysis.

6.3.4 Variables and data sources

6.3.4.1 Behaviour questionnaires

A master version of the Canine Behavioral Assessment and Research Questionnaire (C-BARQ), a validated tool for measuring dog behaviour (http://www.cbarq.org; Hsu and Serpell, 2003), was obtained from the University of Pennsylvania in 2011. C-BARQ is widely used by animal shelters, dog trainers and behaviourists, working dog programmes and scientists (Appendix 5). C-BARQ has also been used by guide dog schools to measure behaviour and predict success in dog training programmes (Duffy and Serpell, 2012; Serpell and Duffy, 2016; Bray *et al.*, 2019). The questionnaire was adapted to suit the study population in consultation with Guide Dogs' training and behaviour and dog health teams. The adapted version contained 42 questions grouped into six factors; 1) Training and obedience, 2) Aggression, 3) Fear and anxiety, 4) Excitability, 5) Attachment and attention-seeking, and 6) Social behaviour (termed 'Miscellaneous' in the original C-BARQ) (Appendix 6). There was a final additional question included (Q43)

asking whether the bitches ever raised their legs during urination, which was included following discussion at the 2013 Association of Pet Behaviour Counsellors Annual Conference regarding masculinisation of urination posture in female dogs.

The questionnaire was designed to be completed by the bitches' owners to gather information about the behaviours observed during the previous few months. Each questionnaire item was completed by selecting an option on a rating scale that was explained within the questionnaire. Responses to items in the Training and obedience, Attachment and attention-seeking and Social behaviour factors were either 'Never', 'Seldom', 'Sometimes', 'Usually' or 'Always'. Responses that could be selected for the Aggression, Fear and anxiety and Excitability factors were numerical on a scale from '0' to '4' where '0' represented a behaviour that had not been observed and '4' represented a high severity of the behaviour (van den Berg *et al.*, 2010). High scores represented less desirable behaviour for all items except four Training and obedience items, scores for which were reversed prior to analysis.

Outcome variables were the six factor scores at one year of age, three years of age and the change in factor scores. Confounding variables were bitch breed, the geographical region that each puppy entered puppy raising (for one-year questionnaires: Scotland and Northern Ireland, North, Wales and West, South and East), the person completing the questionnaire (for three-year questionnaires and change in behaviour scores: boarder, guide dog owner, rehomer, puppy raiser-rehomer) as fixed factors, and age at questionnaire completion and the number of days from neutering surgery as covariates (for one-year and three-year questionnaires). Confounding variable data were extracted from GDIR or from study data collection forms.

6.3.5 Bias and study size

Random effects and confounding factors were included in the statistical analysis to account for the effects of these factors when examining the main effect of trial group on the dependent variables. C-BARQ scores have been shown to vary between breeds of dog (Serpell and Hsu, 2005; Duffy *et al.*, 2008; Serpell and Duffy, 2014; Asp *et al.*, 2015, Tonoike *et al.*, 2015). Dogs in this study were of similar breeds and were not different between trial groups. However, breed was included in all statistical models to control for potential effects on other variables.

While adapted C-BARQ questionnaires were completed by puppy raisers for all bitches at one year of age, at three years of age, there was variation in the person completing the questionnaire. Behavioural assessments and questionnaires completed for animals are known to be subject to variability caused by different observers or participants (Fratkin *et al.*, 2015; Munch *et al.*, 2018). For analysis of three-year behaviour questionnaires, the person completing the questionnaire (guide dog owner, rehomer, boarder or puppy raiser) was included in the models.

Study size was limited by the available cohort of bitches produced by Guide Dogs during the recruitment period for the study that were placed in puppy raising within a suitable distance to travel to one of four national veterinary practices for neutering surgery. Values of alpha were determined use G*Power for each analysis (see Section 4.7). The mean and confidence intervals (CI) were reported where appropriate.

6.3.6 Quantitative variables and statistical methods

6.3.6.1 Adapted C-BARQ internal consistency

Prior to statistical analysis to examine differences between the trial groups, internal consistency for the six behavioural factors in this adapted version of the C-BARQ was tested using Cronbach's alpha that was reported alongside the mean inter-item correlation matrix

(IICM). Data for all bitches from one and three years of age were combined. A Cronbach's alpha of greater than 0.70 was considered to indicate good internal reliability. However, as all scales had fewer than 10 items, a Cronbach's alpha of greater than 0.5 was considered acceptable (van Griethuijsen *et al.*, 2015; Taber, 2018).

6.3.6.2 Adapted C-BARQ factor scores

The mean score for each adapted C-BARQ behavioural factor was calculated for each bitch and used for statistical analysis (Hsu and Serpell, 2003). Histograms for each behavioural factor were examined to investigate score distribution. Mean scores from one and three years of age were used to calculate the change in behavioural factor scores for each bitch between the two time points. Differences between -0.1 and 0.1 were classed as 'No change'. A positive difference represented an increase in mean factor score (move to less desirable behaviour) from one to three years of age while a negative difference represented a decrease in mean factor score (move to more desirable behaviour). For three-year questionnaires, the one boarder response was grouped with rehomer responses for analysis.

Scores for items in Training and obedience, Attachment and attention-seeking and Social behaviour factors were determined by converting the word responses of 'Never', 'Seldom', 'Sometimes', 'Usually' or 'Always' to numerical scores from '0' to '4' where '0' represented the most desirable behaviour and '4' the least desirable behaviour for each question. Mean factor scores calculated for each bitch were examined for normality using Q-Q plots. Scatterplots were produced to visualise any relationship between days from neutering surgery and mean factor scores. Relationships were examined within groups (PPN and Control) and for the combined data using Spearman's rank correlation coefficients.

Alpha and beta were determined using sensitivity analysis. For all behavioural factors for one-year questionnaires, alpha and beta

were 9% and 5% respectively for a sample size of 274 dogs with an effect size of 0.405. For all behavioural factors for three-year questionnaires, alpha and beta were 10% and 5% respectively for a sample size of 193 dogs with an effect size of 0.476. For the change in behavioural factor scores between one and three-year questionnaires, alpha and beta were 10% and 5% respectively for a sample size of 171 dogs, with an effect size of 0.506. These effect sizes should be suitable for C-BARQ data based on those previously reported (Grigg *et al.*, 2016; Bowen *et al.*, 2019; Hare *et al.*, 2021). Differences in mean factor scores between PPN and Control bitches were examined using general (for Training and obedience and Attachment and attention-seeking) or generalized linear models (for a gamma distribution for Fear and anxiety, Excitability, and Social behaviour) depending on the score distribution and model fit. Standardized residuals were examined for general linear models. Where significant effects of confounding factors with more than two groups on behavioural factors were identified from general linear models, Tukey's HSD post-hoc tests were applied to identify where differences were significant. Due to the highly skewed distribution of scores for Aggression (median Aggression factor scores were 0), data were converted to binary responses where '0' represented no aggression observed and scores of '1' or more represented any aggression (Duffy *et al.*, 2014; McMillan *et al.*, 2016; McGreevy *et al.*, 2018). Aggression was analysed using a generalized linear model with a logit link function.

Separate models were used to examine differences in mean factor scores at one and at three years of age. A third set of models were used to examine the change in factor scores between one and three years of age. Bitch breed (three groups) and trial group were included as fixed factors in all models. For models examining behaviour scores at one year of age, puppy raising region was included as an additional fixed factor, and age at questionnaire completion and the number of days from neutering surgery were included as covariates. For models examining behaviour at three

years of age, the person completing the questionnaire was included as an additional fixed factor, and age at questionnaire completion and the number of days from neutering surgery were included as covariates. For the analysis of the change in behaviour scores between time points, inclusion of covariates for age and the number of days since neutering surgery was not possible. The person completing the questionnaire at three years of age was included as a fixed factor. All models were fitted using SPSS version 22 (IBM, USA).

For examination of change in behavioural factor scores, the direction of the change in scores for each behavioural factor between time points was presented graphically by trial group. The number of behavioural factors (out of six) for each bitch with scores that increased, decreased and did not change was determined. This was used to categorise each bitch with an overall direction of behavioural change toward more desirable behaviours (four, five or six scores that decreased by three years of age, none or one that increased, and/ or none to two that remained the same), less desirable behaviours (four, five or six scores that increased by three years of age, none or one that decreased, and/ or none to two that remained the same), or mixed (between one and four scores that increased or decreased and/or between none and four scores that remained the same). The number of bitches with each overall direction of change in behaviour was compared between trial groups using a Chi-square test (XLStat2016, Addinsoft, USA).

6.3.6.3 Leg raising when urinating

The responses for leg raising during urination were examined independently of adapted C-BARQ data and data were described. No statistical analyses were performed due to low incidence of the posture being reported.

6.4 RESULTS

6.4.1 Participants

One-year behaviour questionnaires were completed and returned for 276 bitches (90.5%; 136 PPN, 140 Control), 275 by the puppy raiser and one by a rehomer (Control). One-year questionnaires for two PPN bitches were completed more than two months late and were excluded from analysis. Three-year behaviour questionnaires were completed and returned for 200 bitches (82.3%; 104 PPN, 96 Control), 121 by the guide dog owner, 78 by the rehomer (20 of which were the bitches' puppy raisers) and one by a boarder. Three-year questionnaires were completed more than two months late for seven bitches (2 PPN, 5 Control) and were excluded from analysis. Final adapted C-BARQ data were therefore available for 134 PPN and 140 Control bitches at one year of age and 102 PPN and 91 Control bitches at three years of age, including 171 bitches (89 PPN, 82 Control) at both time points. Three-year questionnaires were completed for 115 bitches that were qualified, 15 of which were working with their second guide dog owner, 77 bitches that were withdrawn, six of which were withdrawn following qualification and one bitch in advanced training after being withdrawn post-qualification.

At the time of one-year behaviour questionnaire completion all 134 PPN bitches with returned questionnaires were neutered. Ninety-one of the 140 Control bitches were entire at the time of questionnaire completion (questionnaires completed 0 to 145 days before neutering surgery) and a further 39 had been neutered in the previous month (questionnaires completed 1 to 30 days after neutering surgery). Only ten Control bitches had been neutered more than a month before one-year behaviour questionnaire completion (questionnaires completed 33 to 92 days after surgery). At the time of the three-year behaviour questionnaires all bitches had been neutered between 617 and 972 days prior to the date of completion.

6.4.2 Adapted C-BARQ internal consistency

Data were included for 476 completed questionnaires. Cronbach's alpha was >0.7 for three factors: Fear and anxiety (0.704, IICM=0.210), Excitability (0.825, IICM=0.486) and Attachment and attention-seeking (0.708, IICM=0.283) and was >0.5 for two factors: Training and obedience (0.681, IICM=0.269) and Social behaviour (0.585, IICM=0.158). Reliability analysis for Aggression items was not possible due to the large number of bitches that had scores of '0' reported for all items (401/476).

6.4.3 Adapted C-BARQ factor scores

6.4.3.1 Questionnaires at one year of age

PPN bitches were neutered between 143 and 228 days before questionnaire completion. Control bitches were neutered 145 days before to 92 days after questionnaire completion. Spearman's rank correlation coefficients (r_s <0.12 for all factors) and Scatterplots did not suggest the presence of any strong relationships between days from neutering surgery and mean behavioural factor scores, PPN and Control bitch scores were clustered in two distinct groups by days from neutering surgery (Figure 6.1).

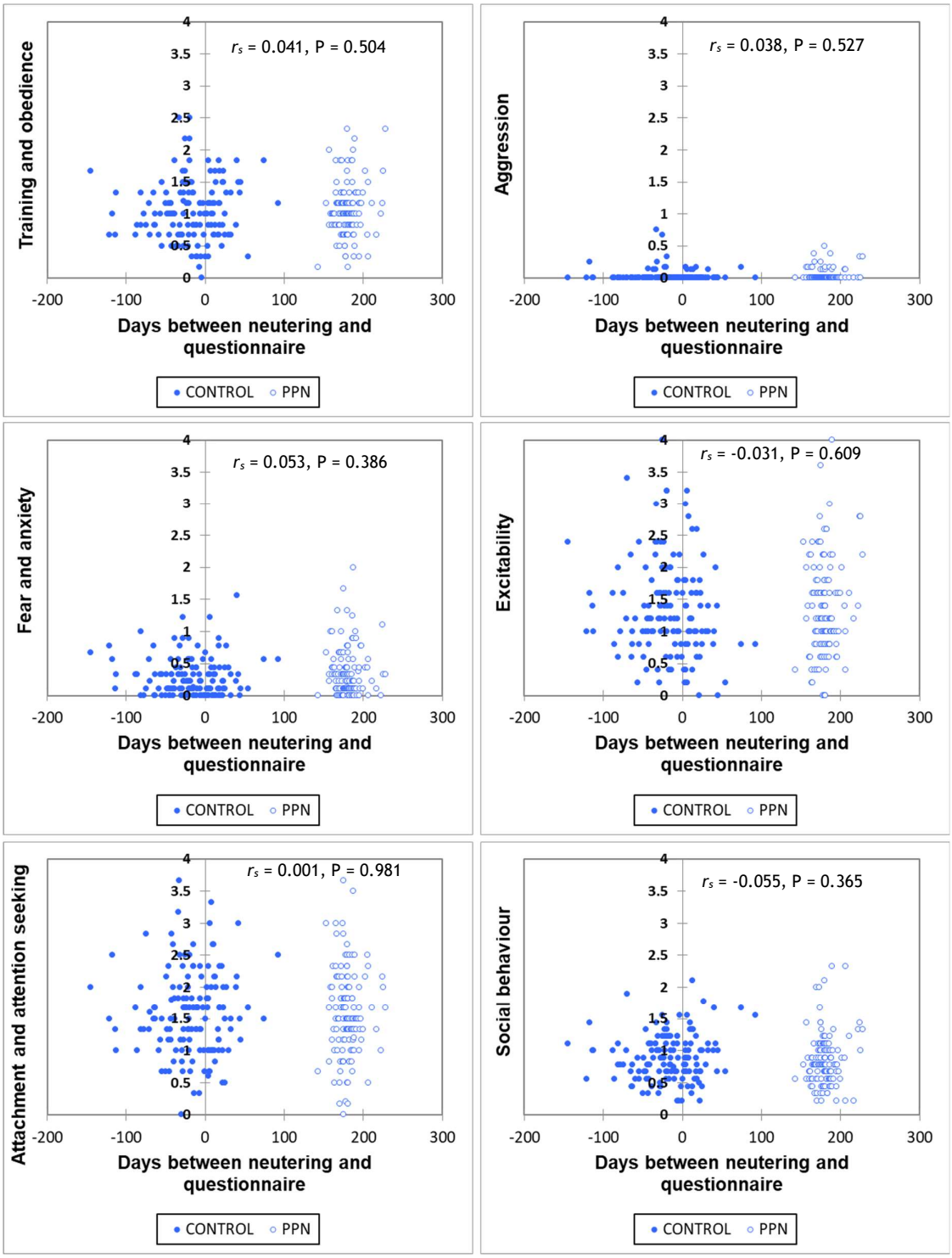


Figure 6.1. Scatterplots showing relationships between the six behavioural factor scores measured using an adapted C-BARQ at one year of age and the days between neutering surgery and questionnaire completion for bitches in a prospective study examining the effect of neutering before (PPN, n=134) or after (Control, n=140) puberty.

6.4.3.1.1 Factors influencing adapted C-BARQ factor scores at one-year questionnaires

None of the six models were significant and for generalized linear models Wald CI for all factors and covariates included zero. Therefore mean scores for Training and obedience (D.F.=1, F=0.661, P=0.417), Fear and anxiety (P=0.657), Excitability (P=0.474), Attachment and attention-seeking (D.F.=1, F=0.052, P=0.819) and Social behaviour (P=0.168) were not different between PPN and Control bitches and were not influenced by any of the fixed factors or covariates included in the models (Tables 6.1; Figure 6.2).

Mean Aggression factor scores were heavily skewed toward '0' and were '0' for 236 of the 274 bitches with completed questionnaires (114 PPN, 122 Control). For the 38 bitches with mean Aggression scores higher than '0', mean scores were low and ranged from 0.13 to 0.75 (Table 6.1). There was no effect of group (P=0.559), or any other fixed factors or covariates on whether bitches scored '0' or greater than '0' for Aggression.

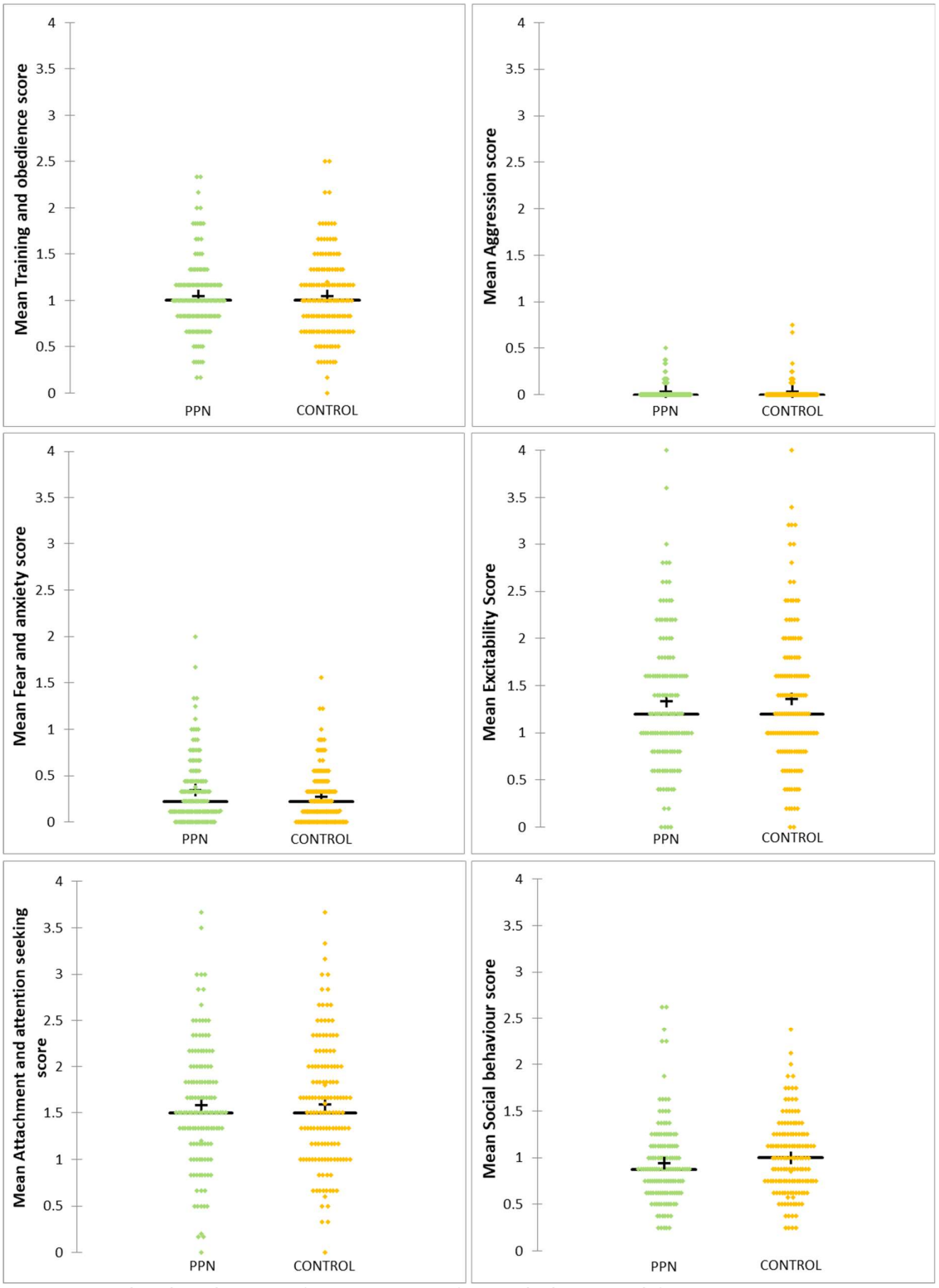


Figure 6.2 The distribution of mean scores for six behavioural factors measured using an adapted C-BARQ at one year of age for bitches in a prospective study examining the effect of neutering before (PPN, n=134) or after (Control, n=140) puberty. Graphs show the mean (+) and median (straight line).

Tables 6.1a and 6.1b. The results from the general (6.1a) and generalized (6.1b) linear models used to examine differences between PPN and Control bitches in six behavioural factors scores from an adapted C-BARQ competed at one year of age. Bitches were neutered either before (PPN, n=134) or after (Control, n=140) puberty in a prospective study. P values and model parameter results shown are for the main effect of neutering before or after puberty, with PPN as the reference group.

6.1a. General linear models for Training and obedience and Attachment and attention-seeking.

Behavioural factor score	PPN mean ± SEM (range min-max)	Control mean ± SEM (range min-max)	Effect size	Degrees of freedom	F	Main effect P
Training and obedience	1.05±0.04 (0.17-2.33)	1.05±0.04 (0.00-2.50)	-0.004	1	0.661	0.417
Attachment and attention-seeking	1.58±0.06 (0.00-3.67)	1.59±0.06 (0.00-3.67)	-0.012	1	0.052	0.819

6.1b. Generalized linear models for gamma distribution for Fear and anxiety, Excitability and Social behaviour and with a logit link function for binary Aggression data.

Behavioural factor score	PPN mean ± SEM (range min-max)	Control mean ± SEM (range min-max)	Effect size	Main effect P	Model parameter estimates for Control compared to PPN			
					B	S.E.	Wald Chi-square	91% Wald CI
Aggression	0.03±0.01 (0.00-0.50)	0.03±0.01 (0.00-0.75)	0.03	0.559	-0.752	1.289	0.341	-2.937, 1.433
Fear and anxiety*	0.34±0.03 (0.00-2.00)	0.28±0.03 (0.00-1.56)	0.22	0.657	-0.145	0.328	0.197	-0.01, 0.411
Excitability	1.33±0.06 (0.00-4.00)	1.36±0.06 (0.00-4.00)	-0.03	0.474	-0.182	0.254	0.513	-0.613, 0.249
Social behaviour	0.94±0.04 (0.25-2.63)	1.00±0.03 (0.25-2.38)	-0.14	0.168	0.262	0.190	1.903	-0.060, 0.584

*model fit was poor with puppy raising region included, therefore results shown are for the model excluding this fixed factor.

6.4.3.2 Questionnaires at three years of age

PPN bitches were neutered between 872 and 972 days before questionnaire completion. Control bitches were neutered between 617 and 822 days before questionnaire completion. Spearman's rank correlation coefficients for combined data suggested weak positive correlations between days from neutering surgery and Aggression (r_s=0.149, P=0.040), Fear and anxiety (r_s=0.191, P=0.008) and Attachment and attention-seeking (r_s=0.156, P=0.032). No relationships were identified between days from neutering and the other three behavioural factors (r_s<0.110) nor when PPN and Control bitch data were analysed separately (r_s<0.194). PPN and Control bitch scores were clustered in two distinct groups by days from neutering surgery (Figure 6.3).

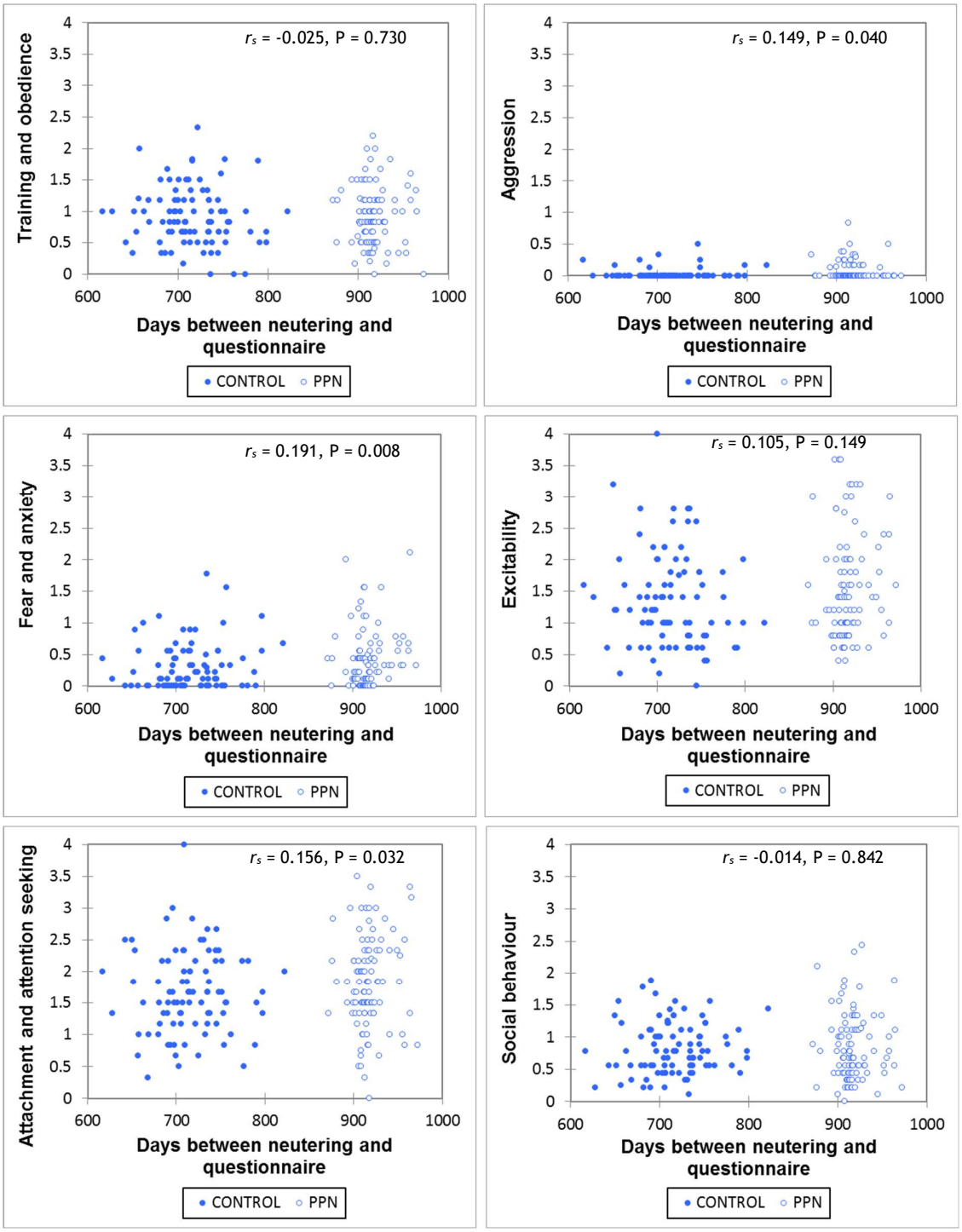


Figure 6.3. Scatterplots showing relationships between the six behavioural factor scores measured using an adapted C-BARQ at three years of age and the days between neutering surgery and questionnaire completion for bitches in a prospective study examining the effect of neutering before (PPN, n=102) or after (Control, n=91) puberty.

6.4.3.2.1 Factors influencing adapted C-BARQ factor scores at three-year questionnaires

Mean behavioural factor scores were not different between PPN and Control bitches in any model and Wald CI for trial group for all factors included zero (Tables 6.2a and 6.2b; Figure 6.4). The person completing the questionnaire affected mean scores for all factors except Aggression (P=0.168) and Attachment and attention-seeking (D.F.=2, F=0.051, P=0.950). Post-hoc Tukey's HSD showed that guide dog owners scored significantly lower than rehomers for Training and obedience (D.F.=2, F=10.985, P<0.001). Guide dog owners were more likely than rehomers to score bitches lower (representing more desirable behaviour) for Fear and anxiety (OR=1.579, 90% CI=1.239 to 2.012, P=0.002), Excitability (OR=1.345, 90% CI=1.167 to 1.551, P=0.001) and Social behaviour (OR=1.610, 90% CI=1.402 to 1.850, P<0.001). Guide dog owners were also more likely to score dogs lower than puppy raiser rehomers for Fear and anxiety (OR=1.687, 90% CI=1.231 to 2.311, P=0.006) and Social behaviour (OR=1.290, 90% CI=1.042 to 1.597, P=0.050). No other fixed effects or covariates influenced any mean behavioural factor scores.

Mean Aggression factor scores were heavily skewed toward '0' and were '0' for 159 of the 193 bitches with completed questionnaires (78 PPN, 81 Control). For the 34 bitches with mean Aggression scores higher than '0', scores were low and ranged from 0.13 to 0.83 (Table 6.2b). There was no effect of trial group (P=0.995), or any other fixed factors or covariates on whether bitches scored '0' or greater than '0' for Aggression.

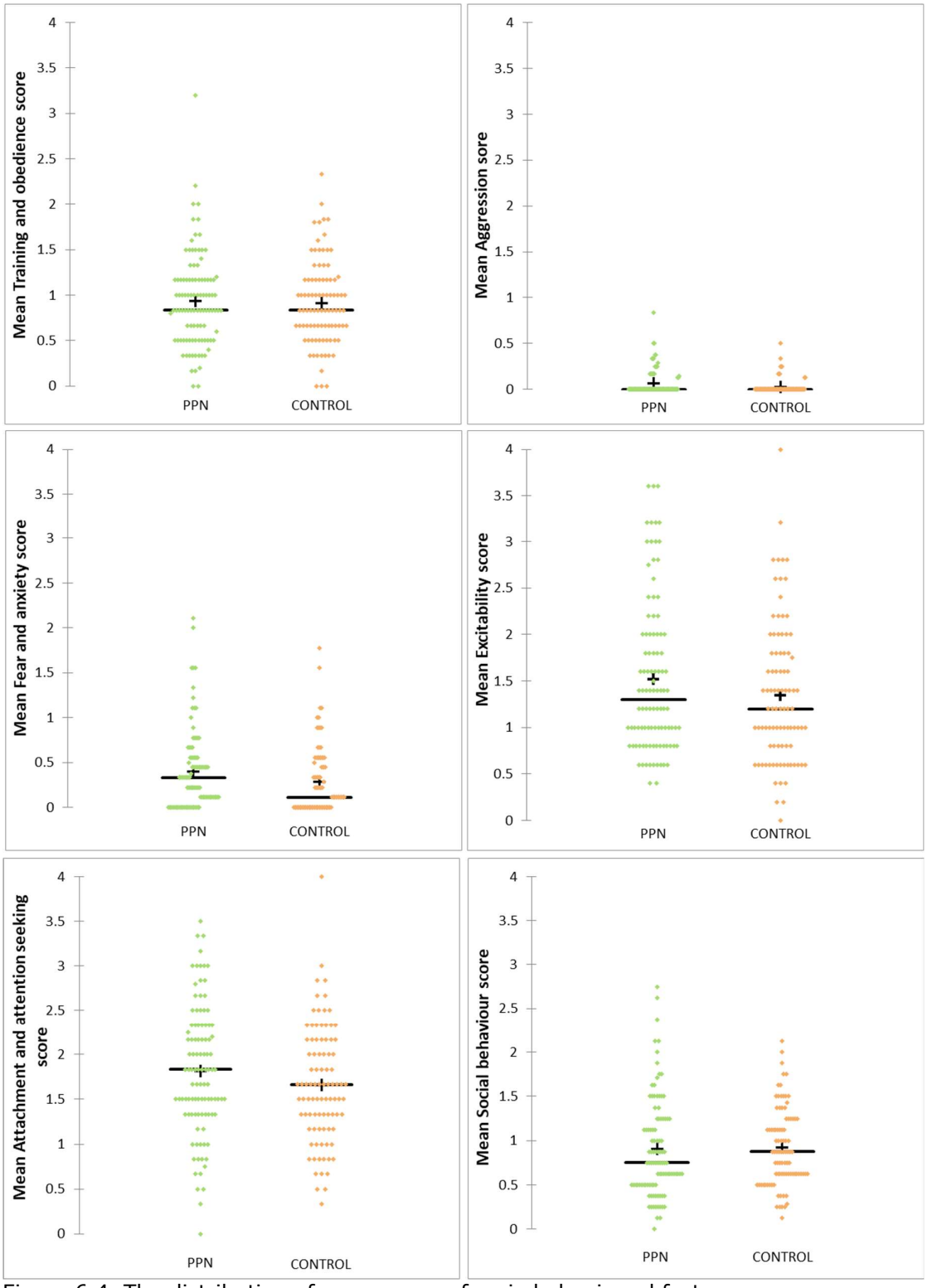


Figure 6.4. The distribution of mean scores for six behavioural factors measured using an adapted C-BARQ at three years of age for bitches in a prospective study examining the effect of neutering before (PPN, n=102) or after (Control, n=91) puberty. Graphs show the mean (+) and median (straight line).

Tables 6.2a and 6.2b. The results from the general (6.2a) and generalized (6.2b) linear models used to examine differences between PPN and Control bitches in six behavioural factors scores from an adapted C-BARQ competed at three years of age. Bitches were neutered either before (PPN, n=102) or after (Control, n=91) puberty in a prospective study. P values and model parameter results shown are for the main effect of neutering before or after puberty, with PPN as the reference group.

6.2a. General linear models for Training and obedience and Attachment and attention-seeking.

Behavioural factor score	PPN mean ± SEM (range min-max)	Control mean ± SEM (range min-max)	Effect size	Degrees of freedom	F	Main effect P
Training and obedience	0.94±0.05 (0.00-3.20)	0.92±0.05 (0.00-2.33)	0.05	1	1.022	0.313
Attachment and attention-seeking	1.82±0.07 (0.00-3.50)	1.66±0.07 (0.33-4.00)	0.25	1	0.164	0.686

6.2b. Generalized linear models for gamma distribution for Fear and anxiety, Excitability and Social behaviour and with a logit link function for binary Aggression data.

Behavioural factor score	PPN mean ± SEM (range min-max)	Control mean ± SEM (range min-max)	Effect size	Main effect P	Model parameter estimates for Control compared to PPN			
					B	S.E.	Wald Chi-square	90% Wald CI
Aggression	0.07±0.01 (0.00-0.83)	0.03±0.01 (0.00-0.50)	0.48	0.995	-0.011	1.815	0.000	-2.997, 2.975
Fear and anxiety	0.40±0.04 (0.00-2.11)	0.29±0.04 (0.00-1.78)	0.30	0.298	0.567	0.545	1.081	-0.330, 1.464
Excitability	1.52±0.08 (0.40-3.60)	1.35±0.08 (0.00-4.00)	0.23	0.383	-0.285	0.326	0.761	-0.821, 0.251
Social behaviour	0.91±0.06 (0.00-2.75)	0.92±0.05 (0.13-2.13)	-0.03	0.420	0.253	0.314	0.649	-0.263, 0.770

6.4.3.3 Change in adapted C-BARQ factor scores between one and three years of age

Data from completed C-BARQ questionnaires were available for 171 bitches (89 PPN, 82 Control) at both one and three years of age. There was no consistent pattern of increase or decrease in mean scores observed for any trial group or behaviour factor. For each behaviour factor there were some bitches with mean scores that increased and some with scores that decreased by three years of age. An increase in score (change to less desirable behaviour) appeared more frequently for PPN than Control bitches for all factors except Excitability. A lower proportion of PPN than Control bitches had scores that decreased (change to more desirable behaviour) between one and three years of age for Training and obedience, Fear and anxiety and Excitability (Figure 6.5).



Figure 6.5. The percentage of bitches in a prospective study examining the effect of neutering before (PPN, n=89) or after (Control, n=82) puberty that had mean factor scores in three-year adapted C-BARQ questionnaires that increased, remained the same or decreased, compared to scores in one-year questionnaires for Training and obedience, Aggression, Fear and anxiety, Excitability, Attachment and attention-seeking and Social behaviour.

6.4.3.3.1 Factors influencing the change in adapted C-BARQ factor scores between one and three-year questionnaires

The change in behaviour factor scores from one to three years of age was not affected by trial group for any factor except Aggression (Tables 6.3a and 6.3b; Figure 6.6). There was no significant effect of any of the factors included in the models for Training and obedience (D.F.=5, F=0.836, P=0.526), Excitability (D.F.=5, F 1.255, P=0.286), Attachment and attention-seeking (D.F.= 5, F=0.249, P=0.940) and Social behaviour (D.F.=5, F=1.644, P=0.151).

Aggression scores for Control bitches were less likely to increase (become less desirable) by three-year questionnaires than scores for PPN bitches (OR=0.959, 90% CI=0.924 to 0.995, P=0.06). Mean scores for Aggression were '0' at both questionnaires for 127 of the 171 bitches (74.3%; PPN=61, Control=66 bitches). For 20 PPN and nine Control bitches mean scores for Aggression increased between one and three-year questionnaires, while Aggression score decreased for only one Control bitch. Effect size was 0.26.

Change in Aggression scores was also influenced by breed. Aggression scores for Golden Retriever cross Labradors (mean change =0.03 ± 0.14) were more likely to increase (become less desirable) by three-year questionnaires than scores for Labrador cross Golden Retrievers (mean change =-0.02 ± 0.20; OR=1.073, 90% CI=1.013 to 1.137, P=0.044).

Change in Fear score was affected by the person completing the three-year behaviour questionnaire; scores reported by rehomers were more likely to increase (become less desirable) than scores reported by guide dog owners resulting in greater positive changes in scores (OR=1.319, 90% CI=1.154 to 1.506, P=0.001).

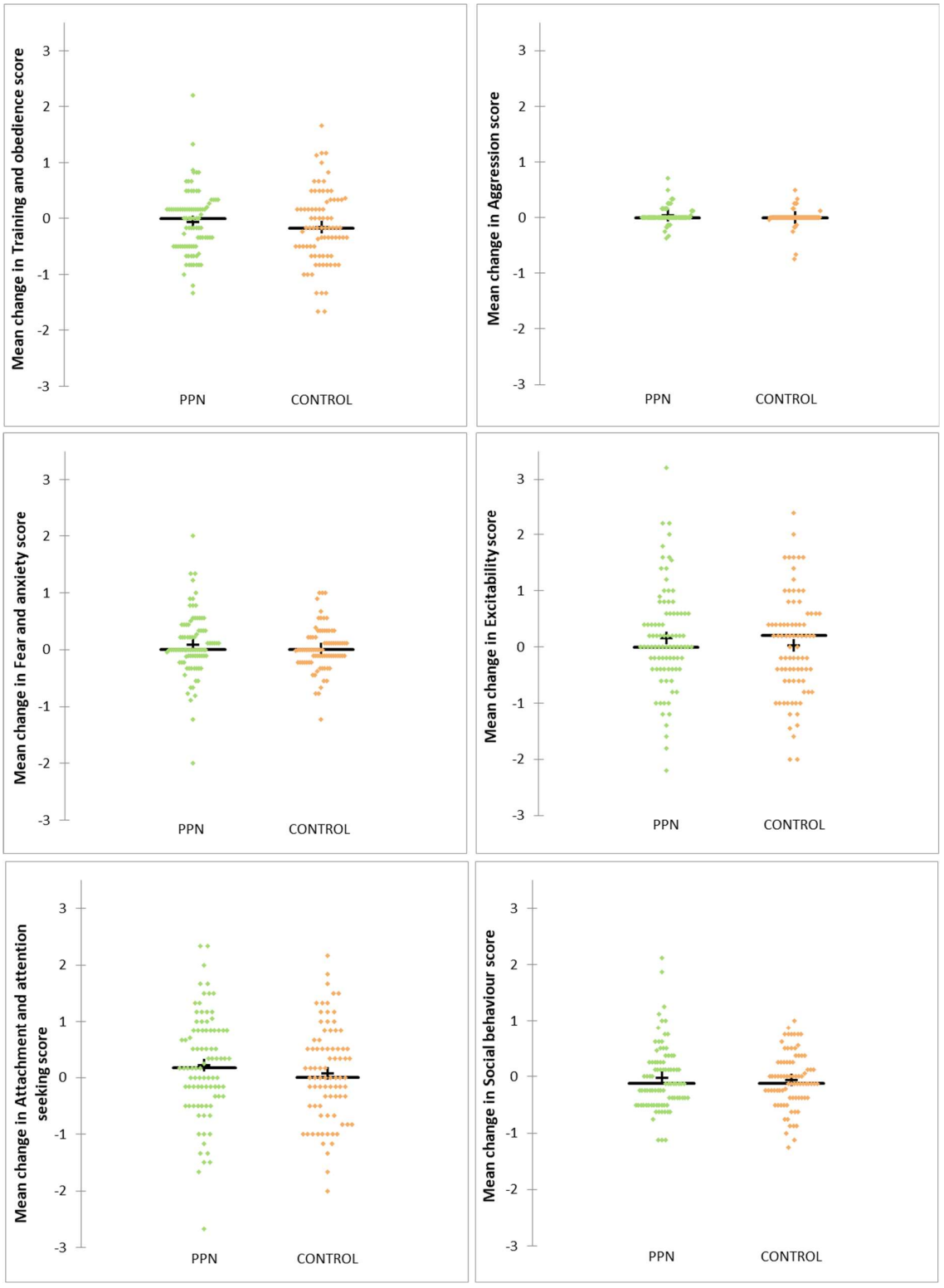


Figure 6.6. The distribution of changes in mean scores between one and three years of age for six behavioural factors measured using an adapted C-BARQ in a prospective study examining the effect of neutering before (PPN, n=89) or after (Control, n=82) puberty. Graphs show the mean (+) and median (straight line).

Tables 6.3a and 6.3b. The results from the general (6.3a) and generalized (6.3b) linear models used to examine differences between changes in six mean behavioural factors scores from an adapted C-BARQ completed at one and three years of age for PPN and Control bitches. Bitches were neutered either before (PPN, n=89) or after (Control, n=82) puberty in a prospective study. P values and model parameter results shown are for the main effect of neutering before or after puberty, with PPN as the reference group.

6.3a. General linear models for Training and obedience, Excitability, Attachment and attention-seeking and Social behaviour.

Behavioural factor score	PPN mean ± SEM (range min-max)	Control mean ± SEM (range min-max)	Effect size	Degrees of freedom	F	Main effect P
Training and obedience	-0.07±0.06 (-1.33-2.20)	-0.16±0.07 (-1.67-1.67)	0.14	1	1.288	0.258
Excitability	0.158±0.10 (-2.20-3.20)	0.03±0.10 (-2.00-2.40)	0.14	1	0.489	0.485
Attachment and attention-seeking	0.22±0.10 (-2.67-2.33)	0.08±0.10 (-2.00-2.17)	0.16	1	1.043	0.309
Social behaviour	-0.02±0.06 (-1.13-2.13)	-0.06±0.06 (-1.25-1.00)	0.08	1	0.204	0.652

6.3b. Generalized linear models for Fear and anxiety and with a logit link function for binary Aggression data.

Behavioural factor score	PPN mean ± SEM (range min-max)	Control mean ± SEM (range min-max)	Effect size	Main effect P	Model parameter estimates for Control compared to PPN			
					B	S.E.	Wald Chi-square	90% Wald CI
Aggression	0.04±0.02 (-0.38-0.71)	0.00±0.02 (-0.75-0.50)	0.26	0.060	-0.042	0.022	3.546	-0.078, 0.005
Fear and anxiety	0.09±0.06 (-2.00-2.00)	0.02±0.04 (-1.22-1.00)	0.19	0.252	0.083	0.072	1.313	-0.035, 0.201

The number of behavioural factors for each bitch with scores that increased, decreased or remained the same between one and three years of age was varied (Figure 6.7). The majority of PPN (74.2%) and Control (73.2%) bitches had scores that did not demonstrate a consistent pattern of increase or decrease between time points across all six factors (Table 6.4) and there was no difference in the number of bitches with overall increases, decreases or mixed changes in factor scores between groups (Chi-square=2.727, D.F.=2, P=0.256).

Table 6.4. The overall direction of change in behaviour scores between one and three years of age for six behavioural factors measured using an adapted C-BARQ for bitches neutered before (PPN, n=89) or after (Control, n=82) puberty in a prospective study.

Overall direction for change in behavioural factor score	PPN n	PPN %	Control n	Control %
Decreased	9	10.1	14	17.1
Increased	14	15.7	8	9.8
Mixed	66	74.2	60	73.2

n=number of bitches

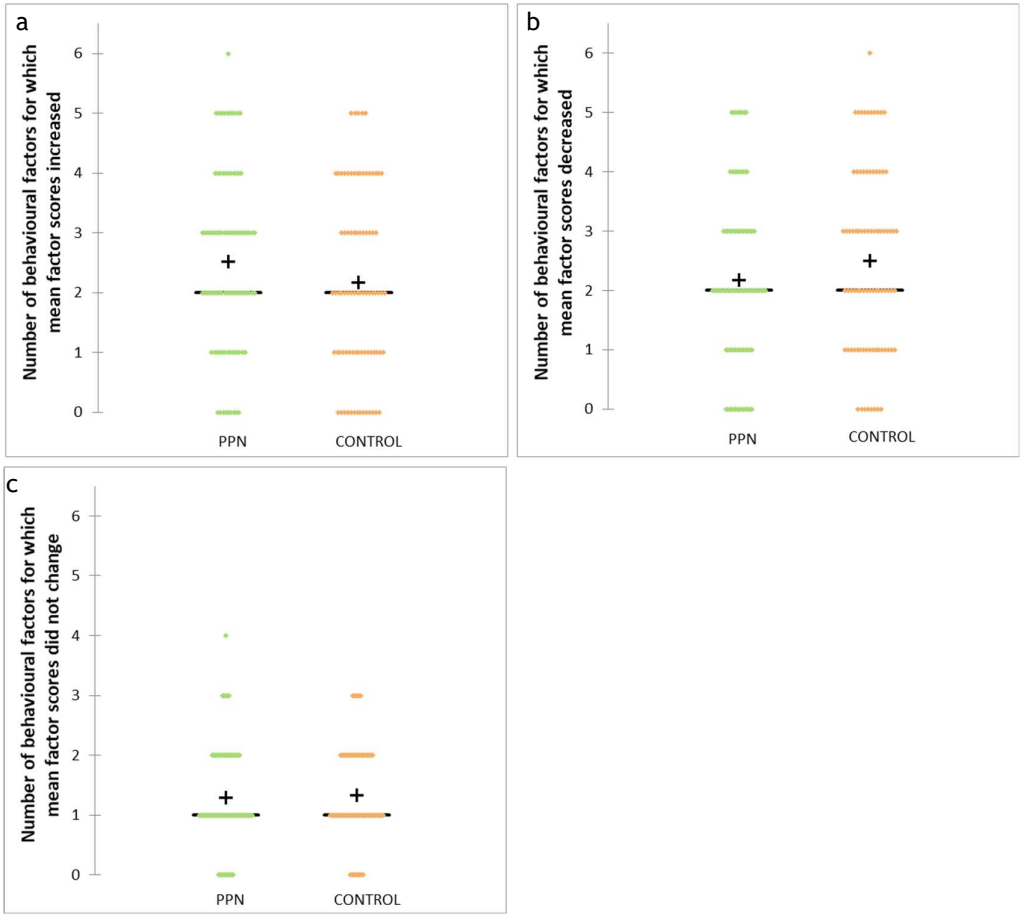


Figure 6.7. The number of mean behavioural factor scores measured using an adapted C-BARQ at one and three years of age, that increased (a), decreased (b) or remained the same (c) at the two time points for bitches neutered before (PPN, n=89) or after (Control, n=82) puberty in a prospective study. Graphs show the mean (+) and median (straight line).

6.4.4 Leg raising when urinating

Data were available for all 274 bitches at the one-year questionnaire and 188 of the 193 bitches at the three-year questionnaire. No PPN bitches and two Control bitches were reported to raise their leg when urinating at the one-year behaviour questionnaire, one 'seldom' and one 'usually'. Questionnaires for both of these bitches were completed before neutering surgery. Six different bitches (4 PPN, 2 Control) were reported to raise their leg when urinating at the three-year behaviour questionnaire, five 'sometimes' and one PPN 'usually'. The remaining 272 at one-year and 182 at three-years did not raise their leg when urinating. Further statistical analysis was not possible due to the low frequency of reported behaviour.

6.5 DISCUSSION

The impacts of neutering before or after puberty on bitch behaviour have not been well studied. Behaviour is an important factor related to becoming a successful assistance dog and one of the main reasons for failure in assistance dog training programmes (Goddard and Beilharz, 1982, 1983; Arata *et al.*, 2010; Tomkins *et al.*, 2011; Duffy and Serpell, 2012). While not studied directly in most cases, some existing research indicates the potential for neutering before or after puberty, and length of gonadal hormone exposure, to impact behaviour as well as health (O'Farrell and Peachey, 1990, Salmeri *et al.*, 1991; Spain *et al.*, 2004; Zink *et al.*, 2014; Balogh *et al.*, 2018; Farhoody *et al.*, 2018; Starling *et al.*, 2019). Therefore, it is important to evaluate the impact that the timing of neutering in relation to puberty can have on the behaviour of bitches in assistance dog programmes. This is not only relevant for developing dogs to be assistance dogs, but also to minimise the dogs needing to be released from programmes and rehomed.

The results presented within our study suggest that for Labrador and Golden Retriever crossbreeds, there are no significant differences in behavioural factors measured using an adapted C-BARQ at one and three years of age for bitches neutered before or after puberty. Additionally, there were no significant differences identified in changes in scores from one to three years of age for adapted C-BARQ behavioural factors between trial groups with the exception of change in Aggression scores, where a significant difference was suggested but requires interpretation in terms of effect size and power of the statistical model. These results address the study hypothesis and objectives and suggest that prepubertal neutering has no detrimental or favourable impact on future behaviour in female dogs of these breeds. This provides important information for organisations such as Guide Dogs to support neutering policies and to potentially allow for neutering when dogs are being cared for in homes by volunteers that provides financial benefits.

6.5.1 Adapted C-BARQ factor scores

No C-BARQ behavioural factor scores were significantly different between bitches neutered before or after puberty at one or at three years of age. There was also no consistent pattern in the change in behaviour scores between one and three years of age for any behaviour factors, or for any trial group. These are important findings that suggest that behavioural development in bitches measured using a validated questionnaire is not impacted by the timing of neutering in relation to puberty. This supports the results from the sole previous study that also examined the effects on behaviour of neutering bitches of similar breeds before or after puberty (Balogh *et al.*, 2018). In that study, 58 Labrador bitches of approximately six years of age were classified as entire (n=12), neutered before (n=17, median age six months) or after (n=29, median age 16 months) puberty. No differences in urination behaviours were observed and no differences in owner-reported urination behaviours or behaviour measured using C-BARQ were identified, although the small number of bitches and the potential for error in classification of timing of neutering due to owner recollection may be considered limitations to the study.

Early neutering has been suggested to increase aggression. O'Farrell and Peachey (1990) reported increased aggression in female puppies neutered at less than one year of age that were already showing signs of aggression, compared to bitches neutered as adults. Farhoody *et al.* (2018) examined data for aggression scores measured using 26 C-BARQ questions for over 13,000 male and female dogs neutered at less than six months of age, seven to 12 months of age, 13 to 18 months of age and entire dogs. No relationship was identified between age at neutering and aggression towards dogs or familiar people. However, the odds of reported aggression towards strangers were higher for neutered than entire dogs. This was heavily influenced by the 26% increased likelihood of aggression in dogs neutered between seven and 12 months of age.

Farhoody *et al.* did not examine differences between dogs neutered at different ages, only between each age group and entire dogs as the referent category. Increased aggressive behaviours were reported for female dogs neutered at earlier ages by Starling *et al.* (2019). Their study investigated relationships between age at neutering and percentage lifetime gonadal hormone exposure and 100 C-BARQ questions for almost 9,000 female dogs. Younger age at neutering was associated with increased aggression toward delivery people (OR=0.999) as well as increased willingness to 'fetch' (OR=0.997; Starling *et al.*, 2019).

The significant difference identified for the change in Aggression scores in the present study suggests that bitches neutered prepubertally were more likely to have scores for Aggression that changed to be less desirable by three years of age. However, the proportion of bitches for which scores changed between the two time-points was small, with the majority scoring '0' at both time points. This is unsurprising, C-BARQ scores for Aggression that are skewed towards zero have previously been reported in the general dog population (Serpell and Duffy, 2014) and in guide dog populations (Hunt *et al.*, 2020). Additionally, at three-year questionnaires, mean scores for Aggression for both groups were less than 0.1 and no bitch scored greater than 0.8, suggesting that only very mild aggressive behaviours were observed. Effect size for change in Aggression score was smaller than the suggested effect size based on power analysis. No difference between PPN and Control bitches in Aggression scores at three years of age was detected, and for that analysis, effect size was larger than the effect size suggested by power analysis. Further investigation of impacts on Aggression with larger numbers of dogs could provide more information. However, aggression in guide dogs is known to be infrequently observed (Murphy, 1998, Hunt *et al.*, 2020) and is likely to have been selected against in this guide dog breeding programme (Goddard and Beilharz, 1983; Murphy, 1998). Together with the points previously highlighted, this suggests that at least for

female dogs of these crossbreeds, there is no considerable detrimental impact of neutering before compared to after puberty on observed aggressive behaviour. This is consistent with the findings of Balogh *et al.* (2018) in bitches neutered before or after puberty, and of those of Flint *et al.* (2017) that identified no difference in the presence or severity of stranger directed aggression for dogs related to age at neutering.

Change in Aggression scores was also influenced by breed group. Aggression scores for Golden Retriever cross Labrador bitches were more likely to change to become less desirable by three-year questionnaires, compared to scores for Labrador cross Golden Retrievers. The reason for this difference is unclear. Breed and crossbreed differences in Aggression scores, as well as scores for other C-BARQ traits such as play, fear, trainability, energy and excitability have been reported (Serpell and Hsu, 2005; Duffy *et al.*, 2008; Serpell and Duffy, 2014; 2016; Asp *et al.*, 2015; Tonoike *et al.*, 2015; Shouldice *et al.*, 2019). However, as previously mentioned, overall Aggression scores were low and most bitches scored '0'. While for Golden Retriever cross Labrador bitches aggression scores were more likely to change to become less desirable, effect size was small and mean Aggression score on the three-year questionnaire for these bitches (0.04) was similar to mean scores for the other two crossbreeds (0.05). There were fewer Labrador cross Golden Retriever bitches, and back cross bitches than Golden Retriever cross Labrador bitches both in the study population, and with complete one and three-year questionnaires for analysis of the change in scores. This difference needs further investigation and may not persist if greater numbers of these less represented breeds were included in future studies.

Fear and anxiety scores reported by rehomers were more likely to change to become less desirable between one and three years of age than those reported by guide dog owners. Fear is one of the major reasons reported for dogs being unsuccessful in guide dog

programmes (Goddard and Beilharz, 1982, 1983; Harvey *et al.*, 2016b). Fear and anxiety was also one of the behavioural factors with scores representing more desirable behaviour more likely to be reported by guide dog owners than rehomers for dogs at three years of age, alongside Training and obedience, Excitability and Social behaviour. Variation in scores between different participant groups could be expected (Fratkin *et al.*, 2015; Munch *et al.*, 2018). However, within the present study it was not possible to determine whether the scores that changed to reflect more desirable behaviour reported by guide dog owners were due to factors relating to the guide dog owners themselves, or due to actual differences in bitch behaviour.

Questionnaires completed by guide dog owners at three years of age were reporting on behaviours observed in their working guide dogs. Thus, it could be reasonable to expect more desirable behaviour in this group than reported by rehomers for their dogs that were either withdrawn from the guide dog training programme, or for the small number that were withdrawn within the first years of qualification and did not requalify. Additionally, guide dog owners frequently work their dogs in varied environments and have support from Guide Dogs' staff that could also contribute to differences in behaviour. Environmental differences between working guide dogs and withdrawn dogs may also influence the opportunity to display behaviours. For example, a guide dog may be more likely to accompany the owner, therefore less likely to remain home alone and have less opportunity to display separation related behaviours. Alternatively, guide dog owners may be less likely to report less desirable behaviours due to a fear of the dog being removed from working with them. Less desirable C-BARQ scores for stranger-directed aggression, stranger-directed fear, non-social fear, attachment and attention-seeking, excitability and energy have been reported for dogs that were unsuccessful in a guide dog programme (Duffy and Serpell, 2008). Conducting a practical behaviour assessment to examine differences in behaviour that

would be unaffected by respondent bias could be considered for future studies. These could include direct measurement of key behaviours before and after neutering. However, this was not feasible within the present study, due to the wide geographic spread of bitches either working with guide dog owners or rehomed in locations throughout the UK.

Few authors account for the reason for neutering when investigating the impacts of neutering on behaviour, with some reporting excluding dogs neutered for behavioural reasons from their studies (Zink *et al.*, 2014; Farhoody *et al.*, 2018; Starling *et al.*, 2019). This is an important consideration when attempting to interpret findings; Podberscek and Serpell (1996) reported that the increased aggression identified in neutered dogs compared to entire dogs disappeared when dogs that were neutered due to aggression were removed from the analysis. Within the present study, no bitches were neutered for behavioural reasons. All bitches were neutered in line with Guide Dogs neutering policy (after their first oestrus) or at six months of age, in line with study design. Neutering groups were assigned in alternating order early in puppy raising. This study therefore contributes to the small number of studies where neutering for behavioural reasons has not been a factor that was overlooked and provides useful insights into the effects of neutering.

Urination postures in dogs are sexually dimorphic from a few months of age. Adult female dogs commonly urinate in a squatting position, sometimes with one rear foot raised (Beach, 1974; Wirant and McGuire, 2004; Balogh *et al.*, 2018). Leg raising while urinating was included in this study following discussion at the 2013 Association of Pet Behaviour Counsellors Annual Conference regarding masculinisation of urination posture in female dogs. This has been proposed to result from the effect of gonadal hormones and has been documented in some bitches exposed to testosterone in utero and postnatally (Beach, 1974; Ranson and Beach, 1985). While no difference in urination posture between neutered and

entire female dogs have been reported (Wirant and McGuire, 2004; Gough and McGuire, 2015; McGuire and Gough, 2017), an effect of the stage of reproductive development at neutering has not previously been considered in a prospective study. Within the present study, very small numbers of bitches were reported to raise their legs during urination such that statistical analysis was not possible. These data suggest that for most of these crossbreed bitches, neutering prepubertally does not cause a notable increase in the propensity to adopt a raised leg posture while urinating.

6.5.2 Study limitations and generalisation

The results of the study are limited by the characteristics of the study sample. These factors are discussed in more depth in the general discussion (see Chapter 9). A selection of papers that have used the C-BARQ to examine dog behaviour were reviewed to determine the most appropriate methods of data analysis for comparison between the two trial groups. The methods of data preparation, analysis, and consideration of covariates varied between these studies (Appendix 5). No studies reported the difference in trait scores that would represent a real-life, observable, significant change in dog behaviour, although Hsu and Serpell (2003) reported graphical representations for distributions of scores for dogs with and without a clinical behaviour problem. In addition, mean behavioural trait scores and S.D., or actual effect size were rarely reported, which reduced understanding of the size of the difference in scores that are reported as significant.

One of the limitations to the present study relates to effect sizes. Power analysis suggested that the detectable effect sizes based on the study population and values of alpha and beta required to balance the likelihood of Type I and II errors for C-BARQ analyses were 0.4 to 0.5. Effect sizes for differences in C-BARQ behaviour factor scores for other study populations vary. Effect sizes, either reported or calculated, from other studies have been identified between 0.1 and 1.2 for Training (mean 0.5), 0.0 and 1.8 for

Aggression (mean 0.9), 0.0 and 1.7 for Fear (mean 0.6), 0.3 and 0.7 for Excitability (mean 0.5) and 0.3 and 0.6 for Attachment/ Attention-seeking (mean 0.4), demonstrating a wide range across different studies (Serpell and Hsu, 2005; Duffy *et al.*, 2008; Grigg *et al.*, 2016; Bowen *et al.*, 2019; Hare *et al.*, 2021). However, it is more common that effect sizes are not reported, and that calculation of them is not possible due to no mean and standard deviation data reported.

Based on effect sizes that have been reported, and with no prior C-BARQ data for the present study population on which to base effect size estimates, the effect sizes from power analysis appeared suitable. However, the actual effect sizes were small; between 0.0 and 0.2 for one-year questionnaires, between 0.03 and 0.30 for three-year questionnaires, with the exception of Aggression that was 0.48, and between 0.08 and 0.26 for the change in scores. The Guide Dogs breeding programme was established in 1960 (Guide Dogs, 2021) and dogs have been selectively bred to be behaviourally well-suited for the guide dog programme since then. In addition, bitches were all reared and socialised following standardised guidelines and had similar life experiences that will impact the range of behaviours observed. Indeed, previous work has highlighted that extreme aggressive behaviour is uncommon in guide dogs, and the range of behavioural displays of aggression reported in other dog populations measured using C-BARQ is not observed for the current population (Murphy, 1998; Hunt *et al.*, 2020). This reduced variation in observable behaviours between dogs in the programme could influence the small effect sizes when compared to other populations.

Dog behaviour can be influenced by their health. Dogs with disease, those experiencing pain or on treatment, or those that experienced an unforeseen event during the study period, such as a dog attack, may display atypical behaviours (Overall, 2003; Camps *et al.*, 2012; Moxon *et al.*, 2016; Lopes Fagundes *et al.*, 2018; Mills *et al.*, 2020).

When completing the behaviour questionnaires, respondents were asked to describe behaviours "in the recent past (i.e. during the last few months)" that their bitches had displayed. However, health or events experienced during the same timeframe were not considered. The incidence of some health problems was influenced by trial group (see Chapter 8); it is possible that there may have been an influence of these on bitch behaviour in the months prior to questionnaire completion. For future studies, aiming to also understand and either control for, or exclude based on, health conditions that may be impacting dog behaviour is advised.

At the time of one-year behaviour questionnaire completion, 91 Control bitches had not yet been neutered and a further 23 bitches were neutered in the two weeks prior to questionnaire completion. The respondents were asked to describe how their puppy had been behaving during the last few months, therefore almost all respondents were providing responses for entire bitches at one year of age. This could be considered as a study limitation and future studies that use a similar design could include additional questionnaires at six months of age.

Questionnaires that require respondents to recall dog behaviour over a period of time may be subject to recall bias (Coughlin, 1990), and more recent behaviours may have been recalled more easily. For behaviour questionnaires at thee-years of age, rehomers and guide dog owners may have lived with their bitches for different lengths of time, a factor that was not considered in analysis and that could be included in future similar studies. Additionally, particularly uncharacteristic or undesirable behaviours may be more memorable than a bitch's more normal behaviour, even if outside of the timeframe being considered (Coughlin, 1990). Respondents may also have been less likely to report undesirable behaviours because the questionnaire was not anonymous, similar to findings in confidential and non-confidential questionnaires completed by owners when relinquishing dogs to animal shelters (Segurson *et al.*,

2005). Although in a similar, larger study using C-BARQ to measure behaviour reported by relinquishing owners, a difference was not observed between anonymised and non-anonymous questionnaires (Duffy *et al.*, 2014). The nature of the present study's design means that these risks could be expected to apply to both groups equally, therefore the risks of this bias limiting the value of the results presented is low.

Bias may also result from the respondents' subjective interpretation of behaviour, experience and knowledge of dog behaviour and body language, and the time that they had known the dog. Reliability in behaviour observation has been shown to be dependent on experience and knowledge (Caro *et al.*, 1979; Diesel *et al.*, 2008; Fratkin *et al.*, 2015). No record of experience was made for respondents in the present study, and bitches' behaviours were measured using a questionnaire completed by one person, with no repeated measures within and between respondents. Guide dog owners may not notice subtle behavioural signs and changes in body language that indicate very mild behaviours; this could have resulted in lower behaviour factor scores. While not previously validated with guide dog owners, C-BARQ is a validated tool for collecting behavioural data from dog owners with good reported reliability.

C-BARQ is a behaviour questionnaire designed for pet dogs, that has also been used to evaluate behaviour in assistance dogs (Duffy and Serpell, 2012; Serpell and Duffy, 2016; Hare *et al.*, 2018; Bray *et al.*, 2019; Bowen *et al.*, 2019; Hare *et al.*, 2021). However, the appropriateness of a pet dog questionnaire for guide dog populations has been questioned (Hunt *et al.*, 2020). Since this project was designed, a new behaviour questionnaire that is more appropriate for guide dogs has been developed (Harvey *et al.*, 2016a; Hunt *et al.*, 2020). This questionnaire measures behaviours at five, eight and 12 months of age and demonstrates better predictive power for outcome than C-BARQ when completed at eight

months of age (Hunt *et al.*, 2020). The present study used questions from the C-BARQ in a shorter questionnaire, which limits direct comparison between the present study and those using the full version of the C-BARQ due to differences in a subscale structure and may limit the conclusions that can be drawn. Despite these limitations, the use of C-BARQ in the present study has allowed owner-reported behaviour to be measured and compared using a validated questionnaire. C-BARQ was not used for predictions of outcome from the training programme, and the differences identified are valid. Future studies in guide dog programmes could consider using the new guide dog specific questionnaire; however, this has not yet been validated in adult dogs.

6.6 CONCLUSION

This is the first time that the effects of neutering bitches before or after puberty on behaviour have been investigated in a prospective study design. Using a validated measure of dog behaviour, the results suggest that there is little to no effect on future behaviour for bitches in an assistance dog programme that were neutered before or after puberty. There are important limitations to consider with regard the effect sizes observed compared to those that could have been expected that should be considered when interpreting the results. However, the study provides important information for other researchers in the field with regard the size of study populations needed for valid statistical analysis. Additionally, impacts on behaviour should not inform neutering timing decisions in isolation, but should be considered alongside the evidence relating to the impacts on health and wellbeing.

These results will be of interest to assistance and working dog organisations, veterinarians and people working in the field of dog behaviour and training, as well as pet dog owners. They provide previously unknown information regarding the impact of neutering relative to stage of reproductive development in bitches on future behaviour.

Chapter 7. A prospective study to investigate the impact of neutering bitches prepubertally or post-pubertally on progression through an assistance dog training programme

7.1 INTRODUCTION

The impact of neutering at different ages or stages of reproductive development, such as before or after puberty, on progression of bitches through a training programme for assistance or working dogs has not been well-studied. Batt *et al.* (2010) conducted a survey of International Federation Guide Dog Schools to examine the relationships between success as a guide dog and puppy management. Within the methodology, the authors stated that age at neutering was included in the survey. However, no results were presented thereafter relating to neutering age.

Only two studies have been identified specifically investigating neutering and the impact on progression though assistance dog training programmes. De Sapio *et al.* (2005) reported the findings from a study of 133 male dogs in a guide dog training programme that were neutered at different ages; seven weeks of age, seven months of age and 12 to 14 months of age. In that study, the authors reported that qualification as a guide dog and behavioural reasons for withdrawal were not affected by age at neutering. However, qualification rates (28.9-44.4%) and the number of dogs withdrawn for behavioural (13-20) reasons were not the same for all groups, therefore the study may have been underpowered due to the small number of dogs included. Zlotnick *et al.* (2019) conducted a retrospective analysis of data from Saint Francis Service Dogs, an organisation training service dogs to assist people with disabilities in the USA. The impact of neutering at different ages on health and behavioural reasons for withdrawal from the programme were examined for 245 dogs (male and female) that were trained between 1996 and 2017. Dogs were grouped as neutered (a) at

younger than seven months of age (suggested as prepubertal), (b) between seven and 11 months of age and (c) older than 11 months of age (suggested as post-pubertal). There were differences identified for both sexes in health and behavioural withdrawals that were attributed to being neutered at different ages. Withdrawal from the training programme and withdrawal for behavioural reasons were reported less frequently for dogs neutered between seven and 11 months of age than dogs in either of the other two age groups, and withdrawals for health reasons were also reported less frequently for dogs neutered between seven and 11 months of age than before seven months of age. Neither study reported clear recommendations for neutering in relation to the timing of puberty, although Zlotnick *et al.* (2019) recommended neutering between seven and 11 months of age.

There remains a lack of evidence to support assistance dog organisations when making decisions about the most appropriate time to neuter their bitches. The impact of timing of neutering in relation to age on certain behaviours and the fact that behaviours such as distraction, excitability, aggressiveness, fearfulness and suspicion have been shown to be important in the success of working dogs (Goddard and Beilharz, 1982, 1983; Arata *et al.*, 2010; Tomkins *et al.*, 2011; Harvey *et al.*, 2016b), imply that the stage of reproductive development at the time of neutering could affect a bitch's progression through training and ultimate likelihood of qualifying as an assistance dog. However, as suggested by Gobello (2014), when considering other published studies that report findings on pre and post-pubertal dogs, the use of age as an indicator is inappropriate and should not be considered as the sole marker for puberty.

This study investigates the effects of timing of neutering in relation to puberty (rather than age at neutering) on progression through an assistance dog training programme in female dogs for the first time. This will provide important information for assistance and working

dog programmes on whether to neuter female dogs before or after puberty for optimum progression through their training programmes.

7.2 AIM AND OBJECTIVES

7.2.1 Hypothesis

Neutering bitches prepubertally impacts the time taken to progress through, or qualification from, an assistance dog training programme compared to bitches neutered post-pubertally.

7.2.2 Aim

To examine data for bitches neutered before or after puberty using a prospective study design, to compare training times, age at entry to training, age at outcome (qualification or withdrawal), withdrawal reasons, qualification rates and success rates.

7.2.3 Objectives

1) To determine whether there were any significant differences in age at entry to training and qualification, or qualification rates for bitches neutered before or after puberty.
2) To determine whether there were any significant differences in training times for bitches neutered before or after puberty.
3) To compare withdrawal reasons and success rates for bitches neutered before or after puberty.

7.3 MATERIALS AND METHODS

7.3.1 Study design

A prospective study was undertaken using 306 Guide Dogs' bitches born between 22/02/2012 and 09/08/2015. Bitches were neutered either prepubertally or post-pubertally (see Section 4.4.4). Data were gathered to examine bitch progression through the guide dog training programme.

7.3.2 Study setting

Within Guide Dogs, all dog movements, including progression through the training programme, qualification, withdrawal from training, withdrawal post-qualification and ultimately retirement and death are recorded in Guide Dogs' electronic database (GDIR). When study bitches were in training (from April 2013 to May 2018), Guide Dogs was transitioning to a new training model that aimed to bring together the early and advanced training stages in the same location. Therefore, some bitches were trained following the traditional (standard) training model (puppy raising - early training in a guide dog training school - advanced training and on class in a guide dog mobility team), some bitches progressed through the new training model (puppy raising - early training, advanced training and on class in a guide dog mobility team) and some progressed through a combination of the two models. The decision to progress dogs from one stage to the next is made by technical staff groups working in consultation with process managers, based on a range of behaviour and health data, and can depend on business needs and available resources. Dogs can also re-enter a previous training stage for reasons related to training performance resulting in more than one period spent in a stage.

For each study bitch, the date that they entered each stage of training, the region of the UK (Scotland and Northern Ireland, North, Wales and West, South and East) and the Guide Dogs training location (which puppy raising scheme, guide dog training school or mobility team), the number of times they had been in each stage of training, their route of progression through training (standard, new or combined) and their outcome (qualified or withdrawn) were recorded in GDIR. In addition, whether the bitch remained qualified with their original guide dog owner (success) for a one-year (short-term) and three-year period (longer-term) was noted.

For dogs that are withdrawn, the reasons for withdrawal are recorded by the member of staff responsible for the dogs in the stage from which they are withdrawn. Reasons can relate to the dogs' health and/or behaviour. For dogs withdrawn after qualification, 'guide dog owner circumstances' is an additional possible cause for withdrawal, with reasons including guide dog owner ill health and deciding a dog is not for them. For study bitches withdrawn from the training programme, the overall withdrawal reason (health, behaviour, health and behaviour) and the detailed withdrawal reasons; the specific health and or behavioural reasons that the bitches were withdrawn, were extracted from GDIR. Bitches withdrawn for behavioural reasons could have been withdrawn for up to four individual reasons; the primary withdrawal reason and up to three additional reasons. All reasons were recorded.

7.3.3 Study participants

The study population included 306 bitches from five different crossbreeds allocated to two groups; PPN (n=155) and Control (n=151) (see Section 4.4.4). Five of these bitches (3 PPN, 2 Control) were transferred to other working dog organisations from puppy raising due to planned exchanges between assistance dog schools. The study population for the examination of progression through the guide dog training programme therefore included 301 bitches; PPN (n=152) and Control (n=149). There was no significant difference in breeds between the two trial groups (Chi-square=2.424, D.F.=3, P=0.489; Table 7.1). All first-generation backcrosses were grouped into one breed group for analysis.

Table 7.1. The number of bitches of each crossbreed (sire first) that were neutered prepubertally (PPN) or post-pubertally (Control) in a prospective study examining the effect of neutering before or after puberty on progression through the guide dog training programme for 301 bitches.

Breed	Number of PPN bitches	Number of Control bitches
Golden Retriever cross Labrador	98	103
Golden Retriever cross (Labrador cross Golden Retriever)*	0	1
Labrador cross Golden Retriever	23	18
Labrador cross (Golden Retriever cross Labrador)*	28	22
Labrador cross (Labrador cross Golden Retriever)*	3	5
Total	152	149

*Breeds grouped as 'first-generation backcrosses' for analysis.

7.3.4 Variables and data sources

Outcome variables examined were 1) the number of days in each stage of training; 2) the age at entry into early training; 3) outcome from the training programme; 4) age at outcome; 5) time from entry to early training to outcome; 6) withdrawal reasons; 7) success as a guide dog to one and three years' post-qualification. All data were extracted from GDIR. For all 301 bitches, movement data were examined to identify the dates, regions and Guide Dogs' training locations that bitches entered puppy raising, early training, advanced training and on class stages as well as the number of times they were in each stage. Whether each bitch qualified as a guide dog and the date; or was withdrawn from the guide dog programme and the date, stage of withdrawal, overall withdrawal reason and detailed withdrawal reasons were recorded using the movement and withdrawal data. Overall withdrawal reasons were recorded as health, behaviour, or health and behaviour. For bitches that qualified as guide dogs, movement data were examined at one and three years post initial qualification date, and whether the bitches remained qualified with their original guide dog owners during that time was recorded. For bitches withdrawn from their original guide dog owners, the withdrawal dates and reasons were noted as well as whether the bitches qualified for a second time with a new guide dog owner or were permanently withdrawn and rehomed. Data to determine success rate to three years post-

qualification were extracted on 27/05/2020 and were available for 298 of the 301 bitches.

Confounding variable data were also extracted from GDIR: the number of puppy raisers, number of puppy development advisors, the main puppy development advisor responsible for each bitch during puppy raising, the geographical region that each puppy entered puppy raising, early training and advanced training, the location that the bitches entered early training and advanced training, and the route of progression through training (training model) were recorded for each bitch.

7.3.5 Bias and study size

Random effects and confounding factors were included in the statistical analysis for days in each stage of training, age at entry to early training and time to outcome to account for the effects of these factors when examining the main effect of trial group. Dog breed has been shown to impact qualification rates (Ennik *et al.*, 2006). While breeds of dog within the study were similar, and were not different between trial groups, there exists potential for variation in rate of progression through training. Thus, bitch breed was included in the statistical models.

Variation in recording behaviour that can be attributed to individual trainers has previously been reported within Guide Dogs (Evans, 2014). Additionally, it is possible that each puppy development advisor, trainer and instructor had different perceptions of the impact of neutering bitches at different times. The effect that this would have on their ability to progress through training may have unconsciously biased the results. For days in puppy raising, the model included main puppy development advisor. For days in early training, advanced training and on class, and qualification rates it was not possible to include Guide Dog Trainer or Guide Dog Mobility Instructor in the models, due to many trainers and instructors each having small numbers of dogs.

Region of the UK and Guide Dogs' training location were included in all models to account for any variation in progression resulting from dogs being trained by staff working in different areas of the UK. The training model (standard, new, combination), although not different between trial groups, was included in the models to account for any difference in progression caused by variations in training methodology. The capacity (staffing and dog numbers) in the next stage of training and guide dog owner demand may have also impacted a bitch's ability to progress. These could not be measured within the present study, however, the confounders could be expected to act on both PPN and Control bitches equally.

Study size was limited by the available cohort of bitches produced by Guide Dogs during the recruitment period for the study that were placed in puppy raising within a suitable distance to travel to one of four national veterinary practices for neutering surgery. Values of alpha were determined use G*Power for each analysis (see Section 4.7). The mean and confidence intervals (CI) were reported.

7.3.6 Quantitative variables and statistical methods

The number of days in puppy raising, age at entry to early training, days in early training, days from entry to early training to outcome (qualified or withdrawn) and days in advanced training (calculated as days in advanced training plus days on class) were calculated using the dates of entry to each training stage. Data for bitches that were identified as being in a training stage more than once were examined manually to ensure the correct number of days in each stage were recorded. No bitches had missing data.

7.3.6.1 Training times and age at entry into training

Data were explored statistically to understand associations between covariates and the dependent variables (days in each training stage and age at entry to early training, reported as mean ± SEM). Data for days in each stage of training (puppy raising, early training, advanced training and on class) and age at entry into training were

imported into MLwiN v.3.04 and were analysed using multilevel models. Each model had three levels: 1) individual bitch ID, 2) puppy raising scheme or training location and 3) puppy raising or training region, along with covariates that were included by forward addition, with trial group added last. Three puppy raising schemes, five early training locations and four advanced training locations had less than five puppies and were grouped as 'Other' in their respective models.

For days in puppy raising, alpha and beta were 10% and 5% respectively with an effective sample size of 64 dogs. Covariates were bitch breed, the training model (standard, new, combination), the number of puppy development advisors, the number of puppy raisers, the main puppy development advisor, bitch outcome (qualified or withdrawn) and trial group (PPN or Control) (Model 1, Appendix 7).

Main puppy development advisors who had less than five puppies (n=13 with between one and four puppies each) were grouped as 'Other' to give 23 different main puppy development advisors.

For days in early training alpha and beta were 10% and 5% respectively with an effective sample size of 264 dogs. Covariates were bitch breed, training model, number of times in early training, age of entry to early training, bitch outcome (qualified or withdrawn) and trial group (PPN or Control) (Model 2, Appendix 7).

For days in advanced training and on class, alpha and beta were 10% and 5% respectively with an effective sample size of 210 dogs. Covariates were bitch breed, training model, number of times in advanced training, number of times on class, early training region, days in early training, age into advanced training, bitch outcome (qualified or withdrawn) and trial group (PPN or Control) (Model 3, Appendix 7).

For age at entry to early training, alpha and beta were 10% and 5% respectively with an effective sample size of 38 dogs. Covariates were bitch breed, early training region, early training location, training model, number of puppy development advisors, number of puppy raisers and trial group. Bitches that had been withdrawn prior to the stage were excluded (Model 4, Appendix 7).

Model progression was reviewed by examining tail area Chi-square *P* values. To determine whether model results were significant for each factor, the t-critical (Z) value for each model was used along with the mean and S.E. from the model for each factor to create CI. Those that included zero were not significant. Standardised residuals were checked for normality and examined graphically to examine model fit and to identify outliers in the data.

7.3.6.2 Outcome from the Guide Dogs training programme

The number and percentage of bitches from each group that qualified as guide dogs were described and examined using two logistic regression models (XLStat2016, Addinsoft, USA). The first model included all 301 bitches with outcome as a binary dependent variable and breed, number of puppy development advisors, number of puppy raisers, and trial group (PPN or Control) as predictor variables. The second model included 264 bitches that entered early training. Outcome was the dependent variable and breed, number of puppy development advisors, number of puppy raisers, training model, region entered early training and trial group were predictor variables. Alpha and beta were 5% and 6% respectively with an effective sample size of 281 dogs. Predictor variables were added to the model by means of forward addition with trial group added last. Changes in the model with each addition were reviewed using the Chi-square value for difference in -2log-likelihood. Goodness of fit statistics (Akaike's Information Criterion (AIC), Schwarz's Bayesian Criterion (SBC) and area under the curve on the generated receiver operating characteristic curve (AUC)) were examined to select the best model.

Kaplan-Meier survival curves and Cox regression analysis (SPSS version 22, IBM, USA) were used to examine the progression of bitches through the training programme to qualification for bitches based on their age (n=301). Prior to early training, bitches were in the puppy raising stage where the intervention (neutering) could have impacted on days in puppy raising for Control bitches. Therefore, a second model examining the time from entry to early training to qualification (n=264) was used to account for this. Risk factors included in the Cox regression models were bitch breed and trial group. For the analysis of time from entry to early training to qualification, early training region was also included as a risk factor and training model was included as a stratum variable due to the violation of model assumptions. Hazard ratios and CI were reported.

7.3.6.3 Bitches withdrawn from the training programme before qualification

Detailed withdrawal reasons were categorised into one of 14 groups dependent on the body function affected (health; as reported by Caron-Lormier *et al.*, 2016a) or for behaviour, into one of nine categories as previously described (Caron-Lormier *et al.*, 2016b). The number of bitches withdrawn for health and behaviour were compared between the groups using a Chi-square test (Power = 90%, alpha = 10%, effective sample size = 96, for an effect size of 0.3). Overall and detailed withdrawal reasons were described and the total number of bitches with behavioural reasons in each category were compared between trial groups using a Chi-square test (Power = 95%, alpha = 5%, effective sample size = 124, for an effect size of 0.4). Behavioural categories were excluded from statistical analysis when n<3 for either one of the trial groups. The number and percentage of bitches from each group that were withdrawn from each training stage were described and examined using a Chi-square test (Power = 90%, alpha = 10%, effective sample size = 96, for an effect size of 0.3). Chi-square tests were performed using XLStat2016 (Addinsoft, USA).

7.3.6.4 Success as a guide dog

The number and percentage of bitches in each trial group withdrawn one year (short-term) and three years post-qualification (longer-term), and the overall reasons for withdrawal (health, behaviour, guide dog owner circumstances) were described and differences between the two groups were examined using Chi-square tests using XLStat2016 (Addinsoft, USA). Power was calculated as 95% and alpha as 4% for an effect size of 0.3 with an effective sample size of 152. The mean number of days between qualification and withdrawal was calculated as withdrawal date minus qualification date, the percentage of bitches withdrawn for each reason (health, behaviour, client circumstances) and the proportion of each that requalified were described.

7.4 RESULTS

7.4.1 Participants

Three hundred and one bitches entered puppy raising (152 PPN, 149 Control), 264 bitches continued into early training (130 PPN, 134 Control), 210 bitches into advanced training (105 PPN, 105 Control) and 195 qualified as a guide dog (97 PPN, 98 Control). One hundred and ninety-four bitches (93 PPN, 101 Control) progressed through Guide Dogs' standard training model, sixty-one (34 PPN, 27 Control) through Guide Dogs' new training model and nine (3 PPN, 6 Control) via a combination of both models. There was no difference in the number of bitches that progressed through the standard or new training model between PPN and Control groups (Chi-square=1.129, D.F.=1, P=0.288). Seven bitches had two periods in early training, 10 bitches had two periods in advanced training and seven bitches had two periods on class recorded in GDIR. Two bitches (1 PPN, 1 Control) had three periods in advanced training recorded and were excluded from analysis for days in advanced training and on class due to the small number of bitches following an unusual route with additional days in the stage before qualifying (Control) or being withdrawn (PPN) that affected data outliers and model fit.

Bitches qualified as guide dogs between 16/10/2013 and 03/05/2018. Data were collected on success on 27/05/2020 by which date three bitches (2 PPN, 1 Control) had qualified less than three years previously. These bitches were excluded from the examination of success to three years post-qualification. Twenty-two bitches (14 PPN, 8 Control) were withdrawn within one year following qualification, 15 of which (8 PPN, 7 Control) requalified. Thirty-five bitches (21 PPN, 14 Control) were withdrawn within three years following qualification, of which 20 requalified (9 PPN, 11 Control). No bitches had missing data.

7.4.2 Days in each stage of training

Multilevel models were used to examine the association between trial group and days in each stage of training for the 301 bitches that entered puppy raising, 264 bitches that entered early training and 208 out of the 210 bitches that entered advanced training (Table 7.2). Two bitches (1 PPN, 1 Control) that entered advanced training three times were excluded from analysis.

Table 7.2. The minimum, maximum, mean (± SEM) number of days in each stage of training for 301 bitches in a prospective study examining the effect of neutering prepubertally (PPN) or post-pubertally (Control) on progression through a guide dog training programme. Superscript letters show significant differences between PPN and Control bitches for each stage. The number of days in each stage includes data for bitches that qualified and were withdrawn.

Stage	Puppy raising		Early training		Advanced training and on class	
	PPN n=152	Control n=149	PPN n=130	Control n=134	PPN n=105	Control n=105
Mean and SEM	387 4.7[a]	406 4.0[b]	122 3.5[a]	120 3.3[a]	97 3.8[a]	108 5.1[b]
Range	149 – 554	196 – 597	25 – 288	4 – 232	12 – 250	25 – 269

n=number of bitches

7.4.2.1 Days in puppy raising

Bitches in the PPN group spent a mean of 386.6 (SEM=4.7) days in puppy raising and Control bitches a mean of 406.3 (SEM=4.0) days (P=0.012; Figure 7.1a). Bitches in the Control group spent

significantly more days in puppy raising (+15.5 days, 90% CI=5.3 to 25.7 days) than PPN bitches, although effect size was -0.400, smaller than that determined by power analysis (0.837). Days in puppy raising were also impacted by breed and the number of puppy raisers for each bitch. First-generation backcross bitches spent significantly more days in puppy raising (+14.4 days, 90% CI=1.2 to 27.7 days) than Golden Retriever x Labrador bitches. Puppies with three puppy raisers spent significantly more days in puppy raising than puppies with one puppy raiser (+50.8 days, 90% CI=10.6 to 90.9 days).

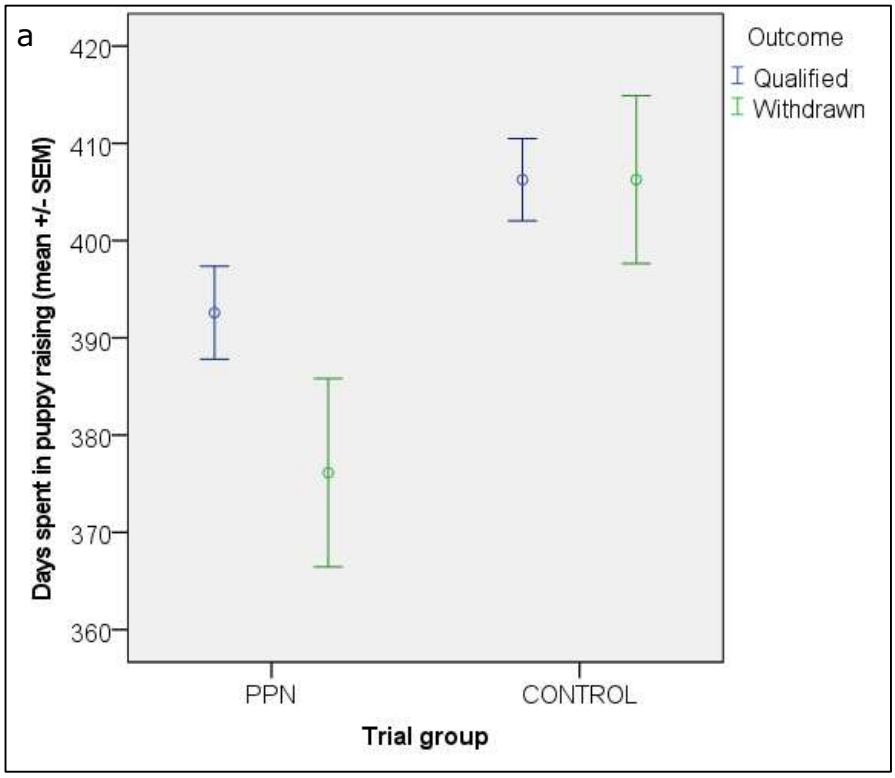
7.4.2.2 Days in early training

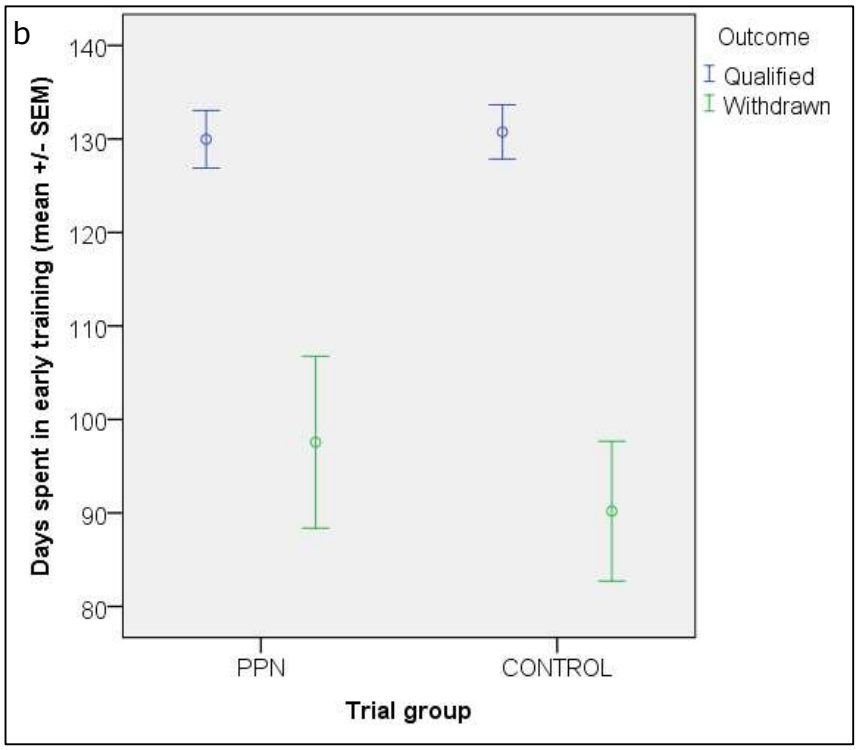
PPN bitches spent a mean of 121.7 (SEM=3.5) days in early training and Control bitches a mean of 119.9 (SEM=3.3) days. The number of days bitches spent in early training was not influenced by trial group (P=0.493; Figure 7.1b); Control bitches -2.8 days (90% CI=-9.6 to 3.9 days) compared to PPN bitches. The number of times in early training, outcome from the training programme and training model all significantly affected the number of days that bitches spent in early training. Bitches spent more days in early training if they had been in the stage twice rather than once (+76.9 days, 90% CI=55.8 to 98.0 days), less days in early training if their outcome from the whole training programme (early training or advanced training and on class) was withdrawal rather than qualification (-37.7 days, 90% CI=-45.3 to -30.0 days) and less days in early training if they were in the new training model than the standard training model (-8.9 days, 90% CI=-16.9 to -0.9 days).

7.4.2.3 Days in advanced training and on class

PPN bitches spent a mean of 96.5 (SEM=3.8) days in advanced training and on class and Control bitches a mean of 108.4 (SEM=5.1) days (P=0.062; Figure 7.1c); Control bitches spent significantly more days in the stage than PPN bitches (+8.3 days, 90% CI=1.0 to 15.7 days). Whether bitches were withdrawn before

entering on class (-97.9 days compared to bitches that entered on class, 90% CI=-158.9 to -37.0 days), the training model (new compared to standard: -11.6 days, 90% CI=-20.5 to -2.6 days; combination compared to standard: +29.2 days, 90% CI=10.9 to 47.7 days), being in advanced training twice (+56.7 days compared to bitches in advanced training once, 90% CI=29.5 to 83.9 days) and being withdrawn from the training programme (+62.4 days compared to bitches that qualified, 90% CI=3.8 to 120.9 days) also affected the number of days bitches spent in advanced training and on class.





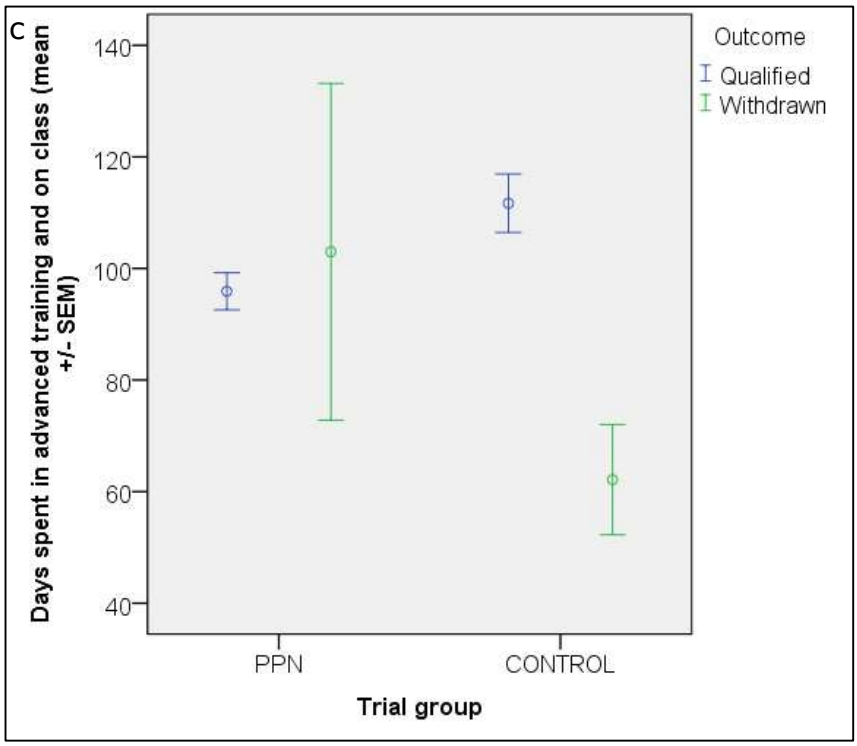


Figure 7.1. The mean (± SEM) number of days that bitches were in a) puppy raising (n=301), b) early training (n=264) and c) advanced training and on class (n=210) in a prospective study of the effect of neutering before (PPN) and after (Control) puberty for bitches that qualified as guide dogs or were withdrawn.

7.4.3 Age at entry into training

PPN bitches entered training at a mean age of 446.9 (SEM=49.0) days of age and Control bitches at 459.1 (SEM=42.2) days of age. Trial group was significantly associated with age at entry to training (P=0.032; Figure 7.2); Control bitches entered training later than PPN bitches (+11.3 days, 90% CI=2.4 to 20.2 days), although effect size was -0.289, smaller than that determined by power analysis (1.095). Bitch breed (first-generation backcross bitches +16.8 days of age compared to Golden Retriever x Labrador bitches, 90% CI=5.3 to 28.3), the region that bitches entered early training (Wales and West + 81.6 days of age compared to Scotland and NI, 90% CI=2.8 to 160.4), the location for early training compared to Forfar guide dog training school (Leamington guide dog training school: -111.8 days of age, 90% CI=-190.4 to -33.2 days; Manchester mobility team: +154.9 days of age, 90% CI=15.9 to 293.9 days), progressing through the new training model compared to the standard model (-87.6 days of age, 90% CI=-164.2 to -10.9 days), having three puppy development advisors rather than one (+26.1 days of age, 90% CI=5.1 to 47.2 days) and having three puppy raisers rather than one (+45.3 days of age, 90% CI=12.4 to 78.2 days) were also associated with age at entry to training. The number of puppy raisers and number of puppy development advisors each puppy had were not related (Cohen's Kappa=0.027).

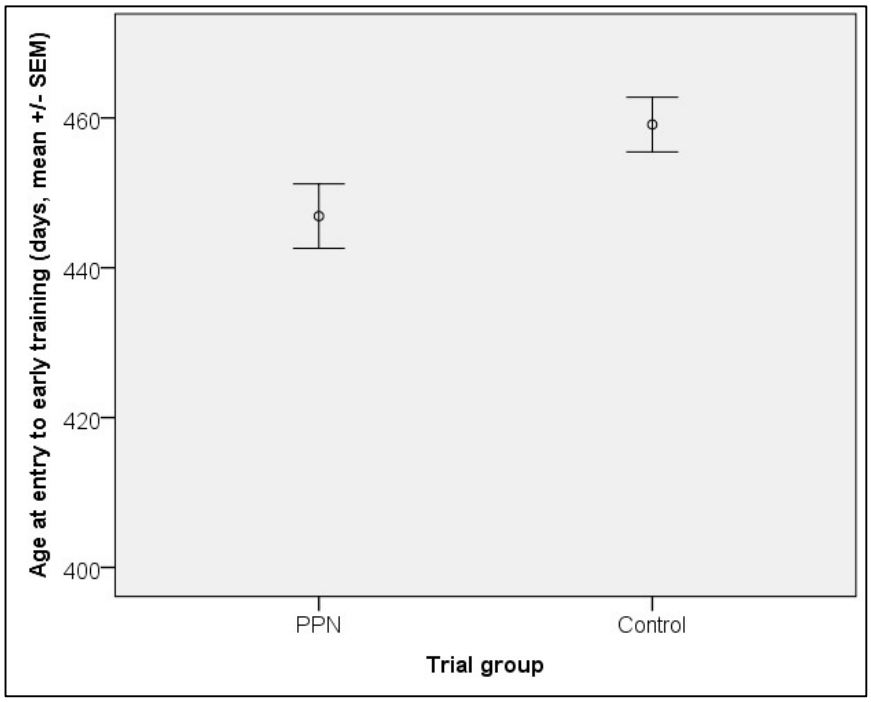


Figure 7.2. The mean ± SEM days of age at entry to training for 264 bitches neutered prepubertally (PPN, n=130) or post-pubertally (Control, n=134) in a prospective study.

7.4.4 Outcome from the Guide Dogs' training programme

One hundred and ninety-five bitches (64.8%) qualified as guide dogs (97 PPN [63.8%], 98 Control [65.8%]) and 106 (35.2%) were withdrawn (55 PPN [36.2%], 51 Control [34.2%]). PPN bitches qualified between 1.5 and 2.2 years of age (mean 1.8, SEM=0.02 years) and Control bitches qualified between 1.5 and 2.5 years of age (mean 1.9, SEM=0.02 years). For all 301 bitches, no variables in the logistic regression contributed significantly to the model (D.F.=7, Chi-square=2.190, P=0.949).

For the 264 bitches that entered early training, and including training model and region entered early training as additional predictor variables, the model was significant (D.F.=12, Chi-square=58.724, P<0.001). All six predictor variables were retained and contributed significantly to the model (Table 7.3a and 7.3b). The AUC was 0.873. The equations for the best fit models are shown in Appendix 7, Models 5 and 6.

Table 7.3a. The model parameters included in the binary logistic regression model to investigate the impact on qualification or withdrawal from the training programme for 264 bitches that entered early training (ET).

Variables	-2 Log (Likelihood)	Pr > LR	Pr > Score	Pr > Wald	Akaike's AIC	Schwarz's SBC
Training model	286.983	0.000	0.135	0.241	292.983	303.711
Training model / Region entered ET	274.338	0.000	0.339	0.493	286.338	307.794
Training model / Region entered ET / No of puppy raisers	265.427	0.000	0.424	0.625	281.427	310.035
Training model / Region entered ET / Number of puppy development advisors / No of puppy raisers	257.080	0.000	0.568	0.770	277.080	312.840
Breed / Training model / Region entered ET / Number of puppy development advisors / No of puppy raisers	248.624	0.000	0.669	0.855	272.624	315.535
Breed / Training model / Region entered ET / Number of puppy development advisors / No of puppy raisers / Trial group	244.601	0.000	0.731	0.897	270.601	317.088

AIC=Akaike information criterion. SBC=Schwarz-Bayes Criterion.

Table 7.3b. The contribution of each predictor variable to the adjustment of the binary logistic regression model.

Predictor variable	DF	Chi-square (Wald)	Pr > Wald	Chi-square (LR)	Pr > LR
Breed	2	0.630	0.730	49.380	<0.0001
Training model	2	2.798	0.247	54.896	<0.0001
Region entered ET	3	1.076	0.783	49.775	<0.0001
Number of puppy development advisors	2	0.594	0.743	49.650	<0.0001
Number of puppy raisers	2	1.021	0.600	49.715	<0.0001
Trial group	1	0.129	0.720	48.885	<0.0001

While each variable contributed significantly to the model, compared to the reference categories, no predictor variable categories had significantly higher or lower odds of qualification (Table 7.3c).

Table 7.3c. The odds ratio (OR) and confidence intervals for each predictor variable category in comparison to the reference category (*).

Variable	Value	S.E.	Wald Chi-Square	Pr > Chi²	OR	OR Lower bound (95%)	OR Upper bound (95%)
Intercept	0.828	0.363	5.212	0.022			
Breed							
*Golden Retriever cross Labrador (n=179)	0.000	0.000					
Backcrosses (n=51)	-0.275	0.358	0.588	0.443	0.760	0.376	1.533
Labrador cross Golden Retriever (n=34)	0.017	0.428	0.002	0.968	1.018	0.440	2.355
Training model							
*Standard (n=194)	0.000	0.000					
New (n=61)	0.332	0.361	0.842	0.359	1.393	0.686	2.829
Combination (n=9)	2.148	1.510	2.024	0.155	8.571	0.444	165.420
Region entered ET							
*Scotland and NI (n=53)	0.000	0.000					
North (n=41)	0.127	0.486	0.068	0.795	1.135	0.438	2.944
Wales and West (n=107)	0.378	0.396	0.913	0.339	1.460	0.672	3.171
South and East (n=63)	0.116	0.421	0.076	0.782	1.123	0.492	2.564
Number of puppy development advisors							
*1 (n=179)	0.000	0.000					
2 (n=72)	-0.100	0.321	0.098	0.755	0.905	0.482	1.696
3 and 4 (n=13)	0.492	0.758	0.421	0.516	1.636	0.370	7.228
Number of puppy raisers	0.000	0.000					

*1 (n=218)							
2 (n=41)	-0.145	0.378	0.147	0.702	0.865	0.413	1.814
3 (n=5)	-0.977	1.022	0.913	0.339	0.377	0.051	2.791
Trial group							
*PPN (n=130)	0.000	0.000					
Control (n=134)	-0.103	0.286	0.129	0.720	0.903	0.515	1.580

Cox regression models were used to examine the differences between PPN and Control bitches in time to qualification based on age and time from entry to early training. PPN and Control bitches that were withdrawn left the training programme at a younger age than those that completed the programme and qualified as guide dogs (Figure 7.3, Table 7.4). There was no significant association between trial group or breed and age at qualification (trial group P=0.072; breed P>0.280; Table 7.4), although PPN bitches took less time to reach outcome (Figure 7.4).

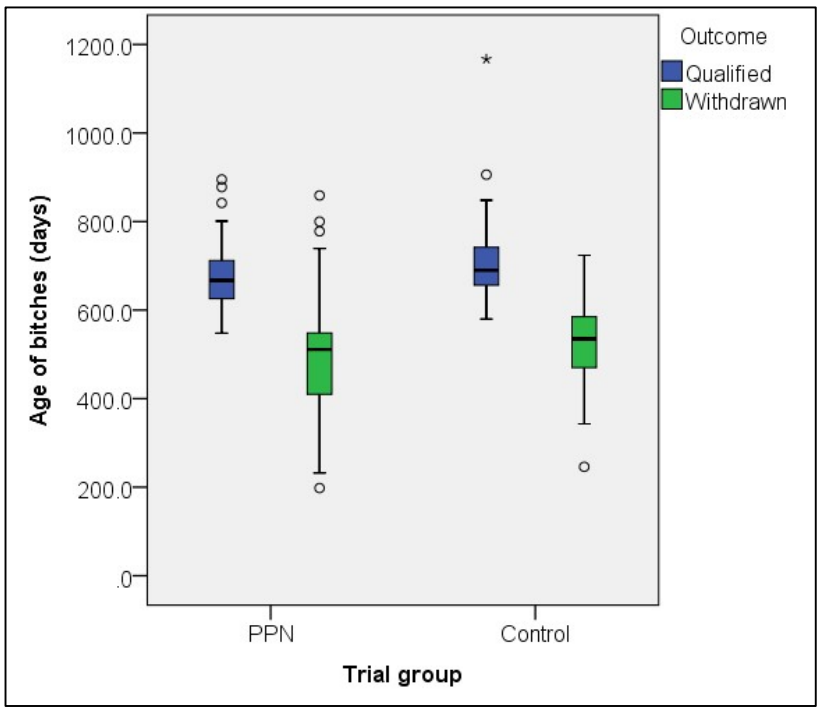


Figure 7.3. Box and whisker plot showing the age at outcome (qualification or withdrawal) for 301 bitches in a guide dog programme that were neutered prepubertally (PPN, n=152) or post-pubertally (Control, n=149) in a prospective study.

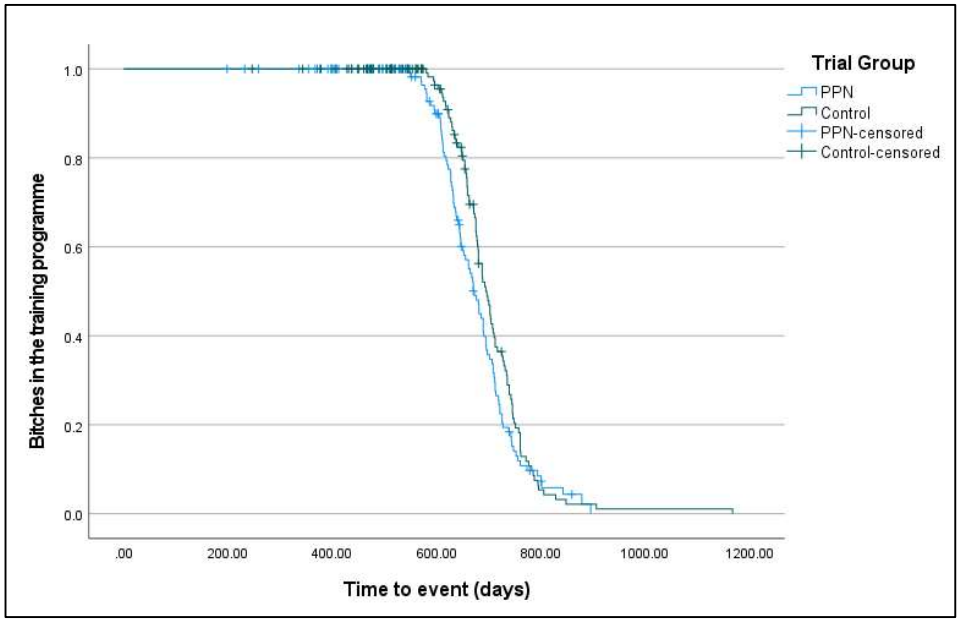


Figure 7.4. Kaplan-Meier curves of survival probability (reaching qualification) for PPN (blue) and Control (green) bitches in a prospective study investigating the effect of neutering before (PPN) or after (Control) puberty on progression through an assistance dog training programme. Censored values (+) indicate bitches that were withdrawn from the training programme. There was no significant association between trial group and age at qualification (P=0.072).

Time from entry to early training to outcome was also less for bitches that were withdrawn (Figure 7.5, Table 7.4). There was no association between any factor and the days from entrance to early training to qualification, although PPN bitches took fewer days to qualify as a guide dog (Figure 7.6, Table 7.4).

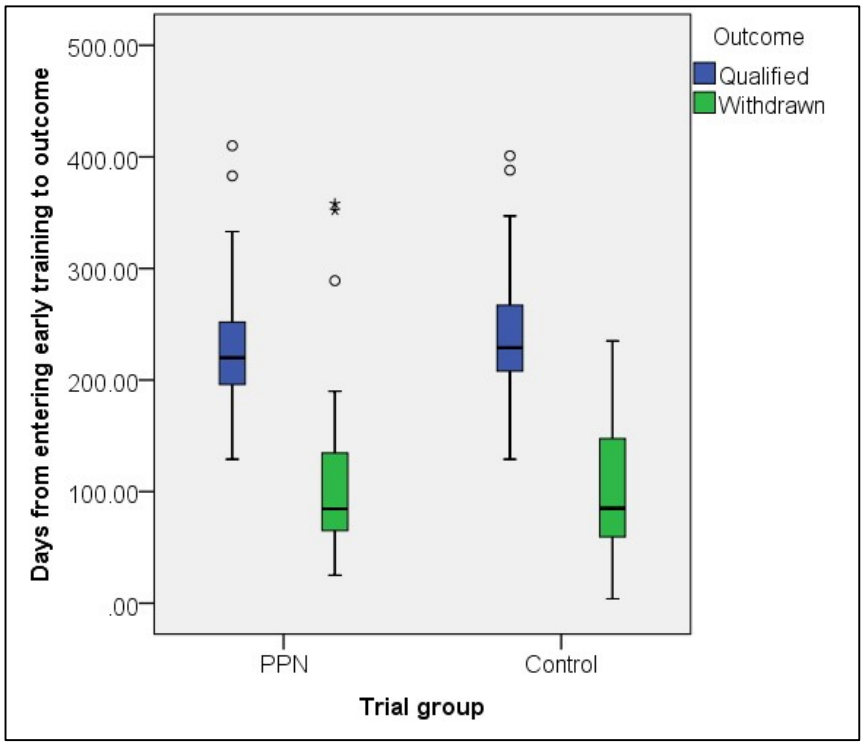


Figure 7.5. Box and whisker plot showing the time in days from entry to early training to outcome (qualification or withdrawal) for 264 bitches in a guide dog training programme that were neutered prepubertally (PPN, n=130) or post-pubertally (Control, n=134) in a prospective study.

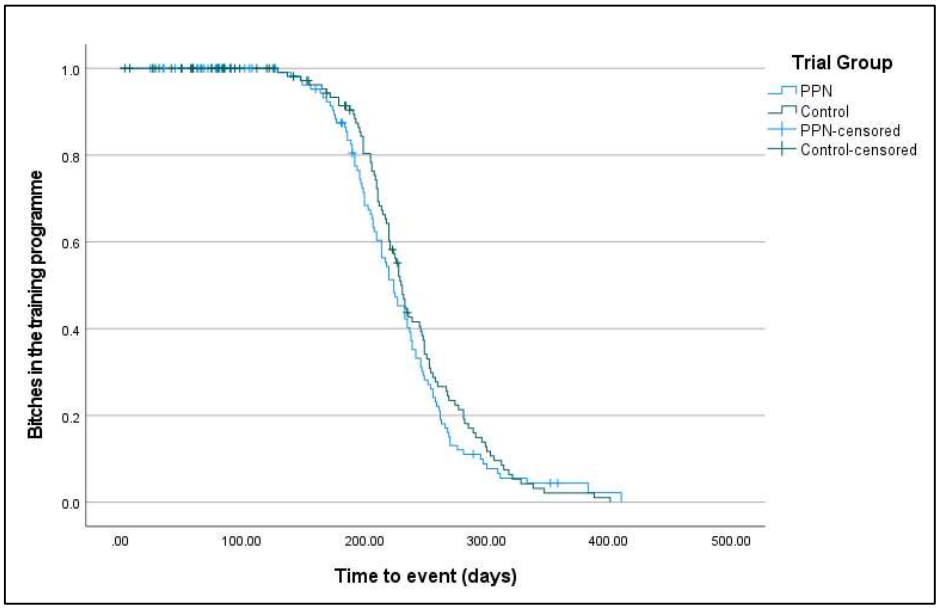


Figure 7.6. Kaplan-Meier curves of survival probability (reaching qualification) for PPN (blue) and Control (green) bitches in a prospective study investigating the effect of neutering before (PPN) or after (Control) puberty on progression through an assistance dog training programme. Censored values (+) indicate bitches that were withdrawn from the training programme. There was no significant association between trial group and time from entry to early training to qualification (P=0.622).

Table 7.4. Results of Cox regression models for risk factors affecting the days of age at qualification and days from entry to early training to qualification from a guide dog training programme for bitches neutered prepubertally (PPN) or post-pubertally (Control) in a prospective study. *R* indicates reference factor.

Risk factor	n	Mean ± S.E.	Hazard ratio	P	95% CI Lower-Upper
Days of age at qualification					
Trial group					
PPN	152	610.9 ± 10.1	*R*	*R*	*R*
Control	149	643.2 ± 9.5	0.771	0.072	0.581-1.024
Breed					
Golden Retriever cross Labrador	201	630.8 ± 7.7	*R*	*R*	*R*
Labrador cross Golden Retriever	41	615.8 ± 24.3	0.893	0.620	0.572–1.395
First-generation backcross	59	621.2 ± 17.1	0.812	0.281	0.556-1.186
Days from entry to early training to qualification					
Trial group					
PPN	129	198.2 ± 6.7	*R*	*R*	*R*
Control	133	201.5 ± 7.0	0.929	0.622	0.692-1.246
Breed					
Golden Retriever cross Labrador	178	204.8 ± 5.7	*R*	*R*	*R*
Labrador cross Golden Retriever	33	196.6 ± 12.2	1.261	0.317	0.801–1.984
First-generation backcross	51	184.8 ± 12.3	1.043	0.844	0.688–1.579
Region entered early training					
North	40	199.4 ± 11.0	*R*	*R*	*R*
Scotland and NI	52	216.8 ± 11.8	0.677	0.139	0.403–1.135
Wales and West	107	196.7 ± 7.8	1.091	0.703	0.696–1.711
South and East	63	191.5 ± 9.1	1.117	0.656	0.687–1.815

n=number of bitches

7.4.5 Bitches withdrawn from the training programme before qualification

The number of PPN and Control bitches withdrawn for health or behavioural reasons was not significantly different (Chi-square=0.159, D.F.=1, P=0.690). One hundred and six bitches were withdrawn from the training programme before qualification as a guide dog (55 PPN, 51 Control). Fifteen (14.2%) were withdrawn for health reasons (7 PPN, 8 Control), 90 (84.9%) for behavioural reasons (47 PPN, 43 Control) and one (0.9%; PPN) for a combination of health and behavioural reasons.

7.4.5.1 Health reasons for withdrawal

There were 11 distinct health withdrawal reasons reported for the 16 bitches (7 PPN, 9 Control) withdrawn for heath or health and behaviour during training (Appendix 8). These affected four body systems: gastrointestinal (1 PPN); musculoskeletal (4 PPN, 4 Control); nervous/sensory (3 Control); and skin (3 PPN, 1 Control). Statistical comparisons between PPN and Control bitches were not possible due to small numbers of bitches diagnosed with disease.

7.4.5.2 Behavioural reasons for withdrawal

Thirty-three bitches had one behavioural reason for withdrawal (17 PPN, 16 Control), 34 had two reasons (19 PPN, 15 Control), 18 had three reasons (seven PPN, 11 Control) and six had four reasons (five PPN, one Control). There were 22 distinct behavioural reasons for withdrawal in total. Seventeen distinct reasons were used to describe the primary reason for behavioural withdrawal. Behavioural withdrawal reasons were grouped into nine behaviour categories (as per Caron-Lormier *et al.*, 2016b). The most commonly reported behavioural reasons for bitches to be withdrawn were in the Environmental Anxiety category (Table 7.5). Behaviours in the Environmental Anxiety category were reported 85 times in total and as the primary withdrawal reason for 48 bitches (52.7% of 91 bitches with a behavioural withdrawal reason). There was no difference between trial groups in the total number of withdrawal

reasons for Attentiveness, Body Sensitivity, Distraction, Environmental Anxiety, Social Behaviour or Willingness/Confidence (Chi-square=4.421, D.F.=5, P=0.491). Statistical analysis for primary behavioural withdrawal categories was not possible due to small frequencies.

Table 7.5. The number of bitches within a prospective study examining the differences in progression through a guide dog training programme for bitches neutered before (PPN) or after (Control) puberty that were withdrawn from the training programme for behavioural reasons.

Behavioural withdrawal reason group	Total number of withdrawal reasons reported		Primary withdrawal reasons	
	PPN	Control	PPN	Control
Attentiveness	7	6	4	2
Body Sensitivity	4	7	2	3
Chasing	8	1	4	1
Distraction	4	4	2	1
Environmental Anxiety	39	46	23	25
Excitability	2	2	0	1
Fear/Aggression	12	1	6	1
Social Behaviour	13	6	5	3
Willingness/Confidence	7	10	2	6

7.4.5.3 Stage of training at withdrawal

The 106 bitches that were withdrawn prior to qualification were withdrawn from puppy raising (22 PPN, 15 Control), early training (26 PPN, 30 Control) and advanced training (seven PPN, six Control). There was no difference in the number of bitches withdrawn from each stage of training between trial groups (Chi-square=1.538, D.F.=1, P=0.463), although a greater proportion of withdrawn bitches in the PPN group were withdrawn in puppy raising (40.0%) and fewer in early training (47.3%) than withdrawn bitches in the Control group (29.4% and 58.9% respectively).

7.4.6 Success as a guide dog

7.4.6.1 Bitches remaining qualified for one year

Twenty-two bitches (11.3% of those qualified) aged 1.9 to 2.8 years of age were withdrawn from working within one year of qualification. There was no difference between trial groups in the number of bitches that were withdrawn post-qualification (14 PPN, 14.4%, 8 Control, 8.2%; Chi-square=1.915, D.F.=1, P=0.166). Seventeen of the 22 bitches were returned to training and 15 requalified with a new guide dog owner; eight of the 14 PPN bitches (57.1%) and seven of the eight Control bitches (87.5%). The mean number of days between qualification and withdrawal was 144.6 ± 22.0 days for PPN bitches and 132.0 ± 38.0 for Control bitches; statistical analysis was not possible due to small numbers of bitches in each group resulting in inadequate power. Bitches were withdrawn from working within one year from the date of qualification for reasons relating to bitch health (1 PPN), behaviour (5 PPN, 3 Control) and guide dog owner circumstances (8 PPN, 5 Control). A higher proportion of bitches that were withdrawn for guide dog owner circumstances requalified (n=11, 84.6%) than those withdrawn for behavioural reasons (n=4, 50.0%). Statistical analysis was not possible due to small numbers of dogs in each group resulting in inadequate power.

7.4.6.2 Bitches remaining qualified for three years

At the time of data collection, three bitches (2 PPN, 1 Control) had remained qualified with their first guide dog owner but had not yet reached qualification date plus three years and were therefore excluded from analysis. For the remaining 192 bitches (95 PPN, 97 Control), 35 (18.2%) were withdrawn from working within three years from the date of qualification (including the 22 withdrawn within one year). These were 21 PPN (22.1%) and 14 Control (14.4%) bitches (Chi-square=1.895, D.F.=1, P=0.169). The additional 13 bitches withdrawn after one but before three years post-qualification were aged 3.0 to 5.0 years of age. Twenty-two of the bitches returned to training and 20 requalified with a new guide

dog owner; nine PPN (42.9%) and 11 Control (78.6%); statistical analysis was not possible due to low numbers of withdrawn bitches comprising the sample resulting in inadequate power. The mean number of days between qualification and withdrawal was 351.1 ± 74.5 days for PPN bitches and 364.9 ± 87.0 for Control bitches; statistical analysis was not possible due to small numbers of bitches in each group. Bitches were withdrawn from working within three years from the date of qualification for reasons relating to bitch health (4 PPN, 1 Control), including two PPN bitches that died, bitch behaviour (8 PPN, 3 Control) and guide dog owner circumstances (9 PPN, 10 Control). There was no numerical difference in the proportion of bitches that were withdrawn and then requalified between those withdrawn for guide dog owner circumstances (n=15, 78.9%) or behavioural reasons (n=5, 45.5%). Numbers were too small to allow statistical analysis. Data on subsequent health withdrawals will be examined in Chapter 8.

7.5 DISCUSSION

Detailed investigation of the impact of neutering bitches before or after puberty on their progression through an assistance dog training programme has not previously been undertaken. In the present study, progression through a guide dog training programme was examined by studying variables relating to 1) the number of days in each stage of training; 2) the age at entry into early training; 3) outcome from the training programme; 4) age at outcome; 5) time from entry to early training to outcome; 6) withdrawal reasons; 7) success as a guide dog to one year and three years' post-qualification. The results showed that bitches neutered prepubertally spent less days in puppy raising and in the advanced training and on class stages, and entered training at an earlier age than those neutered post-pubertally, although the effect sizes and power of the models require consideration when interpreting these results. There was no difference in the number of days spent in early training between the groups. However, overall, there was no difference between groups in time to qualification or

withdrawal and no difference in the number of bitches that ultimately qualified as guide dogs. Additionally, there was no difference in the number of PPN and Control bitches that were withdrawn for health or behavioural reasons during training or that were withdrawn within the three years following qualification. These findings address the main study hypothesis and the results from this part of the study suggest that prepubertal neutering could be considered a suitable strategy for assistance dog organisations.

7.5.1 Progression through training

The number of days that bitches spent in each training stage was associated with trial group for puppy raising and advanced training and on class. Bitches were neutered during puppy raising; PPN bitches at six months of age, some months before the usual intake age for training of 12 to 14 months. However, Control bitches were neutered after their first oestrus, mean day 387.6 (12-13 months) of age (Moxon *et al.*, 2023a). Including the age at neuter in statistical models was not possible due to the high level of relatedness to trial group. However, it is probable that Control bitches that were neutered at an older age due to a later oestrus would have remained in puppy raising for longer due to Guide Dogs requirement to only accept neutered bitches into training. Thus, PPN bitches may have entered training earlier than Control bitches, although the difference in mean ages was only 12 days, effect size was smaller than the suggested effect size based on power analysis and there was no more variation for Control bitch data than data for PPN bitches.

The reasons for PPN bitches spending fewer days in advanced training and on class are less clear. There are many factors known to influence dog responses to stimuli and ability to learn such as early habituation and socialisation, environment, social relationships, experience and genetics (Pfaffenberger and Scott, 1959; Goddard and Beilharz, 1983; 1985; 1986; Battaglia, 2009; Kubinyi *et al.*, 2009; Vaterlaws-Whiteside and Hartmann, 2017).

However, in the current population these factors were relatively consistent between PPN and Control bitches and are therefore unlikely to have led to an increase in required training time for Control bitches. Studies have suggested that the behaviour of bitches can differ for those neutered at different ages (Spain *et al.*, 2004; Zink *et al.*, 2014; Farhoody *et al.*, 2018). However, in the only previous study identified that examined the behaviour of bitches neutered before or after puberty, no differences were identified between groups. In addition, behavioural trait scores for the bitches in the present study were not influenced by trial group at one or three years of age. Therefore, a behavioural explanation for the difference in days spent in advanced training and on class between the two groups seems unlikely. Differences between trial groups were not observed for days spent in early training. It is possible that factors not measured within this study may have impacted Control bitches in the advanced training or on class stage, such as veterinary investigations for atopy or other health conditions or training staff availability. The difference in the number of days spent in advanced training and on class between the trial groups may require further study, including behavioural and health assessments and closer monitoring of bitch progression in order to better understand the influencing factors.

When examined as a whole, there was an association between trial group and the time taken to reach outcome, either based on age at outcome or time from entry to early training. This suggests that overall, timing of neutering in relation to puberty has minimal impact on the time for bitches to reach outcome. However, it is worthy of note that PPN bitches took less days to reach outcome and qualify as a guide dog, mainly influenced by fewer days in the puppy raising stage and entering training earlier. Even small reductions in the time to outcome can result in savings or benefits in terms of resources, including staff and dog care, puppy raiser capacity, the potential to allocate resources to train more dogs, and improved welfare for dogs that are not suited to assistance dog

work (Tomkins *et al.*, 2011; Batt *et al.*, 2008). These efficiencies can have beneficial implications for large assistance dog organisations where most costs are incurred in the stages before qualification (ITV, 2014). Furthermore, reduced likelihood to qualify as a guide dog for dogs that spent longer in puppy raising and early training have previously been identified (Asher *et al.*, 2016), therefore ensuring that an intervention does not increase time spent in these stages is important.

The number of days in each stage of training and the age at entry to early training were influenced by other factors included in the models. First-generation backcross bitches compared with Golden Retriever cross Labradors, and bitches with three puppy raisers compared to those with one, spent more days in puppy raising and entered training at an older age. Additionally, bitches with three puppy development advisors compared to those with one entered training at an older age. Recombination loss (the expression of a breakdown of favourable epistatic relationships; Kinghorn, 1980) with first-generation backcrosses has previously been reported within guide dog programmes both in terms of behaviour and health (Evans *et al.*, 2015; Caron-Lormier *et al.*, 2016b). Recombination loss affecting scores in puppy assessments (Evans *et al.*, 2015) indicates that there may be some underlying difference in temperament within the backcrosses. This may impact learning and behavioural development in such a way as to cause backcrosses to require more days in puppy raising before progressing into early training. Recombination loss estimates associated with poorer assessment scores have previously been identified within the same guide dog training programme (Evans, 2014). This could support the idea that these backcrosses may not perform as well in the programme as first-generation crossbreeds.

Puppies with three puppy raisers may have experienced considerable change during key behavioural development stages, which could have negatively impacted their own development and

learning. While there is much literature relating to the effects of changes experienced by puppies during the critical socialisation period and experiences during the fear emergence period (Scott, 1958; Pfaffenberger and Scott, 1959; Fox and Stelzner, 1966; Pierantoni *et al.*, 2011; Jokinen *et al.*, 2017; Lenkei *et al.*, 2019), there is little information on the impact of changes in handlers or homes on puppy development, learning and trainability. With the exception of dog training programmes and for dogs that are relinquished to animal shelters and then rehomed, it is unusual for pet dogs to experience many different homes during puppyhood, thus limiting the possibility to study this relationship. Alternatively, these puppies may have presented with problems or issues with their behavioural development that caused the need to move to a different puppy raiser. Whether the number of puppy raisers caused, or resulted from, behavioural problems was not investigated within this study. The number of puppy raisers was also not included in statistical models for days in early training or advanced training. However, should the number of puppy raisers have a negative impact on behavioural development it is possible that this impact would continue to be observed into adulthood, especially if the disruption to puppy raisers occurs within the first few months (Fox and Stelzner 1966; Serpell, 1996; Thompson et al., 2010; Wormald *et al.*, 2016).

The number of puppy development advisors that puppies had also affected the age at which they entered training. Those with three supervisors entered training later than those with one. The number of puppy development advisors was not associated with the number of puppy raisers, therefore other factors influenced whether or not bitches had three supervisors. These could include staff turnover or long-term illness, or puppy raisers moving home into a new puppy development advisor's area. These factors are unlikely to occur because of behavioural problems, but could result in a short-term impact on learning and development due to changes in the puppy's life and environment.

Bitches that progressed through the new training model spent fewer days in early and advanced training and entered training at an earlier age. Additionally, time from entry to early training to outcome was significantly lower for bitches in the new training model. This is unsurprising as the new training model was designed to train dogs in a more effective and efficient way, reducing training times and improving capacity for increased partnership production. This is of importance for monitoring the performance of dogs in the new model. It is possible that the capacity to bring dogs into training in this new model was higher or more controlled compared to the standard training model, minimising any effect of delays caused by an excess of dogs already in training. This could have resulted in the lower age at entry to training for dogs in this training model.

Finally, differences in age at entry to training based on region and location of early training were identified in the models. While any impact of this on investigation of the effect of trial group was controlled for, regional variation may be of interest to the guide dog organisation that has aims to standardise training and minimise training times nationally to improve programme performance. Coupled with a high proportion of dogs withdrawn in training, dogs that enter training later may require more financial and resource investment before a decision can be made to remove them from the programme. While not pertinent to this study on the impact of neutering, further investigation of these regional difference and their cause could be beneficial.

7.5.2 Outcome and success as a guide dog

Despite differences in the number of days to progress through certain stages of training, although not length of training overall, there was no difference in the number of bitches that qualified as guide dogs or were withdrawn between trial groups. Additionally, for bitches that were withdrawn from the training programme, there was no difference in the number reported with each overall reason

for withdrawal. This suggests that the timing of neutering did not affect the bitches' health or behaviour in such a way that impacted their likelihood to qualify. It is important to note that this finding does not suggest that health and behaviour are not significantly impacted, outcomes that are addressed in other chapters, only that they are not impacted in ways that affect qualification.

To the authors knowledge there are no published studies investigating success as a working or assistance dog and associations with the timing of neutering in relation to puberty; these results present the first findings. The closest comparisons that can be made are to studies investigating age at neutering. In a retrospective cohort study of 245 dogs of both sexes in a service dog training programme, Zlotnick *et al.* (2019) reported that age at neutering impacted total withdrawals from the programme and withdrawals for health and behaviour, with withdrawals being less common for both female and male dogs neutered between seven and 11 months of age, compared to less than seven months or after 11 months. In contrast, in a study of 133 male Labrador cross Golden Retriever dogs in a guide dog programme, there was no effect of neutering timing (seven weeks of age, seven months of age, 12 to 14 months of age) on qualification rates or on health or behavioural reasons for withdrawal (De Sapio, 2005). In both studies, qualification rates were lower (~40%) than for the present study (65%), which could indicate differences in the criteria for dog withdrawals or in the dogs produced in these programmes compared to the present study population. The qualification rate reported in the present study reflects that which is expected for this guide dog programme (Hunt *et al.*, 2020).

Health withdrawals were low and similar for both groups, with 16 bitches in total. However, this does not correspond to disease diagnosis, rather health conditions that prevented a bitch becoming a guide dog according to the organisation's guidelines. The proportion of male dogs reported to be withdrawn for health reasons

by De Sapio *et al.* (2005) was greater (25.6%) than reported in the present study. This may be due to differences between breeding programmes and population genetics, or between the health criteria that are applied to dogs to determine their suitability to be guide dogs.

Interestingly, half of the withdrawals for health in the present study were for musculoskeletal disease, which includes some of the commonly studied diseases in relation to age at neutering (Spain *et al.*, 2004; Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014, 2016). However, overall, the number of bitches withdrawn for musculoskeletal disease was low and equal across trial groups. Low numbers of dogs withdrawn for musculoskeletal disease were also reported by De Sapio *et al.* (2005) in their study of male guide dogs in training (11/133 dogs). Similar to the present study, Howe *et al.* (2001) reported no difference in the incidence of musculoskeletal disease, and low levels of prevalence, between dogs from humane organisations that were neutered at more or less than 24 weeks of age. While the low level of occurrence supports current observations, here we are primarily focusing on health reasons for dogs being unsuitable to qualify from a guide dog programme rather than true incidence.

Zlotnick *et al.* (2019) reported differences in the withdrawals for health based on age at neutering; dogs neutered at less than seven months of age were more than twice as likely to be withdrawn for health (RR=2.4) and for orthopaedic (RR=2.2) reasons than those neutered at older ages. De Sapio *et al.* (2005) reported no difference in the number of male dogs withdrawn for health or behaviour between neuter groups, but relative risk was not examined. The low number of health withdrawals in the present study is indicative of a well-managed breeding programme that has heavily selected against diseases important to the programme over many years. It is also possible that several health issues that may be affected by neutering occur at a later age after qualification.

Most withdrawals were for behavioural reasons, similar to the findings reported in many other studies where the largest proportion of unsuccessful dogs are for behavioural reasons (Goddard and Beilharz, 1982, 1983; Tomkins *et al.*, 2011; Bray *et al.*, 2019; Zlotnick *et al.*, 2019). The most common behavioural reasons for withdrawal were in the Environmental Anxiety category. Withdrawals in this category were reported four times more frequently than for the next most common category. This is consistent with findings from a separate study that examined withdrawals following qualification in a large cohort of guide dogs from the same organisation (Caron-Lormier *et al.*, 2016b). Specific behavioural withdrawal reasons within the Environmental Anxiety category related to confidence and adaptability, suspicion to sounds, general suspicion, and stress resilience. These behaviour traits are not the most frequently reported in studies of other guide dog populations (Goddard and Beilharz, 1982, 1983; Arata *et al.*, 2010; Tomkins *et al.*, 2011). Importantly, there may be different work requirements for guide dogs in different countries and withdrawal reasons are selected by the member of staff responsible for the dogs in the stage from which they are withdrawn rather than based on validated behavioural measures or criteria. Therefore, there is the potential for human error in behavioural observation and interpretation and selection of the withdrawal reason (Goddard and Beilharz, 1983; Murphy, 1998).

The early identification of dogs not suitable for assistance dog work allows organisations to minimise the resources allocated to the dog. This enables financial savings that are important given that most assistance dog organisations are charities (Tomkins *et al.*, 2011; Batt *et al.*, 2008). Consequently, the ability to identify unsuitable dogs before they enter training, a relatively expensive stage (>£25,000 per dog) in comparison to puppy raising (£5,100 per puppy; ITV, 2014), would be beneficial. In this study, although there was no significant difference in the stage of training from which bitches were withdrawn, it is important to recognise that

more PPN than Control bitches were withdrawn in puppy raising (22 vs 15) and fewer in early training (25 vs 29). If this were due to problems that caused the bitches to be unsuitable for working as an assistance dog appearing earlier in prepubertally neutered bitches, this could indicate an area for further investigation, where time spent in each stage prior to withdrawal should also be considered.

An important measure of success as a guide dog is remaining qualified with the guide dog owner for a period of time (Batt *et al.*, 2010). Being withdrawn from working earlier than anticipated (average working life for dogs that retire = 8.5 years, Caron-Lormier *et al.*, 2016a) can be upsetting for the guide dog owner and result in negative impacts on their quality of life (Nicholson *et al.*, 1995; Whelan, 2016) as well as cause unanticipated demands on the assistance dog programme to provide replacement dogs. Any working practice that increases the number of early withdrawals from working should therefore be cause for concern for assistance dog programmes. Within the present study, there was no significant difference between trial groups in the number of bitches withdrawn in the first three years of working. A three-year timeframe was chosen to reflect previous work within the guide dog organisation (Jones, 2016). However, it is interesting, given the importance of this outcome measure, that 21 PPN compared to 14 Control bitches were withdrawn within three years. The was a trend for more guide dog owner initiated (therefore non-dog related) and fewer health and behaviour related withdrawals for Control bitches, with more PPN bitches being withdrawn for health and behaviour. Additionally, when withdrawn from working, fewer PPN than Control bitches requalified with a second guide dog owner. In depth investigation of the individual dogs that were withdrawn to better understand the circumstances for each case was not undertaken here but could be warranted. This could help to understand whether the withdrawals resulted from problems with matching or whether there were identifiable, distinct commonalities in dogs withdrawn from each trial group (Whelan, 2016; Lloyd *et al.*, 2016).

Overall, the results indicate that the timing of neutering in relation to puberty is associated with the time taken to progress through certain stages of training, with bitches neutered prepubertally ready to enter training at an earlier age. However, age at outcome and qualification rate or the reasons for withdrawal from training were not influenced by pubertal status at neutering. Nor were there any significant differences in the numbers of bitches that were withdrawn from working with three years of qualification, although more PPN than Control bitches were withdrawn. These findings are of interest to assistance and working dog programmes due to the indication that the timing of neutering in relation to puberty will not impact the bitch in such a way that it will affect their suitability for work. Further work would be required to more fully understand the impacts on dogs of different breeds, male dogs and dogs in other working roles.

7.5.3 Study limitations and generalisation

The limitations that apply to all chapters are considered in more detail in the discussion (see Chapter 9). The study was conducted using dogs from one assistance dog organisation. It is possible that across different organisations with different rearing programmes and training methodologies applied, behaviour and learning may be impacted in different ways that could alter the association between neutering timing and progression through training. In the present study, bitches were exposed to consistent rearing protocols. However, some of the observed variation in dependent variables attributable to training model demonstrates the possibility for findings to vary across organisations with different training programmes.

Behaviour was not directly measured in this chapter. The behavioural reasons for withdrawal that were analysed were selected by the person or team responsible for the dog from an existing list of behavioural withdrawal categories, creating the potential for individual subjectivity and bias. This would have been

equally likely across both trial groups, therefore examination of differences remains valid. However, tools to measure behaviour, such as C-BARQ (Chapter 6) or a purpose designed questionnaire (Harvey *et al.*, 2016a, Hunt *et al.*, 2020) rather than subjectively coding behavioural withdrawals would be more appropriate for use in future studies.

Puppies were placed in puppy raising and entered training in different geographical regions of the country and different training schools. The inclusion of covariates in the statistical models attempted to account for variation that could be attributed regional differences in the support and supervision for puppies and in the methods of training. However, this was not always possible due to small numbers, for instance, for the analysis of success. Additionally, some covariates had small numbers of bitches in each group with potential impacts for analysis, for example, for days in each stage of training, the large number of trainers and mobility instructors resulted in too few dogs being trained by each to include the factor in the model. It is possible that other factors not measured as part of this study also impacted bitches' ability to progress through training, such as the number of trainers and instructors available, or the number of dogs in the next stage impacting training capacity. Although again these factors could be expected to have acted upon the bitches in both groups equally.

Finally, it is possible that bitch sire or dam could have had an impact on progression should certain individuals be more likely to produce puppies that would qualify as guide dogs. Qualification rates are higher for guide dog schools with their own breeding programmes compared to sourcing dogs from the public (Goddard and Beilharz, 1982, 1983; Knol, 1988), therefore it is possible to improve the likelihood of producing a puppy that will qualify as a guide dog through selective breeding. While sire and dam were not included in the statistical models due to the small number of puppies produced by each sire and dam in each group, the majority

of sires contributed puppies to both trial groups (42/64 sires) and no bitch contributed more than four puppies to either trial group (data not shown). Thus, the potential for any particular sire or dam to influence the results from one group was low.

7.6 CONCLUSION

This is the first study to report the effect that neutering bitches before or after puberty has on progression of bitches through an assistance dog training programme. The results suggest that bitches neutered prepubertally may enter training earlier and take less time to progress through some stages of training. Despite this, there was no impact on qualification rates or withdrawal reasons. These findings are of interest to assistance and working dog organisations as well as behaviourists and pet dog owners in terms of improving the understanding of how age at neutering in relation to stage of reproductive development in bitches can impact their behavioural development and ability to learn and perform in a working role.

Chapter 8. A prospective study investigating the health outcomes of bitches neutered prepubertally or post-pubertally

8.1 INTRODUCTION

For working and assistance dog programmes, maintaining optimum dog health is important to ensure that, along with dog wellbeing, qualification rates and working life are optimised, and that costs of health problems are limited. The timing of neutering, most frequently related to age at neutering, has been suggested to impact health outcomes for female dogs (Salmeri *et al.*, 1991; Howe *et al.*, 2001; Spain *et al.*, 2004; Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014, 2016, 2020a,b; Simpson *et al.*, 2019). However, the evidence describing impacts is not consistent across diseases (see Chapter 3) and is further complicated by breed differences (Hart *et al.*, 2020a,b) and variation between studies in terms of populations and methodology.

The lack of agreement within the literature is reflected in veterinarian opinion with regard the effect of timing of neutering on bitch health. A survey of 723 UK veterinarians (Berks, 2017) revealed that 42% believed neutering before or after the first oestrus had no impact on the development of future health issues in bitches. However, 17% believed that prepubertal neutering was associated with more health issues, whilst 23% believed that post-pubertal neutering was associated with more health issues. This lack of agreement is unsurprising as there is a lack of evidence regarding the effects of timing of neutering in relation to puberty on female dog health, with questions raised regarding the reliability of some study methodologies and findings (see Chapter 3). That chapter identified only six studies that considered whether bitches were surgically neutered before or after puberty and none investigated common diseases associated with neutering including atopy, musculoskeletal disease, or obesity.

This study aimed to investigate the effects of timing of neutering in relation to puberty (rather than age at neutering) on future health outcomes for bitches from an assistance dog programme. This will provide important information for decision making regarding whether to neuter female dogs before or after puberty.

8.2 AIM AND OBJECTIVES

8.2.1 Aim

To use a prospective study design to compare health data including incidence of disease, withdrawals from working as a guide dog for health reasons and reasons for death, in bitches neutered before or after puberty.

8.2.2 Objectives

1) To determine any significant differences in the incidence and age at diagnosis of specific diseases (categorised as musculoskeletal, malignant neoplasia, urogenital, immune disease and 'other') between bitches neutered prepubertally or post-pubertally.
2) To compare withdrawals from working as a guide dog due to health reasons, and causes of death, for bitches neutered prepubertally or post-pubertally.

8.3 MATERIALS AND METHODS

8.3.1 Study design

A prospective study was undertaken using 306 Guide Dogs' bitches born between 22/02/2012 and 09/08/2015. Bitches were neutered either prepubertally or post-pubertally (see Section 4.4.4). Data were gathered to examine the health of bitches in the study from six months of age to 15/09/2023.

8.3.2 Study setting

Within Guide Dogs, dogs are placed into puppy raising from the nest between seven and eight weeks of age and remain there until they enter training at approximately 14 months of age. During puppy

raising, dogs live with puppy raisers and their families. During training stages dogs are housed in kennels or with boarders in their homes. Between two and three years of age dogs qualify as a guide dog.

Guide Dogs is continually responsible for the dogs' health and any associated veterinary investigations and treatments, including when dogs are working with guide dog owners. A team of Dog Health and Wellbeing Specialists oversee health for all dogs. All health problems and consultations are recorded on Guide Dogs' electronic database (GDIR) and are 'coded' as health items. Health items are only coded for a disease once diagnosis is confirmed using appropriate diagnostic tools in consultation with a veterinarian or specialist, i.e., radiograph or computed tomography for elbow dysplasia, and following referral and work up with a specialist dermatologist for atopy. GDIR is searchable, enabling the identification and extraction of data for any dogs with a particular health code. Additionally, each dog's whole health records can be extracted to allow manual searching of all text entries. Dogs that do not qualify as a guide dog, that qualify but then are withdrawn from working before planned retirement, or that retire, are rehomed with either the guide dog owner or, most likely, new owners (rehomers). Following rehoming, responsibility for dog health is relinquished and health data cease to be gathered. Rehomers are provided with a letter requesting that they inform Guide Dogs should the dog be diagnosed with certain health conditions, however such information is infrequently supplied. When dogs die, their status on GDIR is changed to 'Deceased' and a health record is added to note the cause of death where known.

8.3.3 Study participants

The study population included 306 bitches from five different crossbreeds allocated to two groups; prepubertally neutered (PPN, n=155) and post-pubertally neutered (Control, n=151) (see Section

4.4.4). All first-generation backcross bitches were grouped into one breed group for analysis.

8.3.4 Variables and data sources

8.3.4.1 Diseases

Diseases were included in the study that were determined to be of importance to Guide Dogs by examining withdrawals from the Guide Dogs programme due to health (data not shown) and as advised by Guide Dogs' Chief Veterinary Officer and Breeding Dog Health and Screening Specialist. Diseases were also included that had been cited as being impacted by neutering timing in the scientific literature and/or had been identified in two previous veterinary student projects, including a survey of practicing veterinarians, as potentially impacted by neutering timing (Berks, 2017; Godfrey-Hunt, 2018) (Appendix 9). Outcome variables were (1) being affected with disease and (2) age at first diagnosis.

The complete list of Guide Dogs' health items (n=2,249) that are used to code disease, diagnostic tests and routine treatment on GDIR was obtained. This was reviewed and all codes pertaining to each of the diseases of interest were retained. Health code searches were run in GDIR to generate reports in Excel for all dogs with each health code that were born between 22/02/2012 and 09/08/2015. Data for all health codes that related to one disease were combined onto one excel sheet (e.g., for atopic dermatitis (atopy), health codes included were: 'Acute Atopic Conjunctivitis'; 'Allergic skin disease'; 'Atopic [allergic] Otitis'; 'Atopic conjunctivitis'; and 'Atopic dermatitis') (see Appendix 10). Date of first diagnosis was extracted and used to calculate age at first diagnosis. Only diagnoses that occurred after six months of age were included; diagnoses before six months of age were ignored unless there was a subsequent new episode after six months of age. For being overweight, the date of the first health record where the dog was recorded as being 5% or more over optimum weight was used. Adult body condition scores (BCS) were recorded as the highest BCS for each bitch at ≥2.5

years of age along with the date of the BCS. To ensure that no data were missed, complete health records for each bitch were extracted and manually searched to identify any cases where disease had been described in the veterinary health notes but had not been coded on GDIR. Health data were correct to 15/09/2023. The date of the last available health record was recorded as either the last health record entry for dogs that had left the programme by the time of data collection, or as 15/09/2023 for those that remained working as guide dogs.

8.3.4.2 Withdrawals from working for health reasons and cause of death

The current status of all bitches was extracted from GDIR on 15/09/2023. Any bitches withdrawn from working as guide dogs before reaching retirement at the time of data collation had withdrawal records examined. Withdrawal for health-related reasons were extracted. For all bitches with a status of 'Deceased' the date of death was extracted and used to calculate age at death. Health records were examined to determine cause of death. Outcome variables were the number of bitches that were withdrawn from working for health reasons, the number that had died and age at death.

8.3.5 Bias and study size

Random effects and confounding factors were included in the statistical analysis for Cox regression models when examining the main effect of trial group on the dependent variables (diseases). Dogs in this study were of similar breeds and were not different between trial groups. However, breed was included to control for the potential effect on other variables. Where relevant to the disease being studied, data generated for Chapter 5 relating to physical development, such as change in height for musculoskeletal disease, and data on vulval appearance and development for urogenital disease, were included in the models.

Study size was limited by the available cohort of bitches produced by Guide Dogs during the study recruitment period that were placed in puppy raising within a suitable distance to travel to one of four national veterinary practices for neutering surgery. Values of alpha were determined using G*Power for each analysis (see Section 4.7). The mean, SEM and confidence intervals (CI) were reported where appropriate.

8.3.6 Quantitative variables and statistical methods

Survival analysis was conducted using SPSS version 28 (IBM, USA). Chi-square, Mann Whitney-U and t-tests were run in XLStat (XLStat2016, Addinsoft, USA).

8.3.6.1 Disease

Disease data were grouped into one of five categories: musculoskeletal, malignant neoplasia, urogenital, immune, and 'other' diseases. PPN bitches were neutered before puberty therefore were not able to experience a pseudopregnancy. Pseudopregnancy was reported under urogenital disease, but comparisons were not made between groups and all statistical analysis for urogenital disease excluded pseudopregnancy. For urogenital disease, descriptive data were presented for the number of bitches affected with, or clear from, disease that had different vulval appearance and development data available from assessments at 17 months of age.

The number of bitches diagnosed with each disease, the number that had at least one disease from each category and the number of bitches that had 0, 1 or 2 diseases in each category were compared between trial groups using Chi-square analysis where numbers were sufficient for analysis. Values of alpha were 4 or 5% and beta was 5% for all analyses with effective sample sizes between 39 and 324 dogs (see Section 4.7). The number of Control bitches diagnosed with a disease before neutering was indicated for all diseases where this was observed. The percentage of bitches that were classified as

obese based on having an adult BCS of 7, 8 or 9 was reported and compared between groups using Chi-square analysis. Alpha and beta were both 5% with an effect size of 0.270.

Disease incidence rate was determined for PPN and Control groups for diseases with more than 10 bitches affected. The denominator was the total number of dog-days in the study (the number of days of health record data available from six months of age for all bitches in the group). The total number of episodes of disease diagnosed was the numerator. The rate was multiplied by 1000 to provide a disease incidence rate per 1000 dog-days. The incidence rate ratio (IRR) was calculated using the formula: Disease Incidence Ratio = [PPN disease incidence rate/1000 dog-days] / [Control disease incidence rate/1000 dog-days]. 95% CI were calculated using the formula:

95% CI for IRR = exp{ln(IRR) ± ZySD[ln(IRR)]}
where Zy is 1.96, the critical z-score value for a 95 percent CI (Rothman *et al*., 2008).

For diseases with more than 10 bitches affected, age at first diagnosis was compared between trial groups using t-tests or Mann-Whitney tests dependent on data distribution. Alpha and beta were determined using sensitivity analysis. Values of alpha were between 9% and 13% and beta was 5% for all analyses with effect sizes of between 0.572 and 2.124 (see Section 4.7). For musculoskeletal diseases with more than 10 bitches affected, data for change in height between six and 17 months of age (from Chapter 5) were examined to identify any differences in growth between bitches that were and were not affected with disease using Mann-Whitney tests. Alpha and beta were 9% and 5% for all analyses with effect sizes of between 0.394 and 0.400 (see Section 4.7). For urogenital disease, data for vulval appearance and development were examined to identify associations with urogenital disease using Chi-square tests. Data for vulval appearance at 17 months of age were grouped into

'normal' and 'not normal', data for dorsal fold coverage were grouped as ≤20% and >20%, and data for overall vulval growth were grouped as 'smaller or the same' and 'larger' for analysis. Alpha and beta were 4% and 5% for all analyses with effect sizes of between 0.396 and 1.124 (see Section 4.7).

Kaplan-Meier survival curves and Cox regression analysis were performed to examine differences between PPN and Control bitches in disease survival (remaining free from disease). Differences between curves were examined using log rank tests. Cox regression was used for diseases with more than 10 bitches affected per predictor variable (Peduzzi *et al.*, 1995). Risk factors included in all models were bitch breed and trial group. The models for musculoskeletal disease included data for change in height between six and 17 months of age. The models for urogenital disease included data on vulval appearance (normal, recessed and/or juvenile), percentage dorsal fold coverage at 17 months of age and vulval growth (larger, smaller, no change) between six and 17 months of age. The models for overweight/obesity also included BCS at six and 17 months of age. Statistical power was investigated post-hoc using MedCalc Statistical Software version 19.2.6 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2020). However, the power was so low due to small sample sizes that these analyses were not reported; in order to visualise the data, Kaplan-Meier survival curves were presented.

8.3.6.1.1 Overall health score

The total number of different diseases or health problems of interest that each bitch was diagnosed with was calculated to give each bitch an overall health score. These were compared between trial groups using Chi-square analysis. Alpha and beta were both 5% with an effective sample size of 308 dogs.

8.3.6.2 Withdrawals from working for health reasons and cause of death

The number of bitches in each trial group that were withdrawn from working for health reasons and that had died were compared using Chi-square tests. A t-test was used to examine differences in age at death between PPN and Control bitches. For withdrawals from working, alpha and beta were both 5% with an effective sample size of 195 dogs. For withdrawals from working for health reasons and the number of bitches that died, alpha and beta were 4% and 5% with effective sample sizes of 95 and 236 dogs respectively. For age at death alpha and beta were 10% and 5% respectively with an effective sample size of 32 dogs.

8.4 RESULTS

8.4.1 Participants

Health data were available for bitches until the time they died or were withdrawn or retired from working and rehomed. At the time of data collation (15/09/2023), 82 bitches (33 PPN, 49 Control) remained working as guide dogs, 148 had been withdrawn and rehomed (10 with guide dog owners), 37 had been retired and rehomed (25 with guide dog owners), seven had been transferred to other working dog organisations and 32 had died. Bitches were between 0.5 and 11.1 years of age when they left the programme and bitches that remained on the programme at the point of data collation were between 8.1 and 11.5 years of age. There was no significant difference in the amount of available data (years) between the PPN and Control groups (Chi-square=6.548; D.F.=4, P=0.162; Figure 8.1).

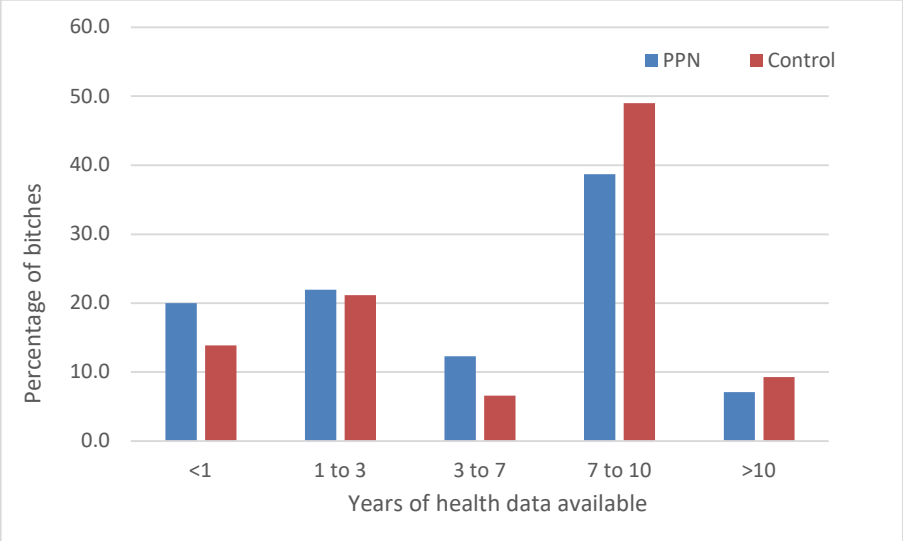


Figure 8.1. The percentage of bitches neutered prepubertally (PPN, n=155) or post-pubertally (Control, n=151) in a prospective study within each group for years of health data available from six months of age to the last health record.

8.4.2 Diseases

The number and percentage of bitches diagnosed with each disease along with descriptive data for age at diagnosis are presented in Appendix 11. No multivariable Cox regression models are presented due to small numbers of bitches with disease and inadequate power.

8.4.2.1 Musculoskeletal disease

There were 82 bitches (47 PPN, 35 Control) diagnosed with at least one musculoskeletal disease (Chi-square=1.990, D.F.=1, P=0.158). There was no significant difference in the number of bitches diagnosed with zero (108 PPN, 116 Control), one (31 PPN, 30 Control) or two or more (16 PPN, 5 Control) musculoskeletal diseases (Chi-square=5.930, D.F.=2, P=0.052). The most frequently reported problem was forelimb lameness (29 PPN, 25 Control [two diagnosed prior to neutering]; Chi-square=0.244, D.F.=1, P=0.621) followed by osteoarthritis (19 PPN, 11 Control; Chi-square=2.139, D.F.=1, P=0.144). Cruciate disease occurred in significantly more PPN (n=11) than Control bitches (n=1; Yates Chi-square=6.784, D.F.=1, P=0.009). ED (3 PPN, 3 Control [one diagnosed prior to neutering]), HD (3 PPN, 0 Control), patella

luxation (1 PPN, 0 Control) and other musculoskeletal diseases (3 PPN, 1 Control) occurred in too few bitches for statistical analysis. No bitches were diagnosed with OCD or juvenile osteochondrosis.

The IRR for CCL disease was 17.80 (95% CI=2.36-134.25), for forelimb lameness was 1.27 (95% CI=0.84-1.92) and for osteoarthritis was 1.92 (95% CI=0.92-4.04).

Age at first diagnosis was not significantly different between PPN and Control bitches for forelimb lameness (PPN 4.6 ± 0.5 years, Control 3.5 ± 0.5 years; Mann-Whitney U=451.5, P=0.125). There was a significant difference for age at diagnosis of osteoarthritis (PPN 8.0 ± 0.4 years, Control 9.2 ± 0.4 years; t=-1.935, P=0.064; Figure 8.2) although effect size was -0.863, smaller than that determined by power analysis (1.345). CCL disease and forelimb lameness were diagnosed multiple times for some bitches throughout their available health history. There were 17 CCL disease episodes in total (16 PPN, 1 Control) and 90 episodes of forelimb lameness (48 PPN, 42 Control).

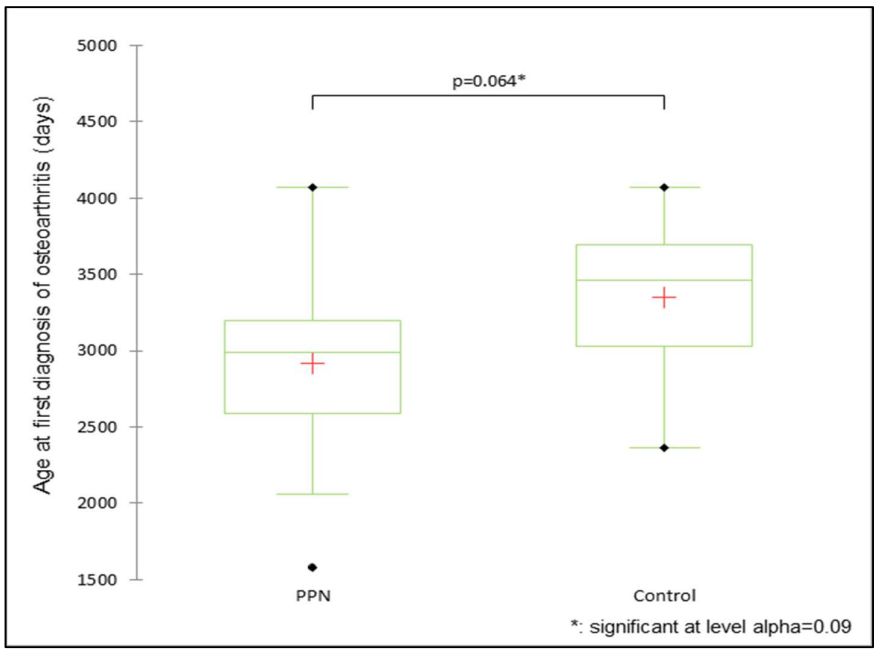


Figure 8.2. The mean, median and range for days of age at first diagnosis of osteoarthritis for bitches neutered prepubertally (PPN, n=18) or post-pubertally (Control, n=11) in a prospective study.

There was no significant difference in growth between six and 17 months of age between bitches diagnosed with or unaffected by CCL disease (affected 5.64 ± 1.09 cm, unaffected 5.45 ± 0.19 cm; Mann-Whitney U=722.0, P=0.811), forelimb lameness (affected 5.71 ± 0.45 cm, unaffected 5.42 ± 0.20 cm; Mann-Whitney U=3431.0, P=0.471), osteoarthritis (affected 5.11 ± 0.59 cm, unaffected 5.50 ± 0.19 cm; Mann-Whitney U=2117.0, P=0.556) or at least one musculoskeletal disease (affected 5.48 ± 0.35 cm, unaffected 5.47 ± 0.22 cm; Mann-Whitney U=4836.5, P=0.889).

For forelimb lameness, Kaplan-Meier survival curves were not different between PPN and Control bitches (χ^2=0.970, D.F.=1, P=0.325). For osteoarthritis (χ^2=5.777, D.F.=1, P=0.016; Figure 8.3) and having at least one musculoskeletal disease (χ^2=5.142, D.F.=1, P=0.023; Figure 8.4) there were significant differences indicated between the curves for PPN and Control bitches.

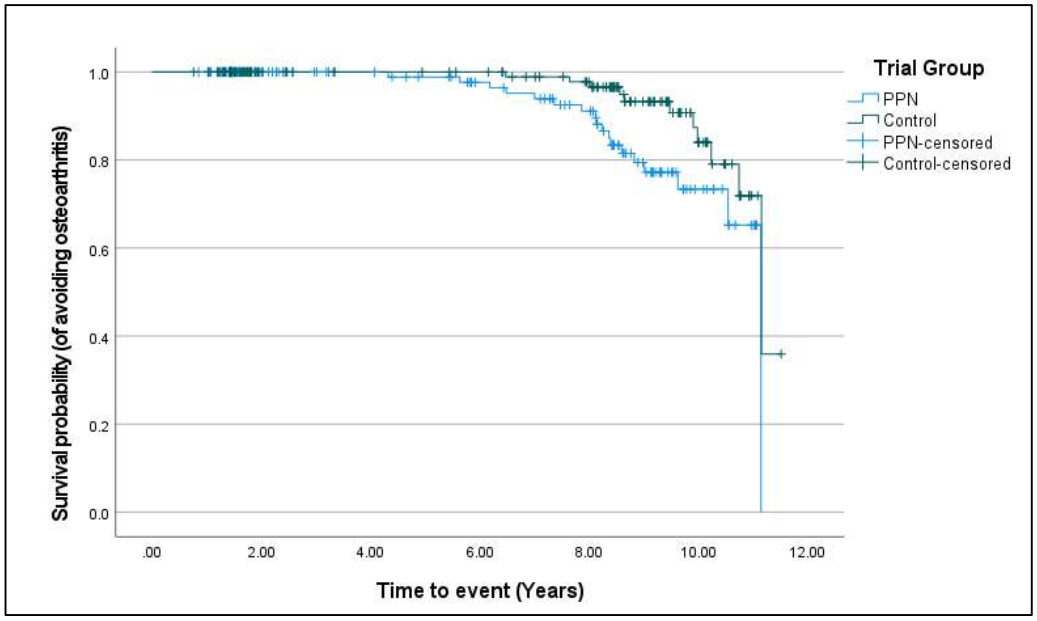


Figure 8.3. Kaplan-Meier curves of survival probability (from having osteoarthritis) for PPN (blue) and Control (green) bitches in a prospective study investigating the effect of neutering before (PPN) or after (Control) puberty on health outcomes. Censored values (+) indicate the time that bitches left the programme.

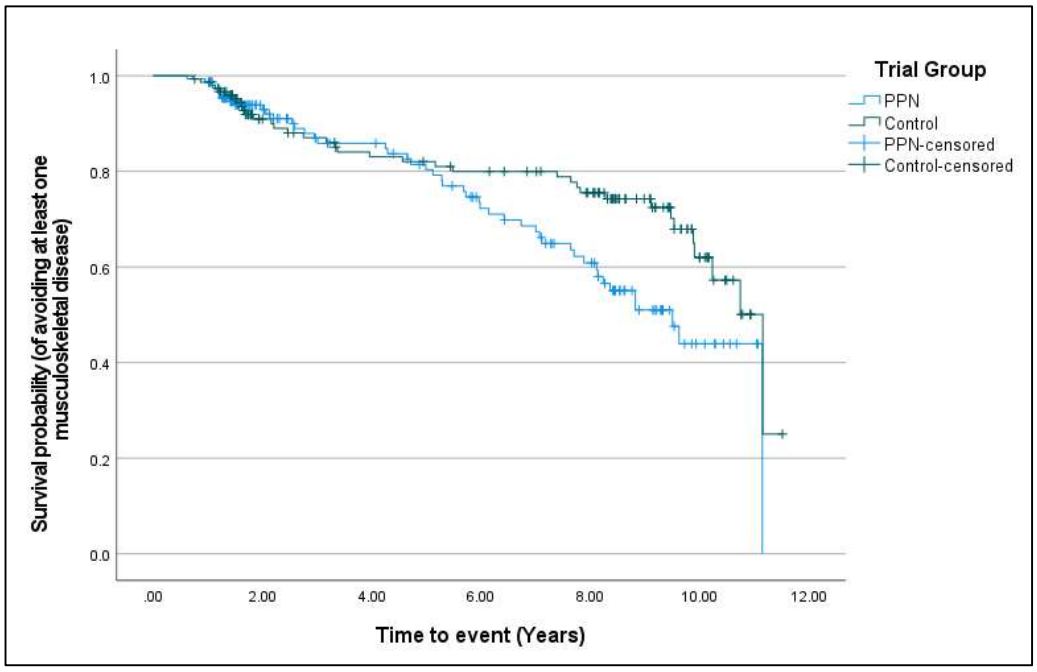


Figure 8.4. Kaplan-Meier curves of survival probability (from having at least one musculoskeletal disease) for PPN (blue) and Control (green) bitches in a prospective study investigating the effect of neutering before (PPN) or after (Control) puberty on health outcomes. Censored values (+) indicate the time that bitches left the programme.

8.4.2.2 Malignant neoplasia

There were 38 bitches (21 PPN, 17 Control) diagnosed with at least one malignant neoplasia (Chi-square=0.369, D.F.=1, P=0.544). Only one bitch (PPN) had two different types of malignant neoplasia diagnosed; a round cell tumour of the proximal tibia and neuroendocrine carcinoma with extensive metastasis. The most commonly reported problem was 'other' malignant neoplasia (12 PPN, 4 Control; Chi-square=4.004, D.F.=1, P=0.045) followed by MCT (4 PPN, 7 Control; Yates Chi-square=0.433, D.F.=1, P=0.510) although for both, there was no significant difference between trial groups. HSA (1 PPN, 2 Control), LSA (2 PPN, 2 Control), melanocytic tumour (1 PPN, 2 Control), and squamous cell carcinoma (1 PPN, 0 Control) occurred in too few bitches for statistical analysis. No bitches were diagnosed with adenocarcinoma, fibrosarcoma, mammary neoplasia, osteosarcoma or transitional cell carcinoma.

The IRR for MCT was 0.87 (95% CI=0.32-2.32) and for 'other' malignant neoplasia was 3.62 (95% CI=1.18-11.09).

Age at first diagnosis was not significantly different between PPN and Control bitches for MCT (PPN 8.4 ± 0.7 years, Control 8.2 ± 0.5 years; Mann-Whitney U=15.5, P=0.824) or 'other' malignant neoplasia (PPN 8.4 ± 0.7 years, Control 8.1 ± 0.3 years; Mann-Whitney U=31.0, P=0.446). MCT were diagnosed multiple times for some bitches throughout their available health history. There were 16 MCT episodes in total (7 PPN, 9 Control).

For having at least one malignant neoplasia (χ^2=1.086, D.F.=1, P=0.297), Kaplan-Meier survival curves were not different between PPN and Control bitches. For 'other' malignant neoplasia there were significant differences indicated between the curves for PPN and Control bitches (χ^2=5.063, D.F.=1, P=0.024; Figure 8.5).

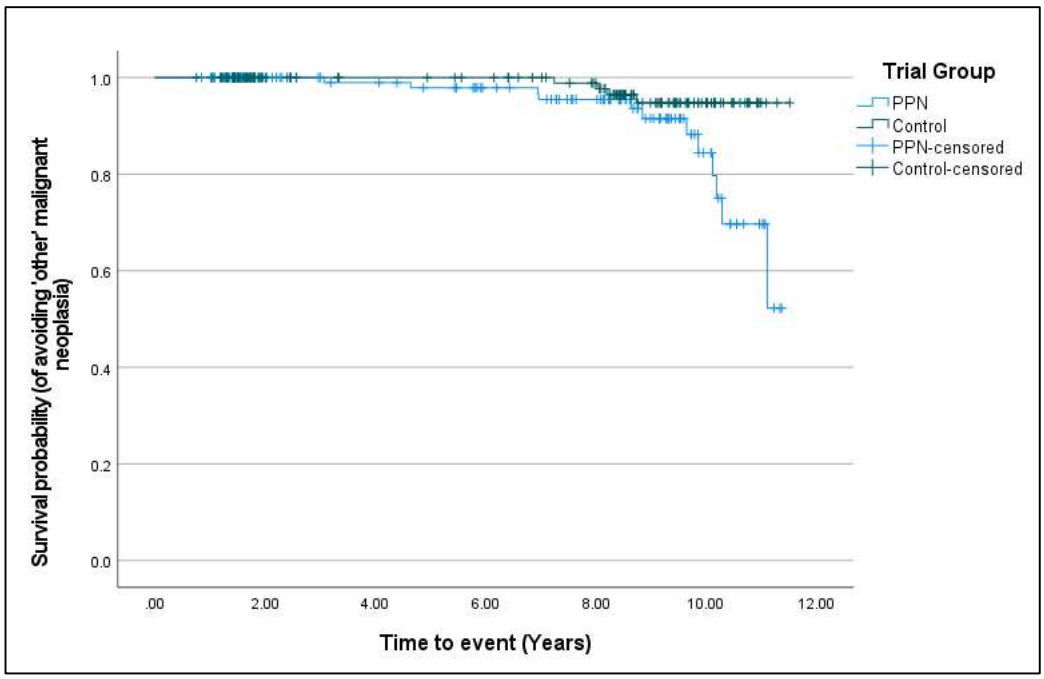


Figure 8.5. Kaplan-Meier curves of survival probability (from having 'other' malignant neoplasia) for PPN (blue) and Control (green) bitches in a prospective study investigating the effect of neutering before (PPN) or after (Control) puberty on health outcomes. Censored values (+) indicate the time that bitches left the programme.

8.4.2.3 Urogenital disease

Excluding pseudopregnancy, there were 54 bitches (25 PPN, 29 Control) diagnosed with at least one urogenital disease (Chi-square=0.498, D.F.=1, P=0.480). There was no difference in the number of bitches diagnosed with zero (130 PPN, 122 Control), one (20 PPN, 24 Control) or two or more (5 PPN, 5 Control) urogenital diseases (Chi-square=0.565, D.F.=2, P=0.754). The most commonly reported problem was UTI/cystitis (12 PPN, 9 Control [four bitches diagnosed prior to neutering]; Chi-square=0.380, D.F.=1, P=0.538), followed by perivulvar dermatitis (8 PPN, 5 Control [four bitches diagnosed prior to neutering]; Chi-square=0.644, D.F.=1, P=0.422), vulval/abnormal discharge (5 PPN, 8 Control [five bitches diagnosed prior to neutering]; Chi-square=0.378, D.F.=1, P=0.538), UI (1 PPN, 6 Control; Yates' Chi-square=2.448, D.F.=1, P=0.118), recessed/inverted vulva (4 PPN, 1 Control [bitch diagnosed prior to neutering]; Yates' Chi-square=0.761, D.F.=1, P=0.383) and vulval/vaginal disorder (1 PPN, 2 Control [one bitch diagnosed prior to neutering]; Yates' Chi-square=0.001, D.F.=1, P=0.975). USMI (0 PPN, 1 Control), and vaginitis (0 PPN, 3 Control [all bitches diagnosed prior to neutering]) occurred in too few bitches for statistical analysis. Pseudopregnancy was reported in no PPN and 23 Control bitches (analysis not possible), one of these was diagnosed post-neutering. No bitches were diagnosed with pyometra, struvite urolithiasis/urinary calculi, urinary/reproductive tract tumours or urinary tract disorders. Only one bitch with perivulval dermatitis had a BCS in their lifetime that represented having obesity. One of the four PPN bitches with a health code for recessed/inverted vulva was categorised as severely affected with recurrent perivulval dermatitis and was also diagnosed with atopic dermatitis. This bitch required vulvoplasty to remove a crescent of skin, which resulted in a resolution of the perivulval dermatitis.

The IRR for perivulval dermatitis was 1.78 (95% CI=0.58-5.44), for UTI/cystitis was 1.39 (95% CI=0.65-2.97) and for vulval/abnormal discharge was 0.70 (95% CI=0.23-2.13).

Age at first diagnosis was not different between PPN and Control bitches for UTI/cystitis (PPN 1.9 ± 0.8 years, Control 2.9 ± 0.8 years; Mann-Whitney U=31.5, P=0.115) or vulval/abnormal discharge (PPN 2.6 ± 1.3 years, Control 1.7 ± 0.9 years; Mann-Whitney U=28.0, P=0.264). There was a significant difference between PPN and Control bitches in age at first diagnosis of perivulval dermatitis (Mann-Whitney U=40.0, P=0.002) with the first diagnosis for PPN bitches (4.7 ± 0.9 years) reported at significantly older ages than for Control bitches (0.8 ± 0.1 years; Figure 8.6). Effect size was 16.82. UI and UTI/cystitis were diagnosed multiple times for some bitches throughout their available health history. There were eight UI episodes in total (1 PPN, 7 Control) and 27 episodes of UTI/cystitis (15 PPN, 12 Control).

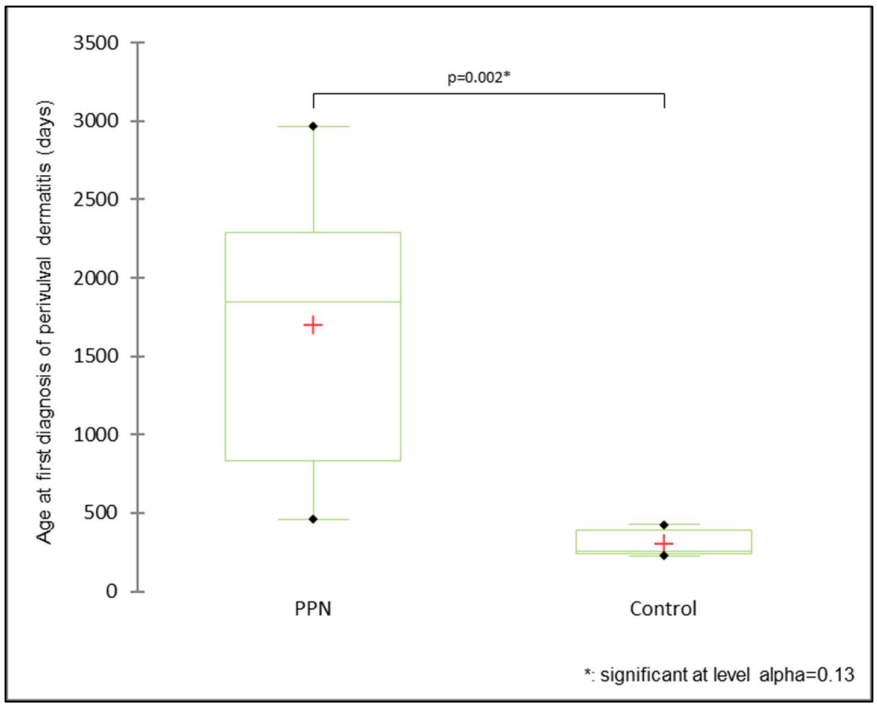


Figure 8.6. The mean, median and range for days of age at diagnosis of perivulval dermatitis for bitches neutered prepubertally (PPN, n=8) or post-pubertally (Control, n=5) in a prospective study.

For UTI/cystitis (χ^2=0.610, D.F.=1, P=0.435) and having at least one urogenital disease (χ^2=0.220, D.F.=1, P=0.639), Kaplan-Meier survival curves were not different between PPN and Control bitches.

There were no significant associations between vulval appearance and development data and urogenital disease (Table 8.1).

Table 8.1. The percentage of bitches with and without a diagnosis of each urogenital disease that also had each type of vulval appearance and percentage dorsal fold coverage at 17 months of age and each descriptor for vulval growth between six and 17 months of age in a prospective study investigating the effect of neutering before or after puberty. Results from Chi-square analyses are shown. Analysis was not possible for dorsal fold coverage for urinary incontinence due to small numbers of bitches with vulval growth data available.

Disease	Bitches diagnosed with the disease	Bitches unaffected by the disease	Chi-square analysis
Perivulval dermatitis (n=13 diagnosed)			
Vulval appearance	Normal = 53.8% Recessed = 46.2% Juvenile = 0 Recessed and juvenile = 0 No image = 0	Normal = 53.2% Recessed = 30.0% Juvenile = 3.1% Recessed and juvenile = 4.1% No image = 9.6%	Chi-square=0.129, P=0.720
Dorsal fold coverage (%)	≤20% = 15.4% 30% = 23.1% 40% = 7.7% 50% = 7.7% No image = 46.2%	≤20% = 24.2% 30% = 17.7% 40% = 8.9% 50% = 3.1% No image = 46.1%	Yates Chi-square=0.216, P=0.642
Vulval growth	Same = 7.7% Smaller = 30.8% Larger = 38.5% Not measured = 23.1%	Same = 11.6% Smaller = 28.3% Larger = 48.5% Not measured = 11.6%	Yates Chi-square=0.001, P=0.982
Urinary incontinence (n=7 diagnosed)			
Vulval appearance	Normal = 71.4% Recessed = 14.3% Juvenile = 0 Recessed and juvenile = 14.3% No image = 0	Normal = 52.8% Recessed = 31.1% Juvenile = 3.0% Recessed and juvenile = 3.7% No image = 9.4%	Yates Chi-square=0.095, P=0.758
Dorsal fold coverage (%)	≤20% = 0 30% = 28.6% 40% = 0 50% = 0 No image = 71.4%	≤20% = 24.4% 30% = 17.7% 40% = 9.0% 50% = 3.3% No image = 45.5%	N/A
Vulval growth	Same = 14.3% Smaller = 0 Larger = 85.7% Not measured = 0	Same = 11.4% Smaller = 29.1% Larger = 47.2% Not measured = 12.4%	Yates Chi-square=1.660, P=0.198
UTI/cystitis (n=21 diagnosed)			

Vulval appearance	Normal = 42.9% Recessed = 42.9% Juvenile = 4.8% Recessed and juvenile = 4.8% No image = 4.8%	Normal = 54.0% Recessed = 29.8% Juvenile = 2.8% Recessed and juvenile = 3.9% No image = 9.5%	Chi-square=1.651, P=0.199
Dorsal fold coverage (%)	≤20% = 19.0% 30% = 19.0% 40% = 4.8% 50% = 9.5% No image = 47.6%	≤20% = 24.2% 30% = 17.9% 40% = 9.1% 50% = 2.8% No image = 46.0%	Yates Chi-square=0.053, P=0.818
Vulval growth	Same = 23.8% Smaller = 23.8% Larger = 42.9% Not measured = 9.5%	Same = 10.5% Smaller = 28.8% Larger = 48.4% Not measured = 12.3%	Chi-square=0.437, P=0.509
Vulval/abnormal discharge (n=13 diagnosed)			
Vulval appearance	Normal = 53.8% Recessed = 38.5% Juvenile = 0 Recessed and juvenile = 7.7% No image = 0	Normal = 53.2% Recessed = 30.4% Juvenile = 3.8% Recessed and juvenile = 3.1% No image = 9.6%	Chi-square=0.129, P=0.720
Dorsal fold coverage (%)	≤20% = 38.5% 30% = 30.8% 40% = 7.7% 50% = 0 No image = 23.1%	≤20% = 23.2% 30% = 17.4% 40% = 8.9% 50% = 3.4% No image = 47.7%	Yates Chi-square=0.002, P=0.960
Vulval growth	Same = 0 Smaller = 30.8% Larger = 61.5% Not measured = 7.7%	Same = 11.9% Smaller = 28.3% Larger = 47.4% Not measured = 12.3%	Chi-square=0.732, P=0.392

n=number of bitches

8.4.2.4 Immune disease

There were 138 bitches (80 PPN, 58 Control) diagnosed with at least one immune disease (Chi-square=5.384, D.F.=1, P=0.020). This was influenced by otitis and was no longer significant if otitis was excluded (at least one immune; 16 PPN, 11 Control; Chi-square=0.877, D.F.=1, P=0.349). There was no difference in the number of bitches diagnosed with zero (75 PPN, 93 Control), one (69 PPN, 50 Control) or two or more (11 PPN, 8 Control) immune diseases (Chi-square=5.385, D.F.=2, P=0.068). The most commonly reported problem was otitis (74 PPN, 55 Control; Chi-square=4.018, D.F.=1, P=0.045) followed by atopy (12 PPN, 9 Control; Chi-square=0.380, D.F.=1, P=0.538). AIHA (1 PPN, 0 Control), hypothyroidism (1 PPN, 0 Control), immune-mediated arthritis (2 PPN, 0 Control), immune-mediated thrombocytopenia (1 PPN, 0 Control) and IBD (0 PPN, 2 Control) occurred in too few

bitches for statistical analysis. No bitches were diagnosed with hypoadrenocorticism or systemic lupus erythematosus.

The IRR for atopy was 1.48 (95% CI=0.63-3.52) and for otitis was 1.47 (95% CI=1.15-1.88).

Age at first diagnosis was significantly different between PPN and Control bitches for otitis (PPN 1.7 ± 0.2 years, Control 2.1 ± 0.3 years; Mann-Whitney U=1676.5, P=0.088). Effect size was -0.219, smaller than that determined by power analysis (0.59). There was a significant difference in age at first diagnosis for atopy with the first diagnosis for PPN bitches (3.9 ± 0.6 years) reported at significantly older ages than for Control bitches (1.6 ± 0.3 years; Mann-Whitney U=90.5, P=0.008; Figure 8.7). Effect size was 2.78. Otitis was diagnosed multiple times for some bitches throughout their available health history. There were 255 otitis episodes in total (145 PPN, 110 Control).

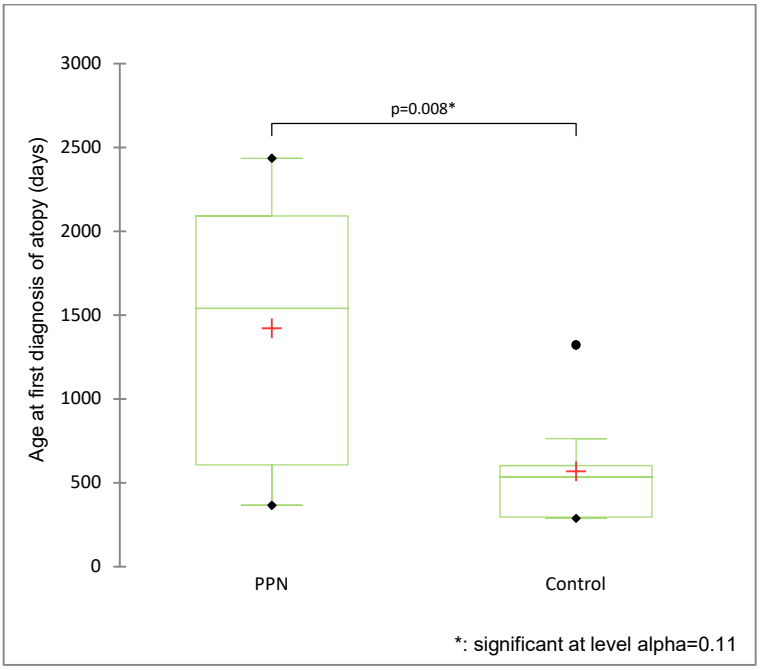


Figure 8.7. The mean, median and range for days of age at diagnosis of atopy for bitches neutered prepubertally (PPN, n=12) or post-pubertally (Control, n=9) in a prospective study.

For atopy, Kaplan-Meier survival curves were not different between PPN and Control bitches (χ^2=0.569, D.F.=1, P=0.451). For having at least one immune disease (χ^2=8.413, D.F.=1, P=0.004) and otitis (χ^2=7.090, D.F.=1, P=0.008; Figure 8.8) there were significant differences between the curves for PPN and Control bitches. The difference between curves for having at least one immune disease was influenced by otitis and was no longer significant if otitis was excluded (χ^2=1.193, D.F.=1, P=0.275).

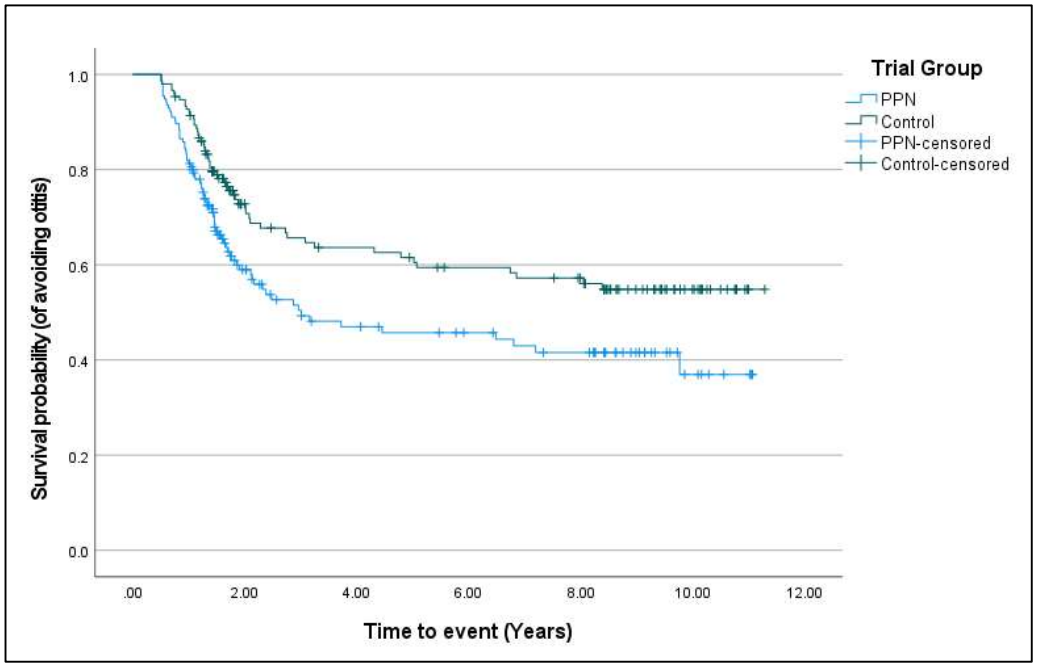


Figure 8.8. Kaplan-Meier curves of survival probability (from having otitis) for PPN (blue) and Control (green) bitches in a prospective study investigating the effect of neutering before (PPN) or after (Control) puberty on health outcomes. Censored values (+) indicate the time that bitches left the programme.

8.4.2.5 Other diseases

The most commonly reported problem was being overweight or having obesity (65 PPN, 69 Control; Chi-square=0.439, D.F.=1, P=0.507). Histiocytoma was diagnosed in seven PPN and three Control bitches (Yates Chi-square=0.851, D.F.=1, P=0.356). For all other diseases of interest, numbers of bitches diagnosed were too small for statistical analysis: aortic stenosis (0 PPN, 2 Control), diabetes mellitus (0 PPN, 1 Control), early-onset cataract (2 PPN, 2

Control), and epilepsy (2 PPN, 0 Control). No bitches were diagnosed with gastric volvulus or geriatric cognitive impairment. The IRR for being overweight or having obesity was 1.05 (95% CI=0.75-1.47). Age at the first health record where a bitch was reported as being overweight or having obesity was not different between PPN (3.4 ± 0.3 years) and Control (2.9 ± 0.3 years) bitches (Mann-Whitney U=2562.5, P=0.155). For being overweight and having obesity, Kaplan-Meier survival curves were not different between PPN and Control bitches (χ^2=0.143, D.F.=1, P=0.706).

One hundred and seventy-nine bitches (90 PPN, 89 Control) had an adult BCS recorded. There were 20 PPN and 24 Control bitches that had an adult BCS of 7, 8 or 9 and would therefore be considered as having obesity (Chi-square=0.543, D.F.=1, P=0.461). The incidence rate ratio (IR) for being obese based on BCS was 0.93 (95% CI=0.51-1.68).

8.4.2.6 Overall health score

Bitches each had a total of between zero and seven different health problems diagnosed. There were more PPN than Control bitches with an overall health score of 4 or more, and no Control bitches with a score greater than 4. Differences between groups were not statistically significant when scores were grouped as 0, 1, 2, 3 and 4+ (Chi-square=7.754, D.F.=4, P=0.101; Figure 8.9). The 13 PPN bitches that had five, six or seven different diseases diagnosed commonly had both musculoskeletal and immune diseases, only one bitch had musculoskeletal diseases with no immune disease, and one had immune diseases and no musculoskeletal disease. Eleven of the 13 bitches therefore had musculoskeletal disease, and 10 had more than one type, including one bitch that had four different musculoskeletal diseases and three bitches that had three different musculoskeletal diseases. Twelve of the 13 bitches had immune disease; six had more than one type.

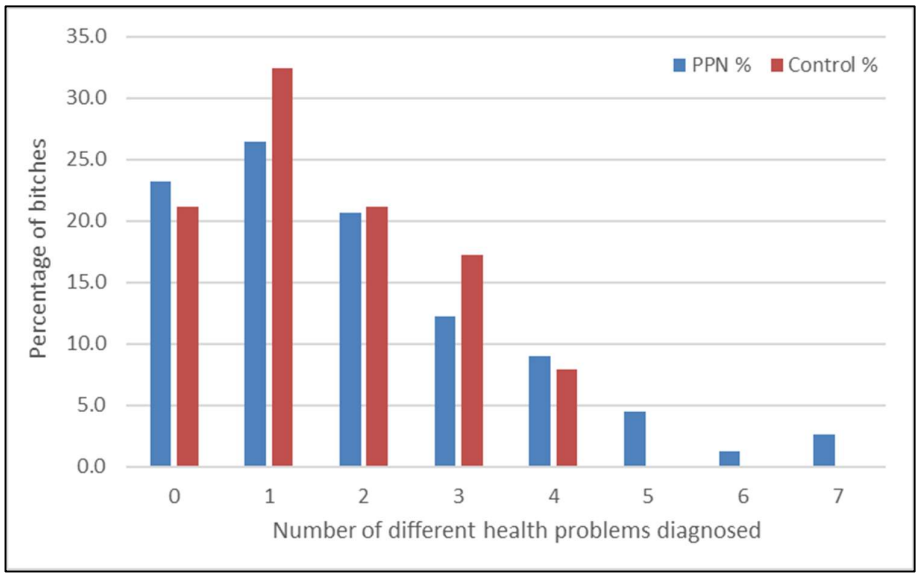


Figure 8.9. The percentage of bitches neutered before (PPN, n=155) or after (Control, n=151) puberty that had each number of different health problems diagnosed.

8.4.3 Withdrawals from working for health reasons

One hundred and thirteen of the 195 bitches that qualified as guide dogs had finished working as of 15/09/2023 (64 PPN, 49 Control, Chi-square=5.108, D.F.=1, P=0.024). Fifteen of these had been withdrawn from working for health reasons (11 PPN, 4 Control). There was a significant difference in the number of bitches withdrawn for health reasons and those still working between PPN and Control groups (Chi-square=5.602, D.F.=1. P=0.018). Bitches were withdrawn for health reasons between 2.4 and 8.9 years of age (PPN bitches 6.0 ± 0.7 years, Control bitches 6.5 ± 1.1 years; Table 8.2). The most common reasons for health withdrawals from working were musculoskeletal (6 PPN, 2 Control) and malignant neoplasia (2 PPN, 1 Control). Two PPN bitches were withdrawn for osteoarthritis and two for CCL rupture. The final four health withdrawals from working were for USMI (Control), auto-immune thrombocytopenia (PPN), idiopathic generalised epilepsy (PPN) and bilateral cataracts (PPN).

8.4.4 Cause of death

As of 15/09/2023, 32 bitches had died (18 PPN, 14 Control; Chi-square=0.448, D.F.=1, P=0.503) between 3.2 and 10.8 years of age (PPN mean = 7.9 ± 0.6 years, Control mean = 8.6 ± 0.4 years; Table 8.2). There was no significant difference in age at death between PPN and Control bitches (Mann-Whitney U=118, P=0.779).

Table 8.2. The minimum, maximum, mean (± SEM) age at withdrawal from working as a guide dog for health reasons for 15 bitches, and age at death for 32 bitches, in a prospective study investigating the effect of neutering before (PPN) or after (Control) puberty on health outcomes.

Descriptive statistic	PPN	Control
Age at withdrawal from working for health reasons		
Number of bitches	11	4
Mean (SEM) (years)	6.2 (0.7)	6.5 (1.1)
Range (years)	2.4–8.9	3.3–8.3
Age at death		
Number of bitches	18	14
Mean (SEM) (years)	7.9 (0.6)	8.6 (0.4)
Range (years)	3.2–10.3	5.2–10.8

The most common cause of death was malignant neoplasia (13 PPN, 9 Control; Appendix 12). Not all bitches were subject to post-mortem examination therefore histology to confirm type of neoplasia was not always available; the body system affected was described where histological examination was not performed. Other causes of death were autoimmune haemolytic anaemia (1 PPN), seizures (1 Control), renal disease (1 Control), severe peritonitis (1 PPN) and one PPN bitch was euthanised due to osteoarthritis. Exact causes of death were unknown for five bitches (2 PPN, 3 Control).

8.5 DISCUSSION

Despite much literature relating to the impact of neutering, and the timing of neutering relative to age, the effect of neutering before or after puberty on bitch health has not been well studied. This prospective study followed a cohort of bitches to examine the impact of neutering before or after puberty on the occurrence of several diseases throughout life. Detrimental associations between prepubertal neutering and musculoskeletal disease were identified

that may be expected based on previous literature relating to the impact of neutering at different ages. There were interesting differences for immune diseases that may be attributable to pubertal status at neutering, such as older age at diagnosis of atopy and lower survival probabilities for remaining free from otitis externa for bitches neutered prepubertally, that have not been reported previously. Unexpectedly, there were no relationships identified between pubertal status at neutering and the incidence of any urogenital disease, with low numbers of bitches diagnosed with diseases such as UI, which is commonly associated with neutering. However, for many diseases, the number of bitches diagnosed was small and did not allow for statistical comparisons. Significantly more pre than post-pubertally neutered bitches had finished working, and more had been withdrawn from working for health at the time of data collation. These results address the study hypothesis and objectives and, for diseases diagnosed in enough bitches to enable analysis, suggest that prepubertal neutering may have a detrimental effect on some future musculoskeletal diseases, immune diseases and on working life in female dogs of these crossbreeds. This provides important information for organisations such as Guide Dogs to support neutering policies, to help maintain optimal dog welfare and to maximise length of working life.

In the scoping review (see Chapter 3), no previous studies were identified that examined the impact of neutering in relation to puberty on musculoskeletal disease. The results of the present study suggest that neutering bitches prepubertally may be negatively associated with musculoskeletal health outcomes, consistent with results reported by others related to neutering age (Spain *et al.*, 2004; Duerr *et al.*, 2007; Torres de la Riva *et al.*, 2013; Ekenstedt *et al.*, 2017; Simpson *et al.*, 2019; Zlotnick *et al.*, 2019). There were significantly more PPN bitches with CCL rupture, although the small number of Control bitches affected may have affected test reliability, and the incidence rate ratio was large (17.8). Greater risk of CCL disease is frequently associated with

earlier neutering (Duerr *et al.*, 2007; Torres de la Riva *et al.*, 2013; Ekenstedt *et al.*, 2017). Ekenstedt *et al.* (2017) reported that 87% of the CCL rupture cases for bitches in their case-control study were neutered at or before 12 months of age. Torres de la Riva *et al.* (2013) reported no CCL disease in Golden Retriever bitches neutered at or after 12 months of age compared to 7.7% for bitches neutered younger than 12 months of age, with a mean age of onset of 4.8 years. Mean age of first diagnosis in the present study for PPN bitches was 7.1 years. In recent review papers, Kutzler (2020; 2023) states that reproductive hormones have been shown to affect CCL laxity in humans. Reproductive hormones have also been shown to affect collagen concentrations and ligament strength in rabbit cruciate ligaments (Slauterbeck *et al.*, 1999; Light *et al.*, 2012). Kutzler (2020; 2023) proposes that the continuous activation of luteinising hormone (LH) receptors (caused by persistently elevated LH) in the cruciate ligament could increase ligament laxity leading to joint instability.

There was a significant difference between survival curves for osteoarthritis and having at least one musculoskeletal disease, with the survival probabilities for PPN bitches for both conditions falling at earlier ages than for Control bitches. Osteoarthritis was diagnosed in PPN bitches at significantly younger ages than in Control bitches, although the effect size was small. While more PPN bitches were diagnosed with osteoarthritis and at least one musculoskeletal disease than Control bitches, the differences in incidence between groups were not significant. There has been limited literature relating to the impacts of neutering age on osteoarthritis, with most authors proposing that the differences in other diseases such as HD and ED may predispose to osteoarthritis later in life. Spain *et al.* (2004) investigated the effect of age at neutering on numerous medical conditions and found no association between age at neutering and arthritis. In comparison to the study by Spain *et al.* (2004) it is possible that bitches in the present study had more regular health checks and were more likely to be

presented to veterinarians for investigation of possible arthritis due to the potential impact on their working ability. The osteoarthritis observed in the present study may not have been associated with HD or ED, with only small numbers of bitches being diagnosed (1% of bitches overall with HD). Hart *et al.* (2020a) reported HD in between 2.1 and 9.6% of Golden Retriever and Labrador bitches. HD and ED are complex diseases that are influenced by many factors and the low proportions observed could be influenced by selectively breeding, and good environmental management and husbandry practices for the bitches in the programme including weight and dietary controls.

Along with musculoskeletal disease, development of neoplasia is a commonly investigated outcome, although only one study was identified that considered pubertal status at the time of neutering (Schneider *et al.*, 1969). Scheider *et al.* (1969) reported increased relative risk for malignant mammary neoplasia in bitches neutered after puberty compared to bitches neutered before puberty. Other studies that have compared bitches neutered at different ages reported similar effects on mammary neoplasia (Torres de la Riva *et al.*, 2013; Beaudu-Lange *et al.*, 2021). Mammary neoplasia was not observed in any bitch in the present study, although only 68 bitches had health data to at least 9.5 years of age, the remaining bitches were either still too young or had been withdrawn and rehomed at younger ages. Mean age of onset for malignant mammary neoplasia in the bitch has been reported as between 9.5 to 14 years (Moulton *et al.*, 1986; Schafer *et al.*, 1998; Sorenmo *et al.*, 2000, 2009; Petrov *et al.*, 2014) and it is most commonly observed in entire bitches and those neutered after experiencing several oestrous cycles (Schneider *et al.*, 1969; Varney *et al.*, 2023). It may be useful to re-examine data for mammary neoplasia once lifetime information is available, though with a low expected incidence and considering the number of bitches in the present study, statistical comparisons may not be possible.

Other types of neoplasia such as appendicular bone sarcoma (Cooley *et al.*, 2002), HSA and MCT (Torres de la Riva *et al.*, 2013) have been associated with age at neutering. However, these associations are not similar across different cancers, with earlier neutering age associated with a decreased risk for some and an increased risk for others. In 242 Golden Retriever bitches, Torres de la Riva *et al.* (2013) reported that those neutered before 12 months of age were significantly less likely to have HSA and MCT compared to bitches neutered when 12 months of age or older. While that study did not consider pubertal status at the time of neutering, the results of the present study were similar for MCT, with more Control bitches being diagnosed, although the numbers of bitches were small (4 PPN, 7 Control) and the difference was not significant. In contrast, for LSA, Torres de la Riva *et al.* (2013) reported that, although not significant, a higher percentage of bitches in the early (5.9%) rather than late neuter group (1.4%) were diagnosed. In the present study, LSA was diagnosed in too few bitches (1.4% in both groups) for analysis, with an incidence similar to that for late neutered bitches reported by Torres de la Riva *et al.* (2013). Cooley *et al.* (2002) also found an increased risk with younger neuter age when investigating bone sarcoma in a study that included 389 female Rottweilers. In the present study no bitches were diagnosed with osteosarcoma.

There were no significant differences in the number of PPN compared to Control bitches diagnosed with any of the individual types of malignant neoplasia studied, and most were diagnosed in too few bitches for statistical analysis. However, for 'other' malignant neoplasia, numerically more PPN than Control bitches were diagnosed (7.7% PPN, 2.6% Control) and the incidence rate ratio was 3.62 with 95% CI of 1.18 to 11.09. There was also a significant difference in survival curves with survival probability for PPN bitches falling to around 0.8 by just after 10 years of age, and continuing to decline thereafter, compared to that for Control bitches which appeared to plateau at ~0.95 from around 9.5 years

of age. Increased incidences of neoplasia have been well documented in neutered compared to entire dogs, with higher circulating LH concentrations considered a potential contributing factor. For the present study, findings related to malignant neoplasia may be influenced by the small number of bitches diagnosed, and it will be useful to re-examine data once more bitches have reached older age. The low incidence combined with the late age of onset means that these diseases may have less impact on the working life of a guide dog. However, the impacts for dog and owner wellbeing should still be considered.

In the present study, the number of bitches diagnosed with urogenital disease was low across all diseases and there were no significant differences in the number of PPN compared to Control bitches diagnosed with any urogenital disease. This finding was unexpected due to urogenital diseases, especially UI, being the most frequently studied in relation to the timing of neutering in female dogs. The scoping review identified four studies that categorised bitches as pre or post-pubertal at surgical neutering and investigated outcomes relating to incontinence. Two of the studies reported no significant effect (Thrusfield *et al.*, 1998; de Bleser *et al.*, 2011) while two did report an effect of neutering before or after puberty, but with conflicting results. These studies have been discussed previously, see Chapter 3. In summary, Stocklin-Gautschi *et al.* (2001) suggested that the incidence of UI in bitches neutered prepubertally was lower compared to those neutered post-pubertally, using data from two different study populations. Lutz *et al.* (2019) reported the opposite, with significantly more bitches neutered prepubertally being reported with UI than those neutered post-pubertally. The method of grouping bitches or data irregularities may however have compromised the findings; the prepubertally neutered group included some bitches aged up to 1.4 years, and some 'post-pubertally neutered' bitches were allegedly aged 0.3 years. Both increased (Spain *et al.*, 2004; Byron *et al.*, 2017; Pegram *et al.*, 2019a) and decreased (Veronesi *et al.*, 2009)

risks of UI have been reported for bitches neutered at earlier ages. Other studies have reported no association between the incidence of UI and bitch neutering age (Howe *et al.*, 2001; Forsee *et al.*, 2013; Pegram *et al.*, 2019b). In the present study, UI occurred in low numbers (2.3% of bitches overall), and more frequently in bitches neutered after puberty (1 PPN, 6 Control). In other studies, rates of UI incidence have been reported from 0 to 20% for neutered bitches (Reichler *et al.*, 2005; Pegram *et al.*, 2019a; Hart *et al.*, 2020a;b) although incidence is known to vary by breed (Reichler *et al.*, 2005; O'Neill *et al.*, 2017). The overall incidence reported in the present study is similar to reports for neutered purebred Golden Retrievers and Labradors by others. Hart *et al.* (2020a) reported no cases in Golden Retrievers neutered at any age and 2-3% UI in Labradors neutered to 12 months of age, and Pegram *et al.* (2019a) reported an incidence of 2.9% for Labradors.

While there were no significant differences in urogenital disease incidence in the present study, some differences were identified between PPN and Control bitches for age at disease diagnosis. Age at first diagnosis of perivulval dermatitis was higher for PPN bitches and most Control bitches affected with perivulval dermatitis were diagnosed before neutering (four out of five bitches). The reason for this difference in age is unclear; although the finding for age at diagnosis of perivulval dermatitis is similar to that observed for atopy. Thus, there is the potential for the same causal factor to be affecting the skin around the vulva as well as the skin elsewhere; although only two of the bitches with perivulval dermatitis were also diagnosed as atopic.

The impact of neutering timing on immune disease has historically received less focus than other health outcomes, yet in the present study there were some interesting findings. Otitis externa was the most commonly reported immune disease, and while there were not significantly more prepubertally neutered bitches diagnosed with otitis at an alpha of 0.04 (74 PPN, 55 Control) there were significant

differences in survival curves. Survival probabilities for PPN bitches by two years of age were approximately 0.6 compared to over 0.7 in Control bitches, and by four years of age were approximately 0.45 for PPN bitches compared to over 0.6 for Control bitches. Age at diagnosis of atopy was significantly higher for PPN than Control bitches and potentially higher than may be expected. Signs of atopy are usually observed in the Guide Dogs population by 12 to 18 months of age, and following a period of clinical work up a diagnosis is normally confirmed within four to six months (Adams, personal communication, 2024). Early identification and diagnosis of atopy is important for Guide Dogs when matching dogs to potential clients. Besides atopy and otitis, other immune diseases were diagnosed in too few bitches for further statistical analysis, including hypothyroidism (1 PPN, 0 Control) that is suggested by some authors to be more common in neutered bitches due to the effect of neutering on thyroid function (Milne and Hayes, 1981; Panciera, 1994).

Atopy is a complex disease influenced by many environmental and genetic factors. It is understood that sex hormones influence immune responses and skin permeability in rats (Kanda *et al.*, 2019). Sundburg *et al.* (2016) reported an increased risk for atopy in neutered compared to entire female dogs and suggested that oestrogens may have a suppressive effect on disease occurrence. However, that would not explain the difference in age at diagnosis reported here, nor would the longer period exposed to unregulated and high LH concentrations explain why bitches neutered earlier were diagnosed at older ages. There may be an effect of experiencing puberty: in humans, before puberty, atopic dermatitis is more common in males than females, while the reverse is true after puberty (Kanda *et al.*, 2019). Bitches in the present study that experienced puberty may have experienced earlier triggers for atopy than those that did not. Serial measurements for circulating gonadal hormones would have been useful to understand associations between these and the development of atopy;

however, they were not undertaken in the present study. Further investigation into the role of gonadal hormones and skin function in the dog, and larger studies involving dogs neutered at different times would be useful to increase understanding about the potential effects of gonadal hormones on canine atopic dermatitis.

For diseases in the 'other' category, there were very few bitches diagnosed with any condition except for being overweight or having obesity. Despite some work suggesting associations between neutering age and the likelihood of dogs becoming overweight (Spain *et al.*, 2004; Simpson *et al.*, 2019), there was no difference between PPN and Control bitches in the number that were overweight or had obesity, in the age at the first report of being overweight or having obesity or between the survival curves. This may be unsurprising but perhaps is due to the small relative difference in neutering age between the PPN and Control groups. Interestingly, although differences were not significant, fewer PPN than Control bitches were found to be overweight or have obesity (41.9% vs 45.7%) or have a BCS indicating obesity (12.9% vs 15.9%). This is similar to the findings of Spain *et al.* (2004) who reported a decreasing risk of overweight body condition with decreasing age at neuter for 1,659 dogs of various breeds, although results were not reported separately for male and female dogs. Simpson *et al.* (2019) examined data for over 2,700 Golden Retrievers and found the opposite relationship, reporting that dogs neutered at 6–12 months had an increased risk for overweight/obesity compared to those neutered at >12 months of age, with no difference in risk for dogs neutered at <6 months of age. There were differences in study design, with all dogs in the study by Spain *et al.* (2004) being neutered between six weeks and 12 months of age. It would be interesting to examine data from the present study in more detail to determine whether bitches were only classed as overweight for a short period or whether being overweight was a longer-term problem. It would also be interesting to examine whether associations that would be expected between

diseases such as being overweight and forelimb lameness were present, and to examine other potential associations such as otitis and being overweight. However, this was not within the scope of the study.

The impact of neutering timing in relation to puberty on future health is of interest for all dogs, however for assistance dogs, where there may be an effect on the ability of the dog to continue working with their human partner, it is particularly important. In the present study, significantly more prepubertally neutered bitches had finished working at the time of data collation. Additionally, significantly more had been withdrawn from working for health reasons, including CCL rupture, and osteoarthritis, the latter occurring at significantly younger ages in PPN bitches. Not all bitches had reached end of life at the time of data collation, and differences in the number that died and age at death between the two groups were not significant. It will be interesting to examine these variables again once whole life data are available, and to see whether the difference in age at death becomes significant once data are available for all bitches.

The differences observed may be related to gonadal hormones and levels of exposure. Bitches neutered after puberty have experienced elevated oestrogen and progesterone and then elevated LH for less time. It is possible that exposure to oestrogen and progesterone and then unregulated and high LH have an impact. Cruciate disease, neoplasia and skin disorders such as otitis and atopy may be influenced by a component of oestrogen and progesterone in terms of the bone or skin itself as well as the potential influence on receptors. Any differences observed would be small in comparison to studies such as those by Hart *et al.* (2014, 2016) that examined data for bitches neutered over a greater age range (less than six months to eight years) with associated greater difference in high LH exposure and are likely linked to whether the bitches have experienced an increase in oestrogen or progesterone (experienced puberty) or not. Concentrations of these reproductive hormones

were not measured in study bitches, and this could be recommended for future studies.

Overall, considering disease incidence and age of diagnosis, there were negative associations between prepubertal neutering and musculoskeletal disease and immune disease, as well as for 'other' malignant neoplasia. Additionally, there were potential detrimental consequences for working life. These findings, combined with recently published recommendations for neutering age (Hart *et al.*, 2020a;b) would suggest that if bitches of these breeds are to be neutered, doing so post-pubertally may be beneficial from a health perspective based on the diseases investigated.

8.5.1 Study limitations and generalisation

As with previous chapters, the results of the study are limited by the characteristics of the study sample. These factors are discussed in more depth in the general discussion (see Chapter 9). The limited number of bitches available to recruit to the study resulted in some of the less common diseases being diagnosed in too few bitches to enable comparison between groups. These were diseases commonly associated with neutering in the literature such as HD (three bitches diagnosed), ED (six bitches diagnosed), HSA (three bitches diagnosed) and LSA (four bitches diagnosed). These diseases are operationally important for assistance dog organisations in maintaining optimum health and working life in their dogs, and the inability to compare diseases incidence between groups is unfortunate. One of the major limitations was that health data were only available for bitches until the time they left the guide dog programme. More PPN bitches had ceased working as guide dogs than Control bitches at the time of health data collation, which could affect the results due to data not being available for the bitches once they were rehomed. Attempts to address this by using Cox regression analysis to deal with censored data were affected by the small number of bitches in the study. Even with the calculated disease rate per 1000 dog-days to standardise the presentation of

disease incidence data, this limits the findings related to diseases that have a later age of onset. For instance, bitches may have been removed from programme when younger and subsequently developed cancer or musculoskeletal diseases such as osteoarthritis later in life. However, the results do provide an indication of the sample sizes that would be required in future prospective studies to adequately study these diseases as well as information about expected age of onset. This limitation applies to both trial groups and there was no difference between groups in the number of bitches withdrawn during training (see Chapter 7) or in the number of PPN and Control bitches within each group for years of health data available from six months of age to the last health record, therefore differences highlighted are valid. Additionally, the time restraints for the study mean that data were not available for all bitches until death. However, future work is planned to investigate whole life and cause of death data for all bitches when they are available. For some diseases, such as elbow dysplasia and atopy, age at first diagnosis will be older than age at first presentation of clinical signs due to the work up period required for a diagnosis to be confirmed.

8.6 CONCLUSION

This is the first time that associations between neutering bitches before or after puberty and a large number of health outcomes have been investigated in a prospective study design. The results suggest that there may be detrimental associations between musculoskeletal disease and neutering bitches before puberty, particularly for CCL disease, and for immune disease, specifically otitis. Additionally, there may be associations with age of diagnosis for diseases that may affect working ability and health management of assistance dogs, such as delayed onset of atopy and earlier onset of osteoarthritis. In contrast, there was little to no association with the individual types of malignant neoplasia studied or on the incidence of urogenital disease. However, there are important limitations to consider with regard to the number of bitches in the study, the

limited breeds used, and the small number of bitches diagnosed with disease. The study provides important information for other researchers in the field in terms of the size of study populations needed for valid statistical analysis.

These results will be of interest to assistance and working dog organisations, veterinarians and pet dog owners, as they provide additional information regarding the future health impacts of neutering bitches at different stages of their reproductive development.

Chapter 9. General discussion and conclusions

Deciding whether to neuter a dog and the related ethical and welfare considerations of neutering have received greater attention in recent years (Palmer *et al.*, 2012; Sandøe *et al.*, 2015; Wongsaengchan and McKeegan, 2019). Once the decision to neuter has been made, the question of the best time to neuter is a complex one. For female dogs, both positive and negative associations between different ages at neutering and future disease and behavioural outcomes have been suggested. The reported findings are often inconsistent across diseases, breeds and sexes, and increasingly authors are recommending that decisions on the best time to neuter should be made on a case-by-case basis, based on the individual dog and owner circumstances, with no broad recommendations to suit all dogs (McKenzie, 2010; Palmer *et al.*, 2012; Hart *et al.*, 2014; Houlihan, 2017; Urfer and Kaeberlein, 2019; Hart and Hart, 2021).

For large assistance dog organisations that are required to neuter their dogs before they commence formal training, the decision regarding the best time to neuter is important due to the need to maximise the dogs' long-term health and behavioural suitability for their role. There is also a need to balance this against the requirements of the organisations' training programmes. At the time of designing the present study, some international guide dog schools, such as Guide Dogs Victoria (Australia) and Irish Guide Dogs, were routinely neutering their dogs before puberty, at five and six months of age respectively. This has the least potential impact on the training programme by ensuring that dogs are neutered well before they are due to enter formal training. However, there was no evidence available from these schools regarding any long-term consequences of their neutering policies. Such populations are almost ideal for such studies due to the high level of consistency and control of many factors compared to other

studies that use diverse populations of animals with varied breeding, rearing methods and previous experience.

Despite many studies reporting an effect of age at neutering on health and behaviour in dogs, and several suggesting an impact of pre or post-pubertal neutering, there has in fact been little data published on the impact of neutering bitches before or after puberty, as highlighted in the scoping reviews in this thesis. This lack of information is at variance with what is often thought to be the situation by veterinary surgeons in clinical practice (Berks, 2017). As well as examining the current evidence relating to the impacts of neutering bitches pre- or post-pubertally, this study aimed to investigate the effect of pubertal status at neutering on several outcomes, including health, behaviour, physical development and factors related to an assistance dog programme, such as progression through training and outcome from the training programme. Neutering before puberty could be undertaken as early as six to 14 weeks of age, commonly referred to as 'early neutering' (Kustritz, 2002). However, in the present study, six months of age was selected so that bitches were at the end of the period of major growth (Von Pfeil *et al.*, 2009a). The prospective study design included bitches from a well-managed population with similar rearing and dog management processes, and comprehensive health monitoring. The benefits of using similar study populations to investigate behavioural outcomes have been mentioned previously (Serpell and Duffy, 2016). This methodology addressed some of the concerns raised in the scoping reviews regarding methodology and potential biases in previously published work.

The findings of the present study suggest that for Labrador and Golden Retriever crossbreed bitches, there was minimal impact of neutering before compared to after puberty on future behaviour and, while bitches neutered prepubertally entered training earlier and took less time to progress though some of the training stages, there was no impact on qualification rates. Considering these results

alone, prepubertal neutering could be considered a suitable strategy for female dogs of these breeds. However, the importance of considering all possible outcomes when making neutering decisions is highlighted when results for physical rather than behavioural variables are reviewed; the findings of the study did suggest associations with physical development and health. Prepubertally neutered bitches had significantly greater change in height measurements and smaller change in measurements of vulval size between six and 17 months of age, and significantly more had vulvas with abnormal appearance observed at 17 months of age than those neutered post-pubertally. Additionally, there were some diseases that were negatively associated with neutering before puberty including CCL rupture, osteoarthritis, otitis and atopy. Finally, at the time that data were collated, significantly more prepubertally neutered bitches had finished working (i.e. they had a shortened working life) and significantly more had been withdrawn from working for health reasons. Based on these results, it is recommended that consideration be given to neutering Labrador and Golden Retriever crossbreed bitches after six months of age, and post-pubertally.

The lack of association between pubertal status at neutering and behaviour, or qualification as a guide dog, was perhaps surprising given the findings of previous authors where an effect of neutering age on behaviour has been reported (O'Farrell and Peachey, 1990, Spain *et al.*, 2004; Starling *et al.*, 2019; Zlotnick *et al.*, 2019), and the knowledge that behaviour is one of the main reasons for failure in assistance dog programmes (Goddard and Beilharz, 1982, 1983; Arata *et al.*, 2010; Tomkins *et al.*, 2011; Duffy and Serpell, 2012). However, other authors investigating the effect of neutering age on behaviour have reported no significant impact (Salmeri *et al.*, 1991; Howe *et al.*, 2001; Edwards *et al.*, 2019). Furthermore, in the only other study identified that examined behaviour in bitches neutered before or after puberty, no differences were identified (Balogh *et al.*, 2018). Despite variation in bitch age at questionnaire completion

between the present study and that of Balogh *et al.* (2018), bitches from both studies were considered 'mature adults' according to the categorisation system for dogs in behavioural studies proposed by Harvey (2021). Therefore, the results reported by Balogh *et al.* (2018) for 58 Labrador bitches and by this study for 306 Labrador and Golden Retriever crossbreed bitches are comparable and appear to refute the assertions of some authors of review papers and conference proceedings (Kustritz, 2002; Thrusfield, 2011; Borstad, 2015; De Cramer and May, 2015; Romagnoli, 2017; Warnes, 2018), and supports the contention that neutering before or after puberty has little impact on behavioural development.

Dog behaviour is influenced by many factors including genetics, environmental factors and experience (Serpell *et al.*, 2016). Reproductive hormones can also affect behaviour and it is well known that behavioural changes around the time of oestrus, urinary behaviour and maternal care behaviours are influenced by reproductive hormones (Christie and Bell, 1971; Beach and Merari, 1970; Beach, 1974, 1975; Lezama-García *et al.*, 2019). Behaviour for bitches in the present study was measured at two time points, the last at three years of age when all bitches had been neutered for at least 617 days before questionnaire completion. Therefore, behaviour at this time would not have been under the direct influence of gonadal hormones. The present study offers improvements in methodology compared to previous studies that have reported an effect of neutering age on behaviour including, a prospective design using dogs from one breeding programme that has been selecting and breeding dogs for certain behaviours for decades, using a validated tool to measure behaviour, and, importantly, similar nest socialisation, rearing, housing and training experiences for all bitches, factors that are known effect adult dog behaviour (Harvey *et al.*, 2016a; Serpell *et al.*, 2016). These study and population characteristics limited the influence of these factors so that the main effect of timing of neutering in relation to puberty could be examined.

While bitch behaviour at one and three years of age was not influenced by pubertal status at neutering, progression through the training programme did appear to be affected. Bitches neutered prepubertally spent less days in puppy raising and entered training at an earlier age than those neutered post-pubertally, however the effect size for these outcomes was smaller than that suggested by power analysis. Given that there were no differences in the number of bitches that qualified between groups, which is logical given that there was no apparent effect on behaviour, it is likely that these differences were influenced mainly by the fact that bitches neutered post-pubertally were required to wait until after their first oestrus to be neutered. Therefore, the timing of the first oestrus and of the subsequent neutering surgery, rather than an effect related to the post-pubertal status of the bitches at neutering, was likely the predominant influencing factor.

The withdrawal of guide dogs from working at an age that is earlier than an expected retirement age can have negative consequences for the guide dog owner (Nicholson *et al.*, 1995; Schneider, 2005; Allen, 2006; Lloyd *et al.*, 2021) as well as for the organisation in terms of 'production planning' for replacement dogs. Therefore, factors that have the potential to increase the likelihood of early withdrawals are important for assistance dog organisations to consider when making changes to their programmes. For the 195 bitches that qualified as guide dogs in the present study, significantly more pre than post-pubertally neutered bitches had finished working and had been withdrawn for health reasons by the time of health data collation (i.e. they had a shorter working life). This study included just over 300 female dogs, however if a policy of prepubertal neutering was adopted by the organisation and these results were applied to the whole population, more than 500 bitches a year would be neutered prepubertally, and approximately 325 of these could be expected to qualify as guide dogs. Over time, such an increase in earlier withdrawals from working, and withdrawals

due to health, as a consequence of neutering prepubertally could have a substantial impact on the guide dog programme.

Health reasons for withdrawals from working were musculoskeletal for six PPN and two Control bitches and four of these PPN bitches were withdrawn for osteoarthritis or CCL rupture. Additionally, of the 16 bitches in the study withdrawn for health reasons before qualification, half were withdrawn for musculoskeletal disease. Musculoskeletal problems were identified as an important factor to consider in this study due to the effect on the organisation caused by withdrawals from the programme for related diseases. Others have also highlighted musculoskeletal disease as a common reason for withdrawal from assistance dog programmes (Caron-Lormier *et al.*, 2016a; Bogaerts *et al.*, 2019; Zlotnick *et al.*, 2019). Thus, these findings are relevant for assistance dog organisations: not only are a large number of the health withdrawals for reasons related to musculoskeletal disease, but the diseases are those that appear to be associated with prepubertal neutering, so they are having an impact on working life.

The detrimental associations identified between prepubertal neutering and development of musculoskeletal disease were not surprising and reflect previously reported findings related to the impact of neutering at earlier ages (Spain *et al.*, 2004; Duerr *et al.*, 2007; Torres de la Riva *et al.*, 2013; Ekenstedt *et al.*, 2017; Simpson *et al.*, 2019; Zlotnick *et al.*, 2019). However, the present study is the first to examine associations between neutering before or after puberty and musculoskeletal disease. Significantly more prepubertally neutered bitches were diagnosed with CCL rupture (although the number of post-pubertally neutered bitches diagnosed was small, n=1), consistent with findings from other authors where increased risk of CCL rupture with earlier neutering age has been reported (Duerr *et al.*, 2007; Torres de la Riva *et al.*, 2013; Ekenstedt *et al.*, 2017). Additionally, there were significant differences between survival curves for bitches neutered before or

after puberty for osteoarthritis and having at least one musculoskeletal disease.

There are few studies examining the impact of neutering on dog growth, however, authors have suggested that differences in growth associated with the timing of neutering may impact future musculoskeletal disease (Salmeri *et al.*, 1991; Spain *et al.*, 2004). Spain *et al.* (2004) proposed that the increased bone length caused by early neutering had secondary effects on joint conformation. Bitches neutered prepubertally in the present study, were taller and perhaps had increased bone length compared to post-pubertally neutered bitches, and could be considered at increased risk of musculoskeletal disease. However, when comparisons were made, musculoskeletal disease did not appear to be associated with growth between six and 17 months of age, although previously discussed issues with method of height measurement may have impacted these data. It is possible that with radiographic measurements of long bone length, such associations may have been apparent. It is also possible that the association between prepubertal or early age neutering and musculoskeletal disease may be mediated in part by another mechanism, and that gonadal hormones, or the earlier absence thereof leading to high circulating LH, have a role to play in future development of other musculoskeletal diseases, as proposed for CCL disease (Kutzler, 2023). LH-receptors have been identified in the femoral head and hyaline cartilage, although differences in expression between entire and neutered dogs have not been demonstrated (Kutzler, 2020). Nevertheless, irrespective of the cause, the findings related to musculoskeletal disease are important for assistance dog programmes due to the potential effect on working ability and working life.

Potential negative associations between neutering before puberty and immune diseases were also observed, such as older age at diagnosis of atopy and lower survival probabilities for remaining free from otitis externa. These results are the first to be presented

examining potential associations between pubertal status at neutering and immune disease, and no previous evidence was identified in the scoping review related to the effect of age at neutering on atopy in female dogs. The later and apparently more variable age of atopy diagnosis for PPN bitches was surprising and concerning, since bitches would likely be qualified and working by this time and the ability of the guide dog owner to manage a dog with atopy could result in the partnership no longer being viable (Caron-Lormier *et al.*, 2016a). The reasons for the difference in age at diagnosis are unclear but would benefit from further investigation due to the importance of the disease for the organisation and the potential negative consequences for the guide dog owner of any impact on the partnership.

The lack of apparent association between urogenital disease and pubertal status at neutering, and between markers of vulval development and future disease, was unexpected. Urogenital disease, especially UI, is perhaps one of the most frequently investigated factors when considering the timing of neutering for female dogs, specifically the appropriate development of the external genitalia and proposed related impacts on future disease. Some authors suggest that the removal of oestrogen at an early age may impact normal vulval development and that this can predispose to urogenital disease (Johnson, 1991; Feldman and Nelson, 2004; Nelson and Couto, 2009). Verstegen-Onclin and Verstegen (2006) reported high proportions of bitches neutered before puberty represented in a population of bitches in Florida presented to a specialist small animal reproduction clinic for urogenital problems, including recessed vulva, vaginitis, vaginal dermatitis and UTI. The authors suggest that these problems may occur at a higher incidence in early neutered bitches. However, it is possible that this method of presenting their data may be open to misinterpretation. It may be correct that for bitches with disease, high numbers were neutered at earlier ages. However, in a prospective study design, where bitches are followed throughout life to examine occurrence of

disease, the incidence may be lower than that which may be expected from solely studying data for dogs with disease. In the present study, the number of bitches diagnosed with all forms of urogenital disease was low and there were no significant differences in the number of PPN compared to Control bitches diagnosed with any urogenital disease. At 17 months of age significantly more pre than post-pubertally neutered bitches had vulvas that appeared juvenile and recessed on veterinary examination and more that were classified as recessed/inverted based on digital image examination. Additionally, post-pubertally neutered bitches had significantly greater change in vulval size between six and 17 months of age. However, these findings related to vulval development were not associated with increased risk of urogenital disease, which was unexpected based on the suggestions of other authors.

It is clear both from the results of the present study and from the many studies in the scientific literature, that the timing of neutering has the potential to influence certain growth and health outcomes. However, greater understanding is required regarding the biology underpinning these differences followed by exploration of potential methods to mitigate any negative impacts. There is evidence that the effect of neutering on dog health outcomes may be related to the absence of gonadal hormones and the consequences for related feedback mechanisms. Removal of the ovaries eliminates the negative feedback mechanism that, in entire bitches, regulates the release of LH and FSH from the anterior pituitary gland in response to pulsatile secretions of GnRH from the hypothalamus. High concentrations of both gonadotrophins have been reported in neutered bitches (Olson *et al.*, 1992; Concannon, 1993; Reichler *et al.*, 2005), although more work has been published related to LH than FSH. High and unregulated concentrations of LH may act on different tissues and increase the risk of disease. Additionally, it is possible that LH may influence some of the behavioural differences observed between entire and neutered dogs, and dogs neutered at

different times, due to its action on the hippocampus and hypothalamus (Kutzler, 2020).

LH-receptors have been identified in smooth muscle and vascular endothelial cells, lymphoid tissues and lymphocytes, and in the skin, and also in HSAs, MCTs, and in cells from neoplastic lymph nodes (Zwida and Kutzler, 2016; Kutzler, 2020; 2023). The potential effect of high circulating LH and activation of these LH-receptors, and the mechanisms by which disease states are influenced are largely unknown. There have been suggestions and proposed associations/mechanisms described relating to the action of LH and LH-receptors in disease processes, including disruption of other receptors in the same tissues, and activation of LH-receptors causing an increase in laxity and subsequent joint instability in the case of CCL rupture (Zwida and Kutzler, 2016; Kutzler, 2023). However, for the most part, the mechanisms by which LH and LH-receptor activation influence disease occurrence are not yet known.

Potential associations between high LH concentrations and disease in neutered dogs have been demonstrated in some studies. Coit *et al.* (2009) found significantly higher levels of mRNA expression for LH-receptor in smooth muscle from the bladder in neutered male and female dogs than in entire dogs, and this was highest in dogs that had acquired UI and had the lowest bladder contractility. UI can be successfully treated in some neutered bitches using methods such as GnRH analogues to reduce LH concentrations. However, whether there is an effect of LH on receptors in the bladder that affects collagen and bladder contractility is not proven (Kutzler, 2023) and other authors have not shown the same relationship with LH and UI in neutered bitches (Reichler *et al.*, 2005). In fact, Ponglowhapan *et al.* (2008) showed reduced LH-receptor expression in the lower urinary tract of neutered compared to entire dogs.

No studies have been identified that have examined differences in circulating LH or FSH concentrations in bitches based on the timing

of neutering in relation to puberty. Therefore, whether there could be an effect of pubertal status at neutering on these gonadotrophins that could explain the differences observed in disease incidence within the present study is not clear. However, while there is little information available regarding the start of neuroendocrine gonadotropin release in the dog (Laffan *et al.*, 2018), concentrations of FSH and LH in bitches before puberty (from four months of age) are described as being similar to those observed for post-pubertal anoestrus bitches (Concannon, 1993). Thus, it may be unlikely that neutering at six months of age compared to after the first oestrus would result in any large differences in the effect on future concentrations of these hormones. The difference in time between bitches neutered prepubertally or post-pubertally in the present study was only months in most cases. Most bitches, irrespective of trial group had experienced high LH for most of their lives. Whether ovary removal prior to experiencing puberty compared to after has an effect on the levels of LH is unknown, and this would be interesting to examine in future work. If LH was at similar, high levels for bitches in both trial groups, it may be unlikely to be the main contributing factor for the differences observed. Serial measurements of LH and FSH, not undertaken for bitches in the present study, would be required to investigate this concept.

The role of high FSH concentrations on the development of disease in neutered bitches has not been the subject of as much work, and Kutzler (2020) suggests that more needs to be done to understand the influence of FSH and FSH-receptors on long-term health impacts, although also mentions that for FSH, unlike LH, there are other extragonadal negative and positive feedback mechanisms in place. Reicher *et al.* (2005) identified an association between FSH and UI in neutered bitches, however reported that neutered incontinent bitches had lower FSH concentrations than those that were continent. Outside of the ovary, FSH-receptors have not been identified as widely as LH-receptors, but more recently there is emerging evidence to suggest their presence in some other tissues,

suggesting a potential effect of FSH on non-gonadal tissues and organs (Kumar, 2014). In humans and mice, FSH-receptors have been found in non-ovarian reproductive tissues, including the cervix, endometrium, myometrium, vascular endothelium and arterial smooth muscle (Stilley and Segaloff, 2018). FSH-receptors have also been expressed in endothelial cells from a variety of tumours (Radu *et al.*, 2010). Associations between FSH and bone density and metabolism, the immune system and body mass have also been proposed (Sun *et al.*, 2006; Colaianni *et al.*, 2013).

The results reported in this thesis address the study hypotheses and objectives and suggest that prepubertal neutering may be associated with growth and physical development, although measures of growth and physical development did not appear to be directly associated with disease later in life. Additionally, prepubertal neutering may be negatively associated with future musculoskeletal disease, immune disease and working life in female dogs of these crossbreeds. This provides important information for assistance and working dog organisations to support neutering policies, to help maintain optimal dog welfare and to maximise length of working life. However, such organisations' training protocols may mean that neutering at older ages may not be possible. For instance, the recommendation by Hart *et al.* (2020a) to delay neutering for German Shepherds until after 23 months of age would not be possible in an organisation such as Guide Dogs, whose dogs enter training at around 14 months of age and are required to be neutered prior to this time. Neutering protocols and the suitability of breeds used to fit with any programme's requirements for neutering could be reviewed to ensure that dog health and wellbeing is not compromised. For instance, if it is not possible to delay the neutering of German Shepherds to 23 months of age, organisations may benefit from reviewing the use of this breed in their programme to balance the potential benefit to the organisation and society against the potential harm to the dog.

This study did not seek to identify an optimum neutering age, but to determine whether neutering before rather than after puberty would be a preferred neutering strategy. Indeed, it is now suggested that an optimal neutering age that will suit all bitches is not a realistic concept (Urfer and Kaeberlein, 2019; Hart and Hart, 2021). Recommendations have been made for neutering female Labradors after 12 months of age and for female Golden Retrievers, the preference if possible would be to leave them entire, or if neutering is essential, to leave this until after 12 months of age (Hart *et al.*, 2020a). For large mixed breed dogs, Hart *et al.* (2020b) recommend neutering at or after 12 months of age. For assistance and working dog organisations that are required to neuter their dogs as routine practice, waiting until after puberty could be recommended based on the results of the present study, and until approximately 12 months of age based on the work of others.

9.1 STUDY LIMITATIONS

There were some limitations that were applicable to all the studies that comprise this thesis. The applicability of study findings may be limited by the crossbreeds used. Controlling the number of breeds used was considered important given the differences in findings related to neutering and health documented by other authors studying similar pure breeds (Torres de la Riva *et al.*, 2013; Hart *et al.*, 2014, 2016). Crossbreeds make up the majority of the population within the study organisation and were therefore able to be recruited to the study more easily, minimising the recruitment period to three years. Furthermore, other studies that have investigated the impacts of age at neutering commonly either include pure breeds, or large numbers of pure and mixed breed dogs from databases. Therefore, findings related to a known first generation crossbreed would be novel and informative for those organisations that regularly use such crosses in their programmes. This may impact the generalisation of findings to all breeds, including pure Labradors, Golden Retrievers, German Shepherds, Standard Poodles, Flat and Curly Coated Retrievers within the study

organisation's population as well as to other assistance dog schools that commonly use similar breeds, along with others such as Cocker Spaniels and Miniature Poodles (Dogs for Good, 2020; Hearing Dogs, 2020). In spite of this, the small number of crossbreeds used, and the more uniform population, removed some of the breed variability seen in other studies that may affect factors such as behaviour and disease prevalence.

The study included only female dogs neutered before puberty and in the months following their first oestrus. All bitches neutered post-pubertally were neutered before two years of age, with all but one being neutered before the second oestrus. This design was required to study the effect of neutering before compared to after puberty while still allowing bitches to progress into the guide dog training programme without delay. However, it did mean that comparisons between bitches that had been exposed to elevated oestrogen and progesterone and then elevated LH for different amounts of time was not possible. As a result, no conclusions can be drawn regarding the effects of neutering on male dogs, or bitches neutered at older ages. However, in the UK the neutering of bitches younger than two years of age is commonly recommended by veterinarians and practiced by owners (Diesel et al., 2010; Da Costa et al., 2022), therefore the present findings are applicable and are not influenced by other factors that could be associated with later neutering, such as previous pregnancies.

The study was conducted using bitches from one assistance dog organisation. It is possible that other organisations will have different breeding programmes and dog supply procedures, potentially purchasing dogs rather than purpose-breeding their own as in the present study, which could impact health, likelihood of qualification or the behavioural response to neutering. Indeed, qualification rates are known to vary amongst international guide dog schools (from 23.3% to 100%) due to factors such as the use of temperament and puppy testing, the breeds used and the

inclusion of staff feedback in decision making (Batt *et al.*, 2010). Therefore, there are variations between organisations that could impact the ability extrapolate results directly, a limitation also noted by Bray *et al.* (2019) relating to their work on outcome prediction. Similar considerations must be given to the applicability of these findings to other types of working dog programmes where dogs may be bred for very different temperamental qualities, such as police and military dogs. However, the use of dogs from one programme limited the variation in variables such as methods of rearing, training, husbandry that may influence results in other studies that use dogs from multiple sources.

In the most part data were not collected directly by the researcher but relied on others – vets, volunteer puppy raisers and staff. This may lead to a risk of bias compared to direct observations. However, the standardised practices used within the assistance dog organisation used in this study may lessen this risk. It is however possible that this limitation affected some data more than other data. Behavioural questionnaires, for instance, that required volunteers or guide dog owners to report behaviour observed in the previous months may be at more risk of recall and response bias compared to veterinary data for disease diagnosis. However, the methods were necessary due to the geographical spread of dogs throughout the UK preventing the possibility of using standardised behavioural tests for direct observations of behaviour, and the validity and reliability of C-BARQ has been thoroughly assessed (Hsu and Serpell, 2003; Duffy and Serpell, 2008). Despite this risk the study design offered potential improvements over other work that has used data from veterinary or behaviour databases that may be impacted by varied dog backgrounds and management practices, and inconsistent reporting by owners.

The geographical spread of dogs throughout the UK, and the need to recruit the number of dogs required for the study in a three-year period meant that bitches were examined and neutered by four

veterinary practices. Limiting this to one veterinary practice would have been preferred, and would be advised in future work based on the findings related to vet practice in this study. Especially reported differences between practices in bitch measurements related to growth and physical development, and differences reported in the peri and post-operative period (Moxon *et al.*, 2023a).

The design of the study was to allocate bitches to study groups in alternating order at approximately eight weeks of age. This was done by the principal researcher with no prior knowledge of the puppies. However, this methodology could have been improved by randomising the first puppy's trial group allocation. During the study, 18 bitches were re-allocated from the PPN to the Control group following six-month physical assessments and data for these bitches were analysed as such. This could have introduced a risk of bias when interpreting the results. An alternative approach, using the intention to treat principle, whereby data for bitches would be analysed in the group that they were allocated irrespective of when they were neutered, could have been adopted. This method would have retained randomisation of allocation to trial groups and may have resulted in smaller differences between groups.

The study only considered one method of surgical neutering, ovariohysterectomy. Other methods of surgical neutering such as hysterectomy, where the ovaries remain, and ovariectomy, where the uterus remains, are available. Ovariectomy is becoming increasingly common in the UK, however the present study adds no information about the effect of alternative methods of reproduction control for female dogs.

Behaviour in dogs was measured at one and three years of age, however no later measures of behaviour were used to examine dog behaviour further into the working guide dog partnerships. Additionally, no potential measures of cognitive decline were used other than examining dogs' health records for mention of cognitive

health. For future long-term prospective studies, considering the use of validated measures for cognitive health throughout adulthood and into older age could provide useful information on the effects of neutering timing on this outcome.

Due to the fixed sample size, power calculations based on Control group data and the size of effect that would be considered important were used to determine values of alpha for each analysis. This is an improvement on using a standard value of alpha = 5% (Mudge et al., 2012; Aidemouni, 2020). However, a priori power analysis would have allowed the number of bitches required to be calculated for a specified effect size and should be considered when designing future studies (Jones et al., 2003).

Finally, the small number of bitches recruited in comparison to retrospective studies that have used data from databases such as C-BARQ (that contains behaviour data for tens of thousands of dogs), may have impacted the study. Despite the number of dogs recruited to this study from the largest assistance dog breeding colony, the number of withdrawals for health reasons, for distinct behavioural reasons and the number diagnosed with some diseases was small, and it was either not possible to analyse these data statistically, or was not possible to include these in more complex models to examine the effect of trial group while accounting for the effects of covariates. While this limited the data exploration that was possible in this study, it also raised important considerations for other authors. There is a need for controlled prospective studies on well-managed populations with known histories and similar environmental experience. However, there are benefits to the present study design whereby a prospective study was conducted in a carefully controlled population which minimised some of the risks that can be associated with retrospective studies and those conducted on larger numbers of animals but with varied or unknown backgrounds.

This study provides useful information to inform sample size decisions that may be required to conduct further work of a similar nature in the future. For instance, for behavioural analyses, based on the effect sizes reported, power analysis suggests that around 2,000 dogs would be needed for each trial group in some cases, and for some behavioural traits, up to 20,000. For analysis of some disease data, over 2,000 animals would have been required. Studying much larger populations of dogs would provide more confidence in the results and allow for analysis of diseases of interest, such as ED and HD, that were not possible in the present study. However, such a large study may not be feasible in a controlled population; the present study utilised dogs from one of the world's largest assistance dog training programmes, and recruitment of large numbers of dogs comparable to those in retrospective studies of veterinary or behavioural databases would not have been possible. Using dog data from existing data sources, such as from C-BARQ (as in Flint *et al.*, 2017) or from large veterinary databases would allow recruitment of the sample sizes required. However, issues such as retrospective examination of data, incomplete data, recall bias, limited information about potential confounding and environmental factors, and inconsistent dog rearing and husbandry, would then impact the quality of the results. Despite this, the results reported in this thesis provide a first indication of what population size would be required in similar studies. Within the present study, the well-managed and monitored population with controlled data recording reduced the likelihood of missed diagnoses.

9.2 FUTURE WORK

For the present study, further work will be undertaken to examine any differences in working life, age at death and causes of death once complete life data are available for all bitches. Outside of the present study, more work is required into the impacts of neutering to inform the growing number of discussions around the ethics and welfare implications of routine neutering of dogs. However, more

focus should be placed on the requirement for increased understanding regarding the impact of neutering, and neutering at different times, on reproductive hormones in bitches and the role of these on development of disease, rather than just reporting the effects of neutering and timing of neutering on outcome variables. It is generally accepted that there are effects of neutering, and neutering at different ages on health and behavioural outcomes. However, the underpinning mechanisms of hormonal influence on the tissues are not well understood and expanding knowledge in this area may assist with clarifying some of the inconsistencies in findings, for instance between studies when dogs are neutered in different age categories and between diseases, when earlier neutering may either favourably or negatively influence the incidence of certain cancers. Additionally, with increased knowledge may come improvements in methods for reducing disease incidence.

More consideration of, and research into, alternative methods to prevent reproduction in dogs are required. Surgical neutering via hysterectomy, therefore removing the potential for unplanned pregnancies and uterine disease, while leaving the ovaries in place and not disrupting the hypothalamus-anterior pituitary-gonad axis may be a useful alternative to surgeries that remove the ovaries. However, there is limited literature available considering the long-term outcomes of hysterectomy for bitches, and there are considerations for owners; behaviours influenced by gonadal hormones would persist and there would be no prevention of ovarian disease or other diseases associated with ovarian hormones, such as mammary disease and pseudopregnancy.

The use of depot GnRH agonists to delay puberty does not have the same effect on gonadotrophins as neutering, maintaining low circulating concentrations of LH and FSH. In a recent review of the current knowledge around the use of long-term GnRH agonists in dogs, Schafer-Somi *et al.* (2022) state that in studies involving the use of deslorelin to delay puberty the evidence suggests that body

development does not appear to be affected, although epiphyseal closure is delayed, the longer term consequences of which are not yet understood. These could be positive findings in terms of the potential to reduce future disease risk while still enabling management of bitch reproduction, although the consequences for future health related to delayed epiphyseal closure may require further study. Alongside greater understanding of the actions of LH and FSH in disease development, further long-term investigations are required to understand the risks and benefits of the long-term use of GnRH analogues as a medical strategy to potentially replace or delay neutering. Studies should be conducted that control for or limit confounding variables and that confirm pubertal status (Schafer-Somi *et al.*, 2022). It is possible that after some years of living with repeated deslorelin implants commencing prior to puberty (to prevent the induction of oestrus observed in post-pubertal anoestrus bitches post-implantation), bitches could be neutered later, at five, six or seven years of age and therefore have fewer years with high and unregulated LH levels. Alternatively, GnRH analogues implanted following ovariohysterectomy or ovariectomy could be used to maintain low gonadotrophin concentrations after surgery. These methods could reduce future risk of developing diseases that may be associated with LH, but prospective cohort studies with large numbers of bitches, or potentially studies involving rodent models, are required to further investigate this (Schafer-Somi *et al.*, 2022; Kutzler, 2023). In addition to any effects on future disease risk, impacts on behaviour also need to be examined due to the possibility that aggression may be increased in dogs with other disease (Pipan and Pavlin, 2023). In comparison to neutering surgery, the use of an implant that would need to be re-implanted every six months at a cost of between £120 and £250 a time (Bilton Vets, 2022) may be prohibitive for many dog owners.

Attention should be paid in future work to the terminology used and method of categorising dogs based on age at neuter. These factors,

along with potential incorrect interpretation of results from previous studies presented in review articles, complicate understanding when reviewing the available literature. As highlighted in previous chapters, studies have made claims about pubertal status of bitches at neutering that are based on age at neutering and not puberty, leading to potential misinterpretation of the results. Studies group age at neutering in different ways that do not allow for easy comparison of results from multiple studies.

9.3 CONCLUSION

This study met its aims by identifying and describing the available literature relating to the effect of neutering bitches before or after puberty on behaviour and certain diseases, and by reporting the results of a prospective study examining the impacts of neutering bitches before or after puberty on physical development, behaviour, progression through an assistance dog programme and long-term health.

The scoping reviews identified a lack of evidence to support understanding of the impact of neutering bitches before or after puberty on behaviour and on five aspects of health (atopy, DOD, neoplasia, obesity and urogenital disease) and a need for further studies.

The prospective study showed that while there was no impact on behaviour or guide dog qualification, neutering prepubertally was associated with growth in terms of height and vulval development, and was negatively associated with some musculoskeletal and immune diseases. This study provides the first information from a prospective study on behavioural outcomes for bitches neutered before or after puberty as well as the first data examining the effects on progression through an assistance dog programme, and on the development of musculoskeletal disease and atopic dermatitis.

Areas for future work have been highlighted and these can be used by others to build the evidence base and improve clarity and understanding. Findings from the study will be of interest to veterinarians, those working in the fields of dog behaviour, other assistance and working dog organisations as well as the dog owning public. The decision to neuter a female dog, and when to neuter is complicated and it is suggested that, in agreement with other authors, there is not one standard recommendation that will suit all dogs and owners. In terms of neutering bitches in relation to pubertal status, waiting until after puberty is advised for bitches of the breeds studied, and potentially until after 12 months of age based on the work of others.

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Appendices

Appendix 1 – Scoping review extraction form (behaviour)

Title	
Authors	
Year of publication	
Source/country	
Aims	
Population and sample size Breeds Source – shelter / pet	
Study design Random allocation or retrospective ask when neutered Know about behaviour before neuter?	
Excluded dogs neutered due to a behavioural problem	
Methods	
Intervention details	
How define prepubertal	
Did they actually note when bitch had first season	
Did they note whether bitches had ever been bred?	
Concept	
Outcomes	
How outcomes measured	
Key findings that relate to review question	

Appendix 2 – Scoping review extraction form (health)

Title	
Authors	
Year of publication	
Source/country	
Aims	
Population and sample size Breeds Source – shelter / pet	
Study design Random allocation or retrospective ask when neutered Consider health before neuter?	
Excluded dogs with a related pre-existing health problem	
Methods	
Intervention details	
How define prepubertal	
Did they actually note when bitch had first season	
Did they note whether bitches had ever been bred?	
Concept	
Outcomes	
How outcomes measured	
Key findings that relate to review question	

Appendix 3 – Six-month physical assessment form

To be completed for all bitches allocated to the prepubertal neutering study (trial and control dogs).

To be completed at **6 months** of age, within the individual timeframe specified by Canine Research. For bitches in the PPN group, this form should be completed prior to surgery.

To be completed by the Centre Vet.

Please return completed forms and digital photographs to canine.research@guidedogs.org.uk

Dog Name:	
Computer Number:	
Trial Group:	
Assessment window:	
Date completed:	

General Physical Assessment

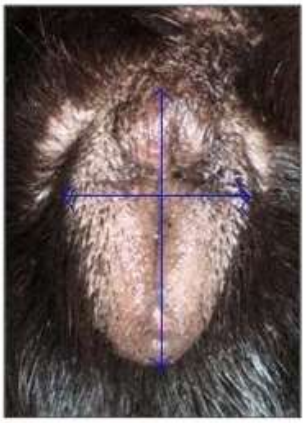
Height at the withers: To be measured using the measuring stick provided by Canine Research	cm
Weight:	Kg
BCS:	1 ☐, 2 ☐, 3 ☐, 4 ☐, 5 ☐, 6 ☐, 7 ☐, 8 ☐, 9 ☐

For 6-month-old puppies please refer to Guide Dogs' Puppy Body Condition Scoring System.

Physical Appearance of the Vulva

Please take a digital photograph of the vulva – to be emailed with this completed questionnaire to the email address above.

The photograph should be taken in portrait orientation at 90º to the vulva. The vulva should occupy the majority of the photograph – please see the photograph below as an example.



This photograph illustrates the measurement points for the required measurements below:

Overall length of the vulva:		cm
Overall width of the vulva:		cm

Does the vulva appear swollen?	Yes ☐, No ☐
Is any vaginal discharge present?	Yes ☐, No ☐
Does the vulva appear juvenile in appearance? (More so than expected for age)	Yes ☐, No ☐
Does the vulva appear recessed/inverted?	Yes ☐, No ☐
Are there prominent perivulval skin folds present?	Yes ☐, No ☐
Is there any perivulval dermatitis present?	Yes ☐, No ☐

Clinical History

To be completed from the dog's clinical history and evidence present at the time of the 6-month physical assessment.

Does the puppy have existing pre-pubertal vaginitis or history of any vulval discharge?	Yes ☐, No ☐
Has the puppy already shown signs of oestrus?	Yes ☐, No ☐
Is there any evidence of mammary enlargement at the time of the physical assessment?	Yes ☐, No ☐
Are there any clinical signs consistent with endocrine disease (including for example symmetrical skin disease, polydipsia, polyuria)?	Yes ☐, No ☐

Appendix 4 – 17-month physical assessment form

To be completed for all bitches allocated to the prepubertal neutering study (trial and control dogs).

To be completed at **17 months** of age.

To be completed by the Centre Vet or Dog Health and Wellbeing Specialist.

Please return completed forms and digital photographs to canine.research@guidedogs.org.uk

Dog Name:	
Computer Number:	
Trial Group:	
Assessment window:	
Date completed:	

General Physical Assessment

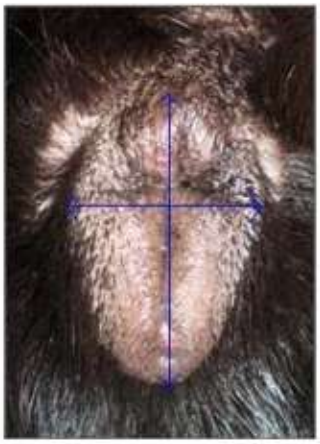
Height at the withers: To be measured using the measuring stick provided by Canine Research	cm
Weight:	Kg
BCS:	1 ☐, 2 ☐, 3 ☐, 4 ☐, 5 ☐, 6 ☐, 7 ☐, 8 ☐, 9 ☐

Please refer to Guide Dogs' Adult Dog Body Condition Scoring System.

Physical Appearance of the Vulva

Please take a digital photograph of the vulva – to be emailed with this completed questionnaire to the email address above.

The photograph should be taken in portrait orientation at 90º to the vulva. The vulva should occupy the majority of the photograph – please see the photograph below as an example.



This photograph illustrates the measurement points for the required measurements below:

Overall length of the vulva:		cm
Overall width of the vulva:		cm

Does the vulva appear swollen?	Yes ☐, No ☐
Is any vaginal discharge present?	Yes ☐, No ☐
Does the vulva appear juvenile in appearance?	Yes ☐, No ☐
Does the vulva appear recessed/inverted?	Yes ☐, No ☐
Are there prominent perivulval skin folds present?	Yes ☐, No ☐
Is there any perivulval dermatitis present?	Yes ☐, No ☐

For dogs that have been withdrawn only (to be used in chapter 9 related to future health):

Has the dog been diagnosed with any health problems since being rehomed?	Yes ☐, No ☐
If 'Yes', please detail	
If 'Yes', does the owner give consent for Canine Research to contact their veterinary practice to obtain further details if required?	Yes ☐, No ☐
If 'Yes', please provide contact details for the veterinary practice	

Appendix 5 – Published C-BARQ studies

A selection of published studies that have used the C-BARQ tool to examine behavioural traits in dogs and relationships with different factors are shown in chronological order. The factors examined, score calculations and methods of analysis used, covariates controlled for in statistical models and reported or calculated effect size (where possible) are reported.

Source	Differences investigated	Behavioural trait scores calculated for analysis	Actual trait scores reported?	Reported what difference would be considered significant?	Statistical model used to test for difference	Covariates included in model?
Hsu and Serpell (2003)	Dogs with a diagnosis compared to other dogs with behaviour problems	Mean	No	Yes, but only in graphs	Mann-Whitney U tests	No. Examined prior using t-tests and x^2.
Serpell and Hsu (2005)	Distribution of 'Trainability' trait scores between dogs of different breed, sex and neuter status	Not clear from methods, mean and median reported in figures for breeds	Yes	No	Mann-Whitney U and Kruskal-Wallis tests	No
Duffy *et al*. (2008)	Breed differences in aggressive behaviour	Mean	Yes	No	Mann-Whitney U and Kruskal-Wallis tests	No
Bosch *et al*. (2009)	Between dogs on different dietary supplements	Mean	Yes (least square means)	No	Linear mixed models using Residual Maximum Likelihood	Yes Week and Dog ID Dog related factors (breed, age etc) not included
Hsu and Sun (2010)	Factors affecting aggressiveness by dog characteristics, owner characterist	Not clear from methods, state 'average'	No	No	Multiple logistic regression	Yes following initial individual analysis to examine effects

	ics, environment and owner-dog interaction					
McMillan *et al*. (2011)	Between dogs removed from commercial breeding establishments and 'typical' pet dogs	Not clear from methods	No	No	Generalized linear models	Yes the presence of other dogs in the home, the length of time in the home and neuter status
Duffy and Serpell (2012)	Differences in behaviour scores between dogs that qualify as guide/service dogs and those withdrawn for behaviour	Mean values converted to z-scores	No	No	Logistic generalized linear models	Yes Dog breed and sex nested within 'organization'
McMillan *et al*. (2013)	Between dogs obtained as puppies from pet stores and non-commercial breeders.	Not clear in methods	No	No	Hierarchic linear and logistic regression models	Yes Other dogs in household, working or recreational roles, breed, sex, bodyweight, age and neuter status
Arata *et al*. (2014)	Association between 'reactivity to stimuli' and aggression	Mean	No	No	ANOVA	No
Duffy *et al*. (2014)	Evaluate shorter C-BARQ as a behavioural screening tool for animal shelters	Average	No	No	Generalized linear models	Yes Breed, sex, age and shelter

Asp *et al.* (2015)	Breed effect on behaviour	Average	Yes	No	Ordinal least squares general linear model	Yes Breed, sex and age
Nagasawa *et al.* (2015)	Behavioural characteristics of dogs in Japan and the USA	Average	No	No	Multiple linear regression analysis and Mann-Whitney U-tests	Yes Sex, neuter status, age and body weight at evaluation, age when acquired, owner's experience of dog-ownership and the source in acquisition of dogs
Tonoike *et al.* (2015)	Behavioural characteristics of dogs in Japan and the USA	Average	Yes, on graphs	No	Generalized linear models	Yes Country, sex, neuter status, age and body weight at evaluation, age when acquired, owner's experience of dog-ownership and the source in acquisition of dogs
Grigg *et al.* (2016)	Behavioural scores between mixed breed dogs in North America and St Kitts 'island dogs'	Mean	Yes	No	Mann–Whitney U tests	No, but dogs matched by source (stray or street) age, sex, neutered status with that of the island dogs
McMillan *et al.* (2016)	Rehomed dogs formerly living in a hoarding	Mean and dichotomized for skewed	No	No	Generalized linear mixed regression models	Yes Breed, sex, weight, and age at

	situation and pet dogs					evaluation, the amount of time owned, and the presence of other dogs and/or children in the household
Flint *et al.* (2017)	Risk factors associated with dogs displaying aggression: age evaluated, age acquired, sex, breed, neuter age and source	Not clear in methods	No	No	Mixed logistic regression models	Yes Dog ID, country
Dodman *et al.* (2018)	Association between owner personality and psychological status, methods of training, and behaviour problems	Not clear in methods	Yes	No	Hierarchical linear or logistic regression analyses	Yes Age, sex, neuter status, owner gender, prior dog ownership, and number of dogs in the household
Farhoody *et al.* (2018)	Between dogs neutered at different ages and entire dogs	Categorised based on aggression scores	No	No	Multivariable logistic regression models	Yes Age at acquisition, at neutering and at evaluation, sex, weight, neuter reason, weeks since neuter, dog source, health problems, activity/role, first dog-owned status,

						total number of dogs owned, dogs owned as a child and other dogs in a household
Hare *et al*. (2018)	Between search and rescue dogs and pet dogs	Mean	Yes	No	Non-parametric models and partial regressions	Breed, sex, neuter status, and age
McGreevy *et al*. (2018)	Between entire and neutered male dogs	Categorised based on question scores	No	5% difference in proportion of dogs between groups	Ordinal logistic regression model	Yes Age at castration, age at evaluation, breed, weight, whether it is the owner's first dog, other dogs in the household, dog role,
Bowen *et al*. (2019)	Between dogs with different Distraction Index from PennHip examinations	Not clear from methods, presume average	Yes	No	Binary logistic regression	sex, breed
Hare *et al*. (2021)	Between dogs that qualified and were withdrawn from a prison puppy raising program	Mean	Yes	No	Multinomial log-linear models	age group, medical category, sex, neuter status within sex

Appendix 6 – Adapted C-BARQ questions

The questions included in the adapted version of the C-BARQ questionnaire including behavioural factor groupings and the answer scales. * Denotes questions for which the scores were reversed prior to analysis.

Behavioural factor	Question	Scale
Training and obedience	When off the leash, returns immediately when called*	**Never, Seldom, Sometimes, Usually, Always**
	Obeys the "sit" command immediately*	
	Obeys the "stay" command immediately*	
	Seems to attend/listen closely to everything you say or do*	
	Slow to learn new tasks	
	Easily distracted by interesting sights, sounds or smells	
Aggression	When approached directly by an unfamiliar person while being walked/exercised on a lead	**5-point scale (0= No aggression, 4= Serious aggression)**
	When toys, bones or other objects are taken away by a household member	
	During grooming or being given treatments by a household member	
	When approached directly by a household member while s/he (the dog) is eating or has a toy/favourite object	
	When approached directly by an unfamiliar dog while being walked/exercised on a lead	
	Toward unfamiliar persons visiting your home	
	When approached while eating by another (familiar) household dog (leave blank if no other dogs)	
	When approached while playing with/chewing a favourite toy, bone, object, etc., by another (familiar) household dog (leave blank if no other dogs)	
Fear and anxiety	When approached directly by an unfamiliar person while away from your home	**5-point scale (0=No fear, 4=Extreme fear)**

	In response to sudden or loud noises (e.g. vacuum cleaner, car backfire, road drills, objects being dropped, etc.)	
	In heavy traffic	
	When examined/treated by a veterinarian	
	During thunderstorms, firework displays, or similar events	
	When approached directly by an unfamiliar dog of the same or larger size	
	When approached directly by an unfamiliar dog of a smaller size	
	In response to wind or wind-blown objects	
	When groomed or bathed by a household member	
Excitability	When you or other members of the household come home after a brief absence	**5-point scale (0 = calm, 4 = extremely excitable)**
	When playing with you or other members of your household	
	Just before being taken for a walk	
	Just before being taken on a car trip	
	When visitors arrive at your home	
Attachment and attention-seeking	Displays a strong attachment for one particular member of the household	**Never, Seldom, Sometimes, Usually, Always**
	Tends to follow you (or other members of household) about the house, from room to room	
	Tends to sit close to, or in contact with, you (or others) when you are sitting down	
	Tends to nudge, nuzzle or paw you (or others) for attention when you are sitting down	
	Becomes agitated (whines, jumps up, tries to intervene) when you (or others) show affection for another person	
	Becomes agitated (whines, jumps up, tries to intervene) when you (or others) show affection for another dog or animal	
Social behaviour	Chases or would chase birds or small animals, such as cats or rabbits, given the opportunity	**Never, Seldom, Sometimes, Usually, Always**

	Rolls in animal droppings or other 'smelly' substances	
	Eats own or other dog's faeces	
	Chews inappropriate objects	
	'Mounts' objects, furniture, or people	
	Begs persistently for food when people are eating	
	Steals food	
	Playful, puppyish, boisterous	
Additional question	Does your dog ever raise their leg during spending [when urinating]	**Never, Seldom, Sometimes, Usually, Always**

Appendix 7 – Equations for Chapter 7

Model 1.

$$\text{TotalDaysAtPW}_{ijk} = \beta_0 + \beta_1\text{Breed\_2}_{ijk} + \beta_2\text{Breed\_3}_{ijk} + \beta_3\text{Number of PTS\_2}_{ijk} + \beta_4\text{Number of PTS\_3}_{ijk} + \beta_5\text{Number of PTS\_4}_{ijk} + \beta_6\text{NoOfPWs\_2}_{ijk} + \beta_7\text{NoOfPWs\_3}_{ijk} + \beta_8\text{MainPTSCAS\_3}_{ijk} + \beta_9\text{MainPTSCAS\_4}_{ijk} + \beta_{10}\text{MainPTSCAS\_6}_{ijk} + \beta_{11}\text{MainPTSCAS\_7}_{jk} + \beta_{12}\text{MainPTSCAS\_9}_{ijk} + \beta_{13}\text{MainPTSCAS\_12}_{ijk} + \beta_{14}\text{MainPTSCAS\_13}_{ijk} + \beta_{15}\text{MainPTSCAS\_14}_{ijk} + \beta_{16}\text{MainPTSCAS\_15}_{ijk} + \beta_{17}\text{MainPTSCAS\_16}_{ijk} + \beta_{18}\text{MainPTSCAS\_17}_{ijk} + \beta_{19}\text{MainPTSCAS\_21}_{ijk} + \beta_{20}\text{MainPTSCAS\_22}_{ijk} + \beta_{21}\text{MainPTSCAS\_24}_{ijk} + \beta_{22}\text{MainPTSCAS\_26}_{ijk} + \beta_{23}\text{MainPTSCAS\_27}_{ijk} + \beta_{24}\text{MainPTSCAS\_30}_{ijk} + \beta_{25}\text{MainPTSCAS\_32}_{ijk} + \beta_{26}\text{MainPTSCAS\_33}_{ijk} + \beta_{27}\text{MainPTSCAS\_34}_{ijk} + \beta_{28}\text{MainPTSCAS\_35}_{ijk} + \beta_{29}\text{MainPTSCAS\_36}_{ijk} + \beta_{30}\text{OriginalOutcome\_2}_{ijk} + \beta_{31}\text{TrialGroup\_2}_{ijk} + e_{ijk}$$

$e_{ijk} \sim N(0, \sigma_e^2)$

*-2*loglikelihood* = 3211.440(301 of 301 cases in use)

Model 2.

$$\text{TotalDaysInET}_{ijk} = \beta_0 + \beta_1\text{Breed\_2}_{ijk} + \beta_2\text{Breed\_3}_{ijk} + \beta_3\text{BusinessModelTraining\_2}_{ijk} + \beta_4\text{BusinessModelTraining\_3}_{ijk} + \beta_5\text{NoTimesInET\_2}_{ijk} + \beta_6(\text{Age into ET-gm})_{ijk} + \beta_7\text{OriginalOutcome\_2}_{ijk} + \beta_8\text{TrialGroup\_2}_{ijk} + e_{ijk}$$

$e_{ijk} \sim N(0, \sigma_e^2)$

*-2*loglikelihood* = 2589.828(264 of 264 cases in use)

Model 3.

$$\text{TotalDaysInATOC}_{ijk} = \beta_0 + \beta_1\text{Breed\_2}_{ijk} + \beta_2\text{Breed\_3}_{ijk} + \beta_3\text{BusinessModelTraining\_2}_{ijk} + \beta_4\text{BusinessModelTraining\_3}_{ijk} + \beta_5\text{NoTimesInAT\_2}_{ijk} + \beta_6\text{NoTimesInOC\_0}_{ijk} + \beta_7\text{NoTimesInOC\_2}_{ijk} + \beta_8\text{RegionEnteredET\_2}_{ijk} + \beta_9\text{RegionEnteredET\_3}_{ijk} + \beta_{10}\text{RegionEnteredET\_4}_{ijk} + \beta_{11}(\text{TotalDaysInET-gm})_{ijk} + \beta_{12}(\text{Age into AT-gm})_{ijk} + \beta_{13}\text{OriginalOutcome\_2}_{ijk} + \beta_{14}\text{TrialGroup\_2}_{ijk} + e_{ijk}$$

$e_{ijk} \sim N(0, \sigma_e^2)$

*-2*loglikelihood* = 2016.157(208 of 208 cases in use)

Model 4.

$$\text{Age into ET}_{ijk} = \beta_0 + \beta_1\text{Breed\_2}_{ijk} + \beta_2\text{Breed\_3}_{ijk} + \beta_3\text{RegionEnteredET\_2}_{ijk} + \beta_4\text{RegionEnteredET\_3}_{ijk} + \beta_5\text{RegionEnteredET\_4}_{ijk} + \beta_6\text{LocationET\_2}_{ijk} + \beta_7\text{LocationET\_3}_{ijk} + \beta_8\text{LocationET\_4}_{ijk} + \beta_9\text{LocationET\_6}_{ijk} + \beta_{10}\text{LocationET\_8}_{ijk} + \beta_{11}\text{LocationET\_14}_{ijk} + \beta_{12}\text{LocationET\_15}_{ijk} + \beta_{13}\text{LocationET\_16}_{ijk} + \beta_{14}\text{LocationET\_17}_{ijk} + \beta_{15}\text{LocationET\_30}_{ijk} + \beta_{16}\text{BusinessModelTraining\_2}_{ijk} + \beta_{17}\text{BusinessModelTraining\_3}_{ijk} + \beta_{18}\text{Number of PTS\_2}_{ijk} + \beta_{19}\text{Number of PTS\_3}_{ijk} + \beta_{20}\text{NoOfPWs\_2}_{ijk} + \beta_{21}\text{NoOfPWs\_3}_{ijk} + \beta_{22}\text{TrialGroup\_2}_{ijk} + e_{ijk}$$

$e_{ijk} \sim N(0, \sigma_e^2)$

*-2*loglikelihood* = 2712.387(264 of 264 cases in use)

Equations for the best fit logistic regression for outcome from the Guide Dogs' training programme for all 301 bitches (Model 5) and for the 264 bitches that entered early training (Model 6).

Model 5.

$$Pred(OriginalOutcome) = 1/(1 + exp(-(0.697 - 0.334 \times B2 - 0.255 \times B3 + 6.863^{-0.2} \times PTS2 + 3.331^{-0.3} \times PTS3 - 0.228 \times PW2 - 0.249 \times PW3 + 0.079 \times TG2)))$$

Where Breed = B, Number of puppy development advisors = PTS, Number of puppy raisers = PW, Trial group = TG

Model 6:

$$Pred(OriginalOutcome) = 1/(1 + \exp(-(0.828 - 0.275 \times B2 + 1.744^{-0.2} \times B3 + 0.332 \times TM2 + 2.148 \times TM3 + 0.127 \times RET2 + 0.378 \times RET3 + 0.116 \times RET4 - 0.100 \times PTS2 + 0.492 \times PTS3 - 0.145 \times PW2 - 0.977 \times\times PW3 - 0.103 \times TG2)))$$

Where Breed = B, Training model = TM, Region entered early training = RET, Number of puppy development advisors = PTS, Number of puppy raisers = PW, Trial group = TG

Appendix 8 – Health withdrawal reasons

The number of bitches neutered before (PPN) or after (Control) puberty that were withdrawn from the training programme for each detailed heath reason.

Health withdrawal reason and body system affected	Number of PPN bitches withdrawn	Number of Control bitches withdrawn
Eyes		
Epiphora	0	1
Geographic retinal dysplasia	0	1
Posterior polar capsular cataract	0	1
Musculoskeletal		
Bilateral muscle myopathy resulting in forelimb lameness	0	1
Disorder of musculoskeletal system	1	0
Elbow dysplasia	2	1
Hindlimb lameness	0	1
Cruciate ligament rupture	0	1
Hip dysplasia	1	0
Skin		
Atopic dermatitis	3	1
Gastrointestinal		
Chronic diarrhoea	0	1
Total withdrawn for health reasons	7	9

Appendix 9 – The diseases that were chosen for inclusion in the study and the reason for inclusion.

Health condition	Health reasons for withdrawal	Guide Dogs Chief veterinary Officer / Breeding Dog Health Specialist	Survey of veterinarians	Scientific literature	Veterinary student projects
Musculoskeletal (joint/orthopaedic)					
Cruciate disease	1			1	
Elbow dysplasia	1				
Forelimb lameness		1	1		
Hip dysplasia				1	1
Juvenile osteochondrosis					
Osteochondritis dissecans		1			
Osteoarthritis				1	
Patella luxation				1	
Malignant neoplasia					
Adenoma / adenocarcinoma			1	1	
Fibroma/fibrosarcoma			1		1
Haemangiosarcoma				1	
Lymphosarcoma/lymphoma				1	
Mammary neoplasia				1	
Mast cell tumour				1	
Melanocytic tumour				1	
Osteosarcoma				1	
Squamous cell carcinoma				1	
Transitional cell carcinoma				1	
Urogenital					
Urinary / reproductive tract tumour				1	
Perivulvar dermatitis			1	1	1

Pseudopregnancy			1	1	1
Pyometra			1	1	1
Recessed/inverted/juvenile vulva					1
Struvite urolithiasis / urinary calculi				1	
Urinary incontinence			1		1
Urinary tract disorders					1
Urinary sphincter mechanism incompetence			1		1
Urinary tract infection / cystitis				1	1
Vaginal / vulval disorder				1	1
Vaginitis			1	1	
Vulval discharge, abnormal discharge			1		1
Immune					
Atopy	1	1	1		
Autoimmune haemolytic anaemia				1	
Hypoadrenocorticism				1	
Hypothyroidism				1	
Immune-mediated arthritis					
Immune-mediated thrombocytopenia				1	
Inflammatory bowel disease (IBD)				1	
Otitis externa		1			
Systemic lupus erythematosus					1
Other					
Aortic stenosis				1	
Bodyweight (obesity)		1	1		
Diabetes mellitus				1	
Early onset cataracts (eyes)				1	
Epilepsy (idiopathic) (CNS)				1	
Gastric volvulus (GI)				1	
Geriatric cognitive impairment / cognitive dysfunction syndrome				1	
Histiocytoma				1	

Appendix 10 – The health code searches run for each health condition.

MUSCULOSKELETAL

Juvenile osteochondrosis
Juvenile osteochondrosis

Cruciate disease
Cruciate Ligament Disease
Rupture of cruciate ligaments

Elbow dysplasia
Canine elbow dysplasia
Short radius syndrome
Medial compartment disease
Short ulna syndrome
Fragmented coronoid process
Medial coronoid process disease
Ununited anconeal process
Canine trochlear notch incongruity
OCD-osteochondritis dissecans of the elbow

Forelimb lameness
Forelimb lameness
Forelimb stiffness

Hip dysplasia
Hip dysplasia
Unilateral dysplastic hip
Bilateral dysplastic hip
Subluxation of hip joint

Osteoarthritis
Arthritis (check for osteoarthritis)
Chronic osteoarthritis
Osteoarthritis
Osteoarthritis of elbow
Osteoarthritis of hip
Polyarthritis (check for osteoarthritis)

Osteochondritis (OCD)
OCD-Osteochondritis dissecans
OCD-osteochondritis dissecans of hock
OCD-osteochondritis dissecans of stifle
OCD-osteochondritis dissecans of the shoulder
OCD-osteochondritis dissecans of the elbow (cases moved to elbow dysplasia)

Patella luxation
Dislocated patella

Subluxation of the patella

MALIGNANT NEOPLASIA

Adenocarcinoma
Adenocarcinoma
Adenocarcinoma of Anal Glands
Adenocarcinoma of bladder
Adenocarcinoma of lung
Adenocarcinoma of stomach
Ceruminous gland adenocarcinoma

Fibrosarcoma
Fibrosarcoma
Fibrosarcoma of spleen

Haemangiosarcoma
Angiosarcoma
Angiosarcoma of spleen
Haemangiosarcoma

Lymphosarcoma/lymphoma
Hodgkin's-like lymphoma of animals
Lymphosarcoma
Lymphoma
Lymphangiosarcoma
Pagetoid reticulosis

Mammary neoplasia
Malignant neoplasm of mammary gland
Neoplasm of mammary

MCT
Mastocytoma
Mast cell tumour

Melanocytic tumour
Limbal melanoma
Melanocytoma
Malignant melanoma
Malignant melanoma of ciliary body
Malignant melanoma of eye
Malignant melanoma of iris
Malignant melanoma of retina
Malignant melanoma of skin of ear

Osteosarcoma
Osteosarcoma
Osteochondrosarcoma

Squamous cell carcinoma
Squamous cell carcinoma

Transitional cell carcinoma
Transitional cell carcinoma of bladder

UROGENITAL
Urinary / reproductive tract tumours
Benign neoplasm of bladder
Benign neoplasm of vulva
Benign neoplasm of urethra
Benign neoplasm of uterus
Benign neoplasm of vagina
Malignant neoplasm of uterus
Malignant tumour of urethra
Malignant tumour of urinary bladder
Malignant tumour of vagina
Malignant tumour of vulva
Neoplasm of bladder
Neoplasm of vulva
Neoplasm of uterus
Neoplasm of urethra
Neoplasm of urinary tract
Neoplasm of urinary system
Neoplasm of vagina
Neoplasm of female genital organ
Sarcoma of bladder
Urinogenital tumour of uncertain behaviour

From above
Transitional cell carcinoma of bladder
Adenocarcinoma of bladder

Perivulvar dermatitis
Vulval dermatitis
Pyoderma (then had search for any around vulva)

Pseudopregnancy
False pregnancy

Pyometra
Pyometra

Recessed/inverted/juvenile vulva
Inverted vulva
Disorder of vulva (search for 'inverted' 'recessed' 'juvenile' only)

Struvite urolithiasis / urinary calculi
Urolithiasis
Urinary bladder stone
Kidney disease and search for 'stone' and 'lithiasis' 'calculus' 'calculi'
Calculus in urethra

UI
Incontinence

Nocturnal incontinence of urine
Post-Micturition Incontinence
Urinary Incontinence

Urinary tract disorders
Dilatation of ureter
Disorder of the genitourinary system
Disorder of the urinary system
Disorder of ureter
Disorder of urethra
Disorder of urinary tract
Pyonephrosis
Ureteritis
Urethral Incompetence
Urethritis

USMI
Urinary incontinence due to urethral sphincter incompetence

UTI / cystitis
Cystitis
Haemorrhagic cystitis
UTI-Urinary tract infection

Vaginal / vulval disorder
Disorder of vagina (discharge cases moved to specific search)
Disorder of vulva (recessed, juvenile, inverted and discharge cases moved to specific search)
Vulval vestibulitis
Vulvitis
Vulval hyperplasia
Vaginal hyperplasia
Vaginal polyp

Vaginitis
Prepubertal vaginitis
Vaginitis
Vulvovaginitis

Vulval discharge, abnormal discharge
Abnormal vaginal discharge

IMMUNE
Immune mediated arthritis
Polyarthritis
Rheumatoid Arthritis

Otitis externa
Acute otitis externa
Chronic otitis externa
Otitis Externa

Atopy
Acute Atopic Conjunctivitis
Allergic skin disease
Atopic [allergic] Otitis
Atopic conjunctivitis
Atopic dermatitis

Autoimmune haemolytic anaemia
Autoimmune haemolytic anaemia
Haemolytic Anaemia (check for autoimmune otherwise no)

Hypoadrenocorticism
Addisons disease
Hypoadrenalism
Hypoadrenocorticism

Hypothyroidism
Hypothyroidism

Immune-mediated thrombocytopenia
Auto-immune thrombocytopenia
Thrombocytopenia (checked notes and included only those noted as immune mediated)

Inflammatory bowel disease (IBD)
Acute ulcerative colitis
Chronic Colitis (only if IBD)
Colitis (only if IBD)
Haemorrhagic Colitis (only if IBD)
Acute and Chronic colitis
Ulcerative Colitis (only if IBD)
Inflammatory bowel disease

Systemic lupus erythematosus
Systemic lupus erythematosus

OTHER
Aortic stenosis
Aortic stenosis

Overweight / obesity
BCS health codes 3,4,5,6,7,8,9
Overweight
Morbid obesity
Obese

Diabetes mellitus
Diabetes Mellitus

Early onset cataracts
Anterior capsular cataract
Anterior cortical cataract

Anterior subcapsular cataract
Bilateral cataracts
Cataract
Cortical cataract
Equatorial cataracts
Mature cataract
Nuclear cataract
Perinuclear cataract
Posterior capsular cataract
Posterior cortical cataract
Posterior polar capsular cataract
Posterior polar subcapsular cataract
Pulverulent cataract

Epilepsy (idiopathic)
Epilepsy (check for idiopathic)
Epileptic seizure (check for idiopathic)
Idiopathic generalised epilepsy
Status epilepticus (check for idiopathic)

Gastric volvulus
Gastric Dilatation-Volvulus-Torsion Syndrome

Geriatric cognitive impairment / CDS
Cognitive dysfunction
Senile dementia
Senile-old age
Senility

Histiocytoma
Benign fibrous histiocytoma of skin
Cutaneous histiocytoma
Dermatofibroma
Histiocytoma

Appendix 11 – Table of diseases

Disease group	Disease	Group	Number bitches diagnosed (>6m of age)	% of bitches diagnosed	Number of cases	Mean +/- sem age diagnosis (years) (for all cases not just first)	Min and max age diagnosis (years) (for all cases not just first)
MSK	Cruciate rupture	All bitches	12	3.9	17	6.8 +/- 0.5	1.6 - 9.5
		PPN	11	7.1	16	7.1 +/- 0.4	5.0 - 9.5
		Control	1	0.7	1	1.6 +/- 0.0	-
	ED	All bitches	6	2.0	6	3.2 +/- 1.2	0.6 - 8.3
		PPN	3	1.9	3	1.6 +/- 0.7	0.6 - 3.0
		Control	3	2.0	3	4.8 +/- 2.1	1.1 - 8.3
	HD	All bitches	3	1.0	3	4.5 +/- 1.7	1.1 - 6.2
		PPN	3	1.9	3	4.5 +/- 1.7	1.1 - 6.2
		Control	0	0.0	0	-	-
	Juvenile osteochondrosis	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Osteoarthritis	All bitches	30	9.8	30	8.4 +/- 0.3	4.3 - 11.2
		PPN	19	12.3	19	8.0 +/- 0.4	4.3 - 11.1
		Control	11	7.3	11	9.2 +/- 0.4	6.5 - 11.2
	OCD	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Patella luxation	All bitches	1	0.3	1	7.7 +/- 0.0	-
		PPN	1	0.6	1	7.7 +/- 0.0	-
		Control	0	0.0	0	-	-
	Forelimb lameness	All bitches	54	17.6	90	4.8 +/- 0.3	0.7 - 10.0
		PPN	29	18.7	48	5.4 +/- 0.4	1.0 - 10.0
		Control	25	16.6	42	4.1 +/- 0.4	0.7 - 9.1
	MSk other	All bitches	4	1.3	4	7.4 +/- 0.8	6.0 - 9.5
		PPN	3	1.9	3	6.6 +/- 0.4	6.0 - 7.3
		Control	1	0.7	1	9.5 +/- 0.0	-
Neoplasia	Adenocarcinoma	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Fibrosarcoma	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Haemangiosarcoma	All bitches	3	1.0	3	8.5 +/- 0.2	
		PPN	1	0.6	1	8.6 +/- 0.0	-
		Control	2	1.3	2	8.4 +/- 0.4	8.0 - 8.8
	Lymphosarcoma/ lymphoma	All bitches	4	1.3	4	7.9 +/- 0.7	5.9 - 9.4
		PPN	2	1.3	2	7.6 +/- 1.8	5.9 - 9.4
		Control	2	1.3	0	8.1 +/- 0.1	8.1 - 8.2
	Mammary neoplasia	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	MCT	All bitches	11	3.6	16	8.5 +/- 0.3	6.6 - 10.2
		PPN	4	2.6	7	8.8 +/- 0.4	6.6 - 10.0
		Control	7	4.6	9	8.3 +/- 0.4	6.6 - 10.2
	Melanocytic tumour	All bitches	3	1.0	3	6.9 +/- 0.7	5.8 - 8.2
		PPN	1	0.6	1	8.2 +/- 0.0	-
		Control	2	1.3	2	6.3 +/- 0.4	5.8 - 6.7
	Osteosarcoma	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Squamous cell carcinoma	All bitches	1	0.3	1	9.6 +/- 0.0	-
		PPN	1	0.6	1	9.6 +/- 0.0	-
		Control	0	0.0	0	-	-
	Transitional cell carcinoma	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Neoplasia other	All bitches	16	5.2	17	8.0 +/- 0.6	3.1 - 11.1
		PPN	12	7.7	13	8.0 +/- 0.8	3.1 - 11.1
		Control	4	2.6	4	8.1 +/- 0.3	7.2 - 8.7

Urogenital	Perivulvar dermatitis	All bitches	13	4.2	13	3.2 +/- 0.8	0.6 - 8.1
		PPN	8	5.2	8	4.7 +/- 0.9	2.1 - 8.1
		Control	5	3.3	5	0.8 +/- 0.1	0.6 - 1.2
	Pseudopregnancy	All bitches	23	7.5	23	1.0 +/- 0.0	0.7 - 1.2
		PPN	0	0.0	0	-	-
		Control	23	15.2	23	1.0 +/- 0.0	0.7 - 1.2
	Pyometra	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Recessed/inverted/	All bitches	5	1.6	5	2.1 +/- 0.6	1.3 - 4.7
	juvenile vulva	PPN	4	2.6	4	2.3 +/- 0.8	1.4 - 4.7
		Control	1	0.7	1	1.3 +/- 0.0	-
	Struvite urolithiasis /	All bitches	0	0.0	0	-	-
	urinary calculi	PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	UI	All bitches	7	2.3	8	4.8 +/- 0.8	2.4 - 7.7
		PPN	1	0.6	1	7.7 +/- 0.0	-
		Control	6	4.0	7	4.4 +/- 0.8	2.4 - 7.2
	Urinary / reproductive	All bitches	0	0.0	0	-	-
	tract tumours	PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Urinary tract disorders	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	USMI	All bitches	1	0.3	1	2.71 +/- 0.0	-
		PPN	0	0.0	0	-	-
		Control	1	0.7	1	2.71 +/- 0.0	-
	UTI / cystitis	All bitches	21	6.9	27	3.1 +/- 0.6	0.5 - 11.1
		PPN	12	7.7	15	2.6 +/- 0.9	0.5 - 11.1
		Control	9	6.0	12	3.7 +/- 0.8	0.6 - 7.2
	Vaginal / vulval	All bitches	3	1.0	3	2.6 +/- 1.1	1.1 - 4.8
	disorder	PPN	1	0.6	1	4.8 +/- 0.0	-
		Control	2	1.3	2	1.5 +/- 0.4	1.1 - 1.8
	Vaginitis	All bitches	3	1.0	3	0.5 +/- 0.0	0.5
		PPN	0	0.0	0	-	-
		Control	3	2.0	3	0.5 +/- 0.0	0.5
	Vulval discharge,	All bitches	13	4.2	13	2.0 +/- 0.7	0.5 - 7.7
	abnormal discharge	PPN	5	3.2	5	2.6 +/- 1.3	0.5 - 7.6
		Control	8	5.3	8	1.7 +/- 0.9	0.5 - 7.7
Immune	Atopy	All bitches	21	6.9	21	2.9 +/- 0.4	0.8 - 6.7
		PPN	12	7.7	12	3.9 +/- 0.6	1.0 - 6.7
		Control	9	6.0	9	1.6 +/- 0.3	0.8 - 3.7
	Autoimmune	All bitches	1	0.3	1	6.9 +/- 0.0	-
	haemolytic anaemia	PPN	1	0.6	1	6.9 +/- 0.0	-
		Control	0	0.0	0	-	-
	Hypoadrenocorticism	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Hypothyroidism	All bitches	1	0.3	1	5.7 +/- 0.0	-
		PPN	1	0.6	1	5.7 +/- 0.0	-
		Control	0	0.0	0	-	-
	Immune mediated	All bitches	2	0.7	2	2.9 +/- 1.7	1.2 - 4.5
	arthritis	PPN	2	1.3	2	2.9 +/- 1.7	1.2 - 4.5
		Control	0	0.0	0	-	-
	Immune-mediated	All bitches	1	0.3	1	2.9 +/- 0.0	-
	thrombocytopenia	PPN	1	0.6	1	2.9 +/- 0.0	-
		Control	0	0.0	0	-	-
	Inflammatory bowel	All bitches	2	0.7	2	1.7 +/- 0.3	1.4 - 2.0
	disease (IBD)	PPN	0	0.0	0	-	-
		Control	2	1.3	2	1.7 +/- 0.3	1.4 - 2.0
	Otitis externa	All bitches	129	42.2	255	2.8 +/- 0.1	0.5 - 10.4
		PPN	74	47.7	145	2.7 +/- 0.2	0.5 - 10.4
		Control	55	36.4	110	2.9 +/- 0.2	0.5 - 8.8

	Systemic lupus	All bitches	0	0.0	0	-	-
	erythematosus	PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
Other	Aortic stenosis	All bitches	2	0.7	2	0.8 +/- 0.3	0.6 - 1.2
		PPN	0	0.0	0	-	-
		Control	2	1.3	2	0.8 +/- 0.3	0.6 - 1.2
	Diabetes mellitus	All bitches	1	0.3	1	9.6 +/- 0.0	-
		PPN	0	0.0	0	-	-
		Control	1	0.7	1	9.6 +/- 0.0	-
	Early onset cataract	All bitches	4	1.3	4	1.2 +/- 0.1	1.1 - 1.3
		PPN	2	1.3	2	1.2 +/- 0.1	1.1 - 1.3
		Control	2	1.3	2	1.2 +/- 0.1	1.2 - 1.3
	Any cataract	All bitches	8	2.6	8	4.3 +/- 1.3	1.1 - 10.2
		PPN	4	2.6	4	5.1 +/- 2.3	1.1 - 10.2
		Control	4	2.6	4	3.5 +/- 1.5	1.3 - 7.7
	Epilepsy (idiopathic)	All bitches	2	0.7	2	4.8 +/- 2.4	2.4 - 7.2
		PPN	2	1.3	2	4.8 +/- 2.4	2.4 - 7.2
		Control	0	0.0	0	-	-
	Gastric volvulus	All bitches	0	0.0	0	-	-
		PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Geriatric cognitive	All bitches	0	0.0	0	-	-
	impairment / CDS	PPN	0	0.0	0	-	-
		Control	0	0.0	0	-	-
	Histiocytoma	All bitches	10	3.3	10	1.6 +/- 0.2	0.6 - 2.8
		PPN	7	4.5	7	1.6 +/- 0.2	0.8 - 2.5
		Control	3	2.0	3	1.6 +/- 0.6	0.6 - 2.8
	Overweight/obese	All bitches	134	43.8	134	3.2 +/- 0.2	0.7 - 11.3
		PPN	65	41.9	65	3.4 +/- 0.3	0.8 - 11.3
		Control	69	45.7	69	2.9 +/- 0.3	0.7 - 9.4
	Obesity	All bitches	44	14.4	44	5.4 +/- 0.3	2.0 - 10.6
	(based on BCS ≥7)	PPN	20	12.9	20	5.1 +/- 0.4	2.0 - 8.3
		Control	24	15.9	24	5.7 +/- 0.5	2.5 - 10.6

Appendix 12 – Types of neoplasia noted as cause of death

The type of neoplasia noted as cause of death for 22 bitches neutered before (PPN) or after (Control) puberty.

Neoplasia noted / primary organ affected	PPN (N)	Control (N)
Haemangiosarcoma	1	2
Intestinal	0	1
Liver	1	1
Lung	2	1
Lymphoma	2	2
Mast cell tumour	1	1
Neuroendocrine carcinoma	1	0
Soft tissue sarcoma	1	0
Spinal cord	0	1
Spleen	2	0
Stomach	1	0
Not reported	1	0