Evaluation of a Burdizzo Castrator for Neutering of Dogs

A Ortega-Pacheco1, ME Bolio-González1, RF Colin-Flores1, CH Sauri-Arceo1, E Gutiérrez-Blanco1, M Jiménez-Coello2 and C Linde Forsberg3

1Faculty of Veterinary Medicine and Animal Science; 2CIR ‘Dr Hideyo Noguchi’, Autonomous University of Yucatan, Yucatan, Mexico; 3Department of Clinical Sciences, Division of Comparative Reproduction, Obstetrics and Udder Health, Swedish University of Agricultural Sciences, Uppsala, Sweden

Contents

A Burdizzo castrator was evaluated for the neutering of dogs. Histological and morphological changes of spermatic cells and peripheral serum testosterone after challenge with a GnRH-analogue (gonadorelin) were assessed. There was a control group (G1), a surgically castrated group (G2) and a Burdizzo group (G3) divided in two, G3a receiving two crunches in each spermatic cord and G3b receiving one crunch in each spermatic cord. Sixteen days after application of the Burdizzo blood samples were taken from the dogs at 30 min interval during 2 h; after the second sample the dogs were treated with 1 mg/kg body weight of gonadorelin i.v. The same protocol of gonadorelin challenge was performed in G1 and G2 dogs. The G2 dogs were surgically castrated after the second blood sample, before the gonadorelin treatment, and the G1 dogs after the last blood sample. The excised gonads were examined histologically, and sperm smears were prepared from the caudae epididymis. The testes and plexus pampiniformis of the G1 and G2 dogs had a normal histological appearance, and they had morphologically normal epididymal sperm cells. In all G3 dogs, there was an acute fibrosis with an inflammatory reaction in the plexus pampiniformis. The testes from the G3a dogs showed diffuse areas of infarction and degeneration of the parenchyma. Similar but less diffuse lesions were seen in group 3b dogs. The deferent ducts from all G3 dogs showed vasitis and/or sperm granulomas. Azoospermia or sperm malformations were observed in the epididymal smears from the G3 dogs. Testosterone concentration in the G1 dogs increased after gonadorelin application (p < 0.0001). The G2 dogs had basal testosterone levels after castration (p < 0.001) and did not respond to gonadorelin. Groups 3a and b showed a slight but non-significant increase in testosterone concentration after gonadorelin challenge, supposedly due to the reduction of testicular blood flow and loss of testicular interstitial tissue.

Introduction

Dog overpopulation remains a serious problem in many developing countries despite local efforts to control population growth. In these countries, free roaming dogs are a source of ecological and social problems, attacking other animals and people, causing road accidents, frightening the public and fecal and urine contamination. Therefore, the development of effective control measures has a high priority. Traditionally, surgical sterilization and mass euthanasia campaigns are used but the impact has proved to be low. Besides, the high cost of the campaigns is prohibitive and the often inhumane handling of the dogs is against international animal welfare regulations.

Several contraceptives have been developed to control the growth of dog populations, but have been primarily directed towards the female of this species (Pineda 1986). However, a single intact male is reproductively active around the year and may sire many more pups than a single female is capable of producing in a lifetime. Castration of male dogs would not only decrease the birth of unwanted litters, but also reduce the testosterone levels, and consequently both reduce the risk of developing prostatic disease and avoid undesirable male behaviours such as roaming, aggression, mating and urination marking (Neillson et al. 1997). The sterilization of a large number of males particularly through the use of non-surgical, inexpensive methods would effectively contribute to curb the growth of the pet population. The ideal contraceptive in the male should be effective, safe, readily available and acceptable to owners and have a few side effects (Pineda 1986). Although a new locally injectable product for sterilization in dogs is available (i.e. Neutersol®, Addison Biological Laboratory, Fayette, MI, USA), it is too expensive to be used in sterilization campaigns in which hundreds of dogs are sterilized. Besides, this product was developed for use only in prepubertal animals. Presently, the more commonly used means of contraception in the male dog are confinement and surgical castration. Non-surgical ablation of the testicular artery using the Burdizzo clamp is routinely used in lambs (Molony et al. 1993). A successful case report in a human has also been published, in which no physical side effects in the patient were observed (Herzog and Santucci 2002).

The objective of this work was to evaluate the Burdizzo clamp for non-surgical neutering in dogs, as assessed by histological and spermatological findings and serum levels of testosterone after intravenous administration of a gonadorelin analogue 16 days after neutering.

Material and methods

Animals

Thirty healthy, 3–5-year-old mongrel dogs weighing between 13 and 15 kg were divided into three groups: group 1, control group (n = 10); group 2, surgically castrated group (n = 10); and group 3, Burdizzo group (n = 10). The later group was, in turn, divided in two; G3a (n = 5) and G3b (n = 5). An andrological examination of the dogs was performed before the start of the study to assess the normality and size of their gonads.

Burdizzo procedure

The Burdizzo clamp used (Supervet™, CHIFA, Nowy, Tomysł, Poland), also known as ‘baby Burdizzo’, was...
obtained from a veterinary supply company (SyrVet, Waukee, IA, USA). The clamp has a width of jaws of 42 mm and an overall length of 23 cm and is suitable for bloodless castration in lambs and goat kids by severing the testicular cord without injury to the scrotum. Previous to the application of the Burdizzo clamp in group 3, the animals received an epidural injection of ketamine (5.0 mg/kg). The dogs were, thereafter, placed in a supine position and the Burdizzo jaws were placed over each spermatic cord and were held closed for 60 s (Fig. 1). In the five group 3a dogs, the Burdizzo was applied over two areas of each spermatic cord, one close to the testis above the epididymal head and the other in the middle part of the spermatic cord. In the group 3b dogs, the Burdizzo was applied in the same manner, but only over one area of each spermatic cord, close to the testicles above the epididymal head. Special care was, in all cases, taken to clamp as little of the scrotum as possible to avoid excessive tissue damage and inflammation. All group 3 dogs were examined daily for general condition and to evaluate on a behaviour-based scale the post-operative pain (Holton et al. 2001) and need for analgesic treatment.

**Gonadorelin challenge**

Blood samples were taken from all dogs from the cephalic vein into sterile tubes. Samples were taken at intervals of 30 min during 2 h. Immediately after taking the second blood sample, all dogs were treated i.v. with 1 μg/kg body weight of a GnRH analogue (Gonadorelin, Fertagyl®; Intervet, Edo, Mexico). Blood samples were centrifuged at 400 × g for 15 min; serum was separated and stored at −20°C until hormone assay.

**Testosterone assay**

Sixteen days after application of the Burdizzo, serum testosterone concentrations were determined by radio-immunoassay (Coat-a-count® Total testosterone; DPC, Los Angeles, CA, USA). The detection limit of the assay was 0.08 nmol/l. The intra-assay coefficients of variation for low (3.49 nmol/l), medium (12.03 nmol/l) and high (19.63 nmol/l) controls were 1.4%, 5.1% and 10.4%, respectively.

**Collection and evaluation of tissue samples**

Group 2 dogs were surgically castrated following standard procedures (Johnston 1985) after the second blood sample, just before the gonadorelin challenge, while group 1 and group 3 dogs were similarly castrated 1 h after application of the gonadorelin.

Testicles were after castration dissected free from the scrotum, tunica and epididymis and each testicle was measured and weighed to the nearest 0.1 g. Sperm smears for morphological evaluation were made from the contents of the two caudae epididymidis after slicing them with a scalpel into a physiological saline solution. Samples were dried, fixed and stained with carbol fuchsin and 500 spermatozoa evaluated for the presence of abnormal sperm heads. A total of 200 spermatozoa in a drop of formol-saline were evaluated in search for other sperm abnormalities under a phase-contrast microscope at ×100 magnification (Axnér et al. 1998). A sample of each testicle (1 cm³), cauda epididymis, vas deferens and spermatic cord was placed in 10% neutral buffered formalin. Samples were also sliced at 2 mm thickness and embedded in paraffin. Five-micrometer sections were cut and stained with haematoxylin and eosin according to standard histology techniques.

**Statistical analysis**

Differences between pre-treatment and response values of testosterone were tested using a paired t-test. The level of significance was set at p < 0.05. Values are given as means ± SD.

**Results**

Testicles and plexus of group 1 and group 2 dogs were macroscopically and microscopically normal. Assessment of sperm morphology showed <20% abnormal cells. Minimal damage was noted on the skin of the group 3 dogs. A slight bruising of the skin and inflammation of the scrotum was noted in all the group 3a dogs. After recovering from epidural blockage, dogs were able to move easily around their cages and no signs of acute pain were observed during the 16-day follow-up time. There were no significant changes in scrotal diameter from day 1 to day 16. The testicles recovered from the group 3 dogs on day 16 had a hard consistency and there were macroscopically visible lesions in the plexus pampiniformis and vas deferens (Fig. 2). When incised, a bloodstained fluid came from the testicles and the tissue was seen to be macerated (Fig. 3). Histological examination of the plexus pampiniformis revealed in all group 3 cases, an acute fibrosis with inflammatory reaction and reduction of the arterial luminal area (Fig. 4). Histological findings in the testicles varied from normal to diffuse areas (from one-third to the whole of the testicle) of infarction and a degenerated parenchyma. Seminiferous tubules had disappeared, showed degrees of degeneration or were normal in different areas of the same testicle. Interstitial tissues revealed in six cases (five in G3b and one from G3a) vacuolated Leydig cells, lymphocytic infiltration and diffuse fibrosis.

**Fig. 1.** Burdizzo clamp crushing the spermatic cord close to the testicle

© 2006 Blackwell Verlag
or total absence of Leydig cells (Fig. 5). These lesions were more severe in the group 3a dogs that had received two crushes on the same spermatic cord than in group 3b having received one crush. Spermatogenesis was still present in non-damaged areas of six testicles. Microscopic evaluation of the deferent ducts from all dogs from group 3 showed vasitis at the level where the vas had been crushed. Six dogs developed sperm granulomas in at least one vas deferens. Sperm granulomas were seen as yellow nodular cystic masses. They consisted of a pool of spermatozoa undergoing phagocytosis surrounded by a layer of macrophages, fibroblasts, lymphocytes, neutrophils and giant cells. Three of the five group 3a dogs were azoospermic, and the remaining two had spermograms with up to 95% double bent tails. The group 3b dogs showed semen pictures with high percentages of midpiece abnormalities (40.2 ± 12.3%) and coiled tails (27.5 ± 17.3%). The testosterone concentrations after gonadorelin challenge are shown in Fig. 6. The dogs of the control group (G1) showed a significant increase (p < 0.0001) in testosterone concentrations after gonadorelin application from 4.3 ± 3.4 to 10.2 ± 3.7 nmol/l. Group 2 dogs showed pre-treatment values of 2.2 ± 2.3 nmol/l which rapidly
inevitable ischaemic necrosis or infarction of the testicles depending on the severity of the hypoxia produced. An to the testicles producing different degrees of damage present study, vascular damage decreased the blood flow to the testes with a subsequent quick hypoxic response of the testicular cells ending in coagulative necrosis. Lesions in dogs receiving one crush in each spermatic cord were similar but less intense, but were severe enough to produce irreversible damage. In the light of these results, two crushes to each spermatic cord may be recommended to ensure good results.

After various periods of testicular vascular occlusion, it has been shown that the duration of the hypoxia markedly influence the extent of damage to the germinal epithelium and consequently the sperm quality (Dixit 1977). Additionally, lesions produced in the spermatic cord also reduce the blood supply to the epididymides. Ischaemia in the caput of the epididymis may also affect testicular function by producing dilation of the vasa efferentia which, in turn, produces stasis of the sperm flow, testicular distension and dilated seminiferous tubules and subsequent degeneration of the seminiferous epithelium (Steinberger 1970). In the present study, absence of spermatozoa in the epididymis or the presence of spermatozoa with a high percentage of abnormalities was supposedly due to damage to the germinal cells and an abnormal environment for spermatogenesis.

Sperm granulomas can be found in 15% of men undergoing repairation of the vas after vasectomy, but are usually small and harmless and may go unnoticed (Schmidt 1975). Presence of vas granulomas in this work as a consequence of the Burdizzo procedure was considered as a harmless side-effect. The optimal amount of time for application of the Burdizzo and the optimal pressure required for castration in dogs has yet to be determined, especially when dogs of different sizes are presented. The application time of 60 s used in the present study was, however, appropriate to produce irreversible damage to the testicles. In lambs, closing the Burdizzo clamp over the spermatic cord for 10 s was enough for adequate castration (Kent et al. 1993). Excessive damage to the vas may be prevented by reducing the time of the Burdizzo crushing the plexus. In humans, instead of performing open vasectomy, a method using the Burdizzo to crush the vas while preserving the plexus has been tested (Zufall 1958). This less invasive procedure may also be useful in dogs, although it would not reduce testosterone levels and thus male aggressive and sexual behaviour (Pineda 1986). In lambs, no anaesthesia is provided when using the Burdizzo clamp to castrate dogs. The use of the Burdizzo clamp to crush the spermatic cord resulted in testicular lesions of various degrees after a 16 day observation period. In a study by Smith (1955), it was shown that after 4 h of induced testicular ischaemia in dogs, spermatogenesis had ceased and in some testicles the Sertoli and the Leydig cells were also damaged, while 10 h or more of ischaemia resulted in elimination of all Leydig cells and the testicular elements were replaced by connective tissue. In a later study, also in dogs, it was demonstrated that after 12 days of vascular occlusion, seminiferous tubules were replaced by an ‘amorphous mass’ and had no basement membrane. Leydig cells at this time had become atrophied (Dixit 1977). Local infarction in the testicles and infertility has also been reported in rams after experimental ischaemia of the testicular artery (Markey et al. 1994, 1995), and in dogs with naturally occurring testicular torsion (Pearson and Kelly 1975). In the present study, vascular damage decreased the blood flow to the testes producing different degrees of damage depending on the severity of the hypoxia produced. An inevitable ischaemic necrosis or infarction of the testicles

Discussion
The Burdizzo clamp to crush the spermatic cord has been used in veterinary medicine to castrate primarily lambs and goat kids with minimal post-operative complications (Molony et al. 1993). It has also occasionally been used to successfully vasectomise humans by crushing the vas deferens with minimal side effects (Zufall 1958). A case of supervised medical neutering in humans has been reported using a Burdizzo clamp and its use has been recommended in cases of advanced human prostatic cancer (Herzog and Santucci 2002). This is the first documented study about the use of the Burdizzo clamp to castrate dogs.

The use of the Burdizzo clamp to crush the spermatic cord resulted in testicular lesions of various degrees after a 16 day observation period. In a study by Smith (1955), it was shown that after 4 h of induced testicular ischaemia in dogs, spermatogenesis had ceased and in some testicles the Sertoli and the Leydig cells were also damaged, while 10 h or more of ischaemia resulted in elimination of all Leydig cells and the testicular elements were replaced by connective tissue. In a later study, also in dogs, it was demonstrated that after 12 days of vascular occlusion, seminiferous tubules were replaced by an ‘amorphous mass’ and had no basement membrane. Leydig cells at this time had become atrophied (Dixit 1977). Local infarction in the testicles and infertility has also been reported in rams after experimental ischaemia of the testicular artery (Markey et al. 1994, 1995), and in dogs with naturally occurring testicular torsion (Pearson and Kelly 1975). In the present study, vascular damage decreased the blood flow to the testes producing different degrees of damage depending on the severity of the hypoxia produced. An inevitable ischaemic necrosis or infarction of the testicles is produced either when occlusion of the arterial supply or the venous drainage of the tissue occurs (Slauson and Cooper 2002). Application of two crushes on the same spermatic cord apparently rapidly decreased the blood flow to the testes with a subsequent quick hypoxic response of the testicular cells ending in coagulative necrosis. Lesions in dogs receiving one crush in each spermatic cord were similar but less intense, but were severe enough to produce irreversible damage. In the light of these results, two crushes to each spermatic cord may be recommended to ensure good results.

After various periods of testicular vascular occlusion, it has been shown that the duration of the hypoxia markedly influence the extent of damage to the germinal epithelium and consequently the sperm quality (Dixit 1977). Additionally, lesions produced in the spermatic cord also reduce the blood supply to the epididymides. Ischaemia in the caput of the epididymis may also affect testicular function by producing dilation of the vasa efferentia which, in turn, produces stasis of the sperm flow, testicular distension and dilated seminiferous tubules and subsequent degeneration of the seminiferous epithelium (Steinberger 1970). In the present study, absence of spermatozoa in the epididymis or the presence of spermatozoa with a high percentage of abnormalities was supposedly due to damage to the germinal cells and an abnormal environment for spermatogenesis.

Sperm granulomas can be found in 15% of men undergoing repairation of the vas after vasectomy, but are usually small and harmless and may go unnoticed (Schmidt 1975). Presence of vas granulomas in this work as a consequence of the Burdizzo procedure was considered as a harmless side-effect. The optimal amount of time for application of the Burdizzo and the optimal pressure required for castration in dogs has yet to be determined, especially when dogs of different sizes are presented. The application time of 60 s used in the present study was, however, appropriate to produce irreversible damage to the testicles. In lambs, closing the Burdizzo clamp over the spermatic cord for 10 s was enough for adequate castration (Kent et al. 1993). Excessive damage to the vas may be prevented by reducing the time of the Burdizzo crushing the plexus. In humans, instead of performing open vasectomy, a method using the Burdizzo to crush the vas while preserving the plexus has been tested (Zufall 1958). This less invasive procedure may also be useful in dogs, although it would not reduce testosterone levels and thus male aggressive and sexual behaviour (Pineda 1986). In lambs, no anaesthesia is provided when using the Burdizzo and discomfort is observed during the first 3 h after treatment (Molony et al. 1993; Kent et al. 1995). However, damage to the nerves of the spermatic cord and serotum by the application of the Burdizzo clamp may have blocked the nervous pathways thereby reducing pain and discomfort in lambs (Kent et al. 1995). In the present study, epidural blockade provided surgical anaesthesia against acute pain during the application of the Burdizzo which lasted approximately 25 min. Similar periods of regional anaesthesia have been reported in dogs after epidural administration of ketamine. Additionally, the systemic redistribution of
ketamine after epidural application may provide posterior analgesic effects (60–720 min) after the motor blockade of the hind limbs is over (Duke et al. 2004). Methods for assessment of acute post-operative pain in dogs are well developed (Holton et al. 2001). Although in this study, it was not the objective to quantified the pain after the application of the clamps, the method for pain assessment employed gave a good indicator of the status of the dogs and no indications of that pain management were observed.

Testicular stimulation of normal, entire dogs with GnRH analogues produce a significant dose–response increase in plasma concentrations of testosterone (Knol et al. 1993; Purswell and Wilcke 1993; Kawakami et al. 1997), similarly as observed in the control group in this study. The lack of testosterone response to gonadorelin in the group of castrated dogs (G2) demonstrates the lack of interstitial tissue. Although LH was not measured, an immediate and significant increase is expected to occur after GnRH stimulation (Olson et al. 1992; Knol et al. 1993; Günzel-Apel et al. 1994; Kawakami et al. 1997). The various degrees of vascular and/or testicular damage induced in the group 3 dogs were reflected in the low response to gonadorelin stimulation of the systemic testosterone concentration. After application of LH in rams with induced testicular ischaemia, concentrations of testosterone were not increased, whereas, in contrast, the normal control animals responded with a significant rise. The lack of response in the ischaemic animals apparently was due to a reduction in the blood flow through the damaged spermatic vessels (Markey et al. 1994). In the present study, a similar reduction may have occurred since no significant response to gonadorelin was seen in group 3 dogs. Loss of interstitial tissue was greater in group 3a than in group 3b, and as a consequence the testosterone response was lower in the former group. The pre- and post-treatment differences in groups 3a and 3b were due to the normal episodic secretory pattern of testosterone which showed low and non-significant variations in both groups. The lower response after gonadorelin challenge in group 3 dogs 16 days after the Burdizzo treatment compared with group 1 dogs was considered as a sign of effective blocking of the spermatic cord vessels. The dogs from group 3 were for practical purposes followed for only 16 days, which turned out not to be long enough for the development of complete testicular atrophy in these animals. The induced damages were, however, of such magnitude that Burdizzo clamping safely can be expected to induce complete testicular degeneration in the dog, like previously demonstrated in other species. However, because of the low number of animals in the two Burdizzo groups, results should be interpreted with caution. More studies involving bigger groups of dogs are indicated.

This preliminary study demonstrated that ‘mechanical sterilization’ in male dogs using the Burdizzo clamp is a safe, easy, readily available and inexpensive method that can be used both in individual cases and in large-scale sterilization programs. However, long-term studies are required to evaluate the time required to induce testicular atrophy and to determine any possible further side effects.

Acknowledgements

The authors thank MVZ Francisco Carrillo (SERVIVET S.A DE C.V) for kindly donating the Fertagyl and the dog pound from the municipality of Merida, Yucatan for providing the dogs used in the present study. The financial support of PROMEP (Programa de Mejoramiento del Profesorado de Educacion Superior), Mexican Ministry of Education, is also acknowledged.

References


Submitted: 30.08.2005

Author's address (for correspondence): Dr Antonio Ortega-Pacheco, FMVZ-UADY AP 4-116 Itzimna Merida, Yucatan, Mexico. E-mail: opacheco@tunku.uady.mx

© 2006 Blackwell Verlag