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Evaluation of Pinhole Castration Technique Compared With Traditional Method For Castration In Dogs

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Key words

pinhole, castration, prescrotal, dogs, cortisol estimation.

ABSTRACT:

Castration is the most common routine surgical procedure in animals.The present investigation aimed to compare between two techniques of surgical castration (prescrotal and pinhole) in dogs. The study was performed on nine clinically healthy dogs weighing 20-30 kg and aged from 1-2 years, these animals were divided into three groups (prescrotal, bilateral pinhole and unilateral pinhole) and each group consists of three animals. Castration was done under the effect of xylazine –ketamine anaesthesia. Intra-operative and post-operative complications together with cortisol concentration and histopathological examination were reported. The result of this study revealed that no major intra and post-operative complications were observed in prescrotal technique. Healing score is better in prescrotal than pinhole technique while cortisol level was significantly higher in pinhole technique. It was concluded that prescrotal castration technique proved advantageous than pinhole one as it was less painful, simple and effective for male dog sterilization.

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1. INTRODUCTION

Surgical sterilization of dogs is one of the most commonly performed procedures in veterinary practice, and is done as a method of contraception to aid in the pet overpopulation problem, as well as to prevent diseases of reproductive system, such as benign prostatic hyperplasia (Brendler et al., 1983) and to modify undersirable behavior, such as urine marking, intermale aggression, and mounting of other dogs (Hopkins et al., 1976).Despite castration is almost the sole method for control of pet's overpopulation globally (Brenda, 2002) it has disadvantages and postoperative complications of surgical methods of sterilization such as hemorrhage, wound dehiscence, infections, and scrotal swellings (Gizawiy et al., 2004; Adin, 2011) but it has remained the mainstay for castration in dogs (Howe, 2006).

In situ spermatic cord ligation (pinhole castration) has been described as a novel minimally invasive technique without cutting or involving the scrotum for induction of complete ischemic necrosis and atrophy testes for calf, kid goat and male dog sterilization (Ponvijay, 2007; Okwee-Acai et al. , 2008 ; Okwee - Acai et al., 2012; Baba et al., 2013).

The present experimental investigation aimed to compare between two common techniques (prescrotal and pinhole) for surgical castration in dogs by evaluation of intra and post operative complications, costs and managemental value and assaying cortisol level as a pain marker.

2. MATERIALS AND METHODS:

The present study was performed on 9 apparently healthy adult male a stray dogs (weighing 20-30 kg and aged from 1 - 2 years old). At the time of procurement, the animals were subjected to complete clinical examination including palpation of the scrotum to confirm the presence of two normally descended testes and they were dewormed once using Noromectine injection (1.5% w/v). The animals were kept off food for 24 hours and water was withheld for 12 hours before anesthesia.

2.1. Anaesthesia

The dogs were sedated using 2% Xylazine HCl (1mg/kg body weight intramuscularly then the

anaesthesia was induced five minutes later by intramuscular injection of Ketamine HCl (5 mg/kg body weight).

2.2. Experimental Design

Dogs were randomly divided into 3 groups (3 dogs per group), and each group was subjected to the following experimental castration techniques: (1) prescrotal castration; (2) bilateral pinhole castration (in-situ spermatic cord ligation) and (3) unilateral pinhole castration. The animals were placed in dorsal recumbency and prepared routinely for aseptic surgical procedure.

2.3. Castration Techniques

2.3.1. Prescrotal Technique

Prescrotal castration of the selected three dogs was performed according to the technique described by Booth (2003) and Tobias (2010). Skin stitches were removed 7 - 10 days following surgery.

2.3.2. Pinhole Technique

Bilateral pinhole was achieved by passing a suture (silk No.1) threaded to a suture needle adjacent to the medial side of the spermatic cord at the neck of the scrotum followed by reinsertion in reverse direction (through the original holes) along its lateral side and completed by a subcutaneously buried square knot (Fazili et al., 2009 and Baba et al., 2013). The technique was repeated on the contra lateral spermatic cord. While in group (3) unilateral pinhole technique was adopted the same steps as bilateral ligation but in one testis according to the technique described by Baba et al., (2013). Not use postoperative analgesic in all treated animals (Figs., 1 and 2). Assessment

2.3.3. Intra-operative Complications

Complications detected during the surgical procedures, especially bleeding , were recorded.

2.3.4. Post-operative Complications

Post-operative appearance of the site of skin incision in all dogs was evaluated 10 days after prescrotal technique and 28 days after pinhole technique depending up on the scores described by Al-Gizawiy et al. (2004).

Fig 1 : A silk No. 1 on a suture needle is passed over and below the spermatic cord to make ligation

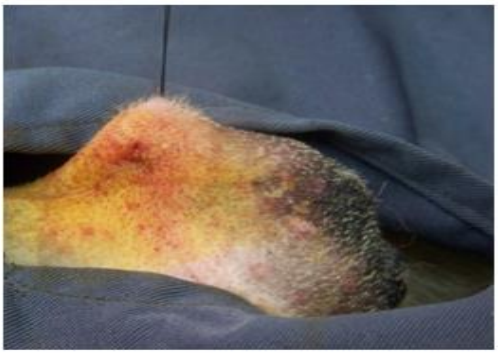

Fig 2 : spermatic cord after ligature

Table (1): Post-operative wound healing scores

Score	Description
A	- Wound was almost closed.
++++	- Good healing, wound was slightly swollen, open, dry and clean.
+++	- Good healing, wound was swollen, open, wet and clean.
++	- Fair healing, wound was grossly swollen, open, wet and clean.
+	- Poor healing, wound was grossly swollen, open and wet with blood or pus oozing.

2.4.3. Economic Value and Managemental Care

All consumable materials used in each prescrotal and pinhole castration case were priced and calculated in Egyptian pounds. The effort of labour after each surgery was taken into account.

2.4.4. Evaluation of cortisol

Post-operative pain following each operation (prescrotal and pinhole) was evaluated depending on the serum cortisol concentration according to the technique described by Okwee-Acai et al. (2012). Four blood samples (about 2-3 ml) were collected; before operation and 24 hours, 5 days and 10 days after each operation for measuring of plasma cortisol. Cortisol concentration (ug / dl) was evaluated in a commercial laboratory via electro-chemi-luminescence (ECL) technology (Cobas e 411, ECL-based immunoassay analyser, Roche Diagnostics, England).

2.4.5. Scrotal Dimensions

The testicular length (L) and testicular width (W) were measured following pinhole castration using a ruler. Observations were recorded at day; 0, 5, 7, 16, 23 and 28 according to the advisors of Baba et al. (2013).

2.4.6. Histopathological Examination

After 28 days from in situ spermatic cord ligation, both of the testes, epididymis and spermatic cord were removed under general anesthesia from all pinhole castrated animals, tissue specimens immediately fixed in 10% neutral phosphate-buffered formalin for at least 24 hours then embedded in paraffin. Sections of 5 µ thickness were stained with hematoxylin and eosin (H&E) according to the method described by (Culling ,1983) and were examined under light microscope.

2.4. Statistical Anlysis

The analysis of variance for the data obtained in pre and postoperative measurements was performed using a statistical analysis system (SAS,2002).

3. RESULTS

3.1. Intra-Operative Complications

No major intra-operative complications were observed in dogs subjected to prescrotal castration. Following pinhole (bilateral and unilateral) castration, spermatic cords were efficiently ligatedin all cases. In bilateral pinhole castration, only one dog showed minor bleeding at site of needle insertion. This bleeding did not require any intervention and spontaneously arrested**.**

3.2. Post-Operative Complications

Healing scores in all cases of prescrotal varied between score (A) and score (++++), where the wound was almost closed, was slightly swollen, dry and clean. While in the dogs underwent pinhole castration either bilateral or unilateral, showed fair to poor healing score (+), the wounds were inflamed, grossly swollen, wet, with pus oozing at site of needle penetration also from lower end of scrotum with stretched scrotal skin which reach to penis in two cases (Fig.3 a,b). The dog no.1 of bilateral pinhole castration showed adhesion between scrotum and the surrounding tissues after 28 days from ligation. Only one case of each pinhole surgery (one unilateral and one bilateral) showed good healing score (A) characterized by contraction of skin wound size and the wounds were visible after 28 days post-operative. Wound healing scores following pinhole castration appeared significantly different and larger than those of the wound healing scores following prescrotal castration. Four of the six cases showed score (+) and only two cases showed score (A)**. 3.3. Economic Value (Cost) and Managemental Care**

Each case of pinhole castration required Xylazine, ketamine, hypodermic needle, and a strand of suture material. Meanwhile, materials needed for the standard prescrotal and scrotal castration included Xylazine, ketamine, antibiotic, antiseptic, dressings and sutures (catgut and silk). The average cost (75-90 Egyptian pounds) for one case of prescrotal approach was nearly three times higher than pinhole approach (20-25 Egyptian pounds). But the effort of labour after pinhole castration care was more than prescrotal castration.

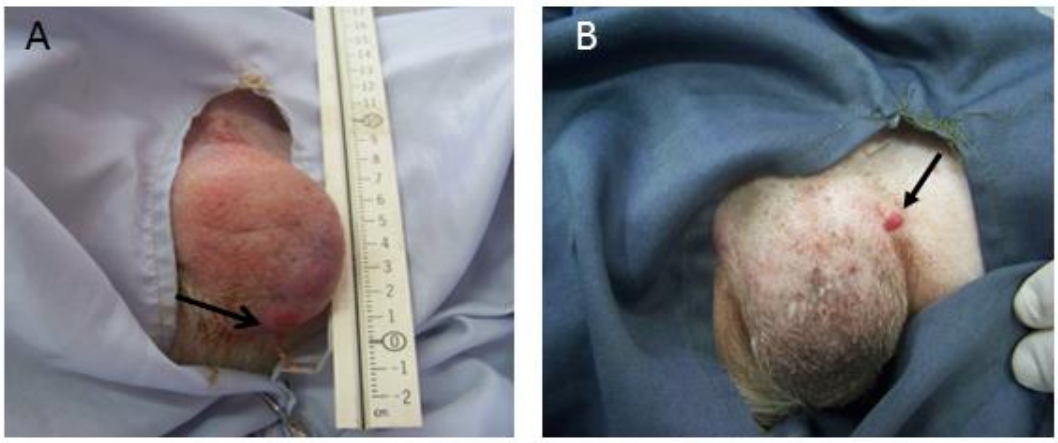

Fig 3: Pus oozing from lower end of testis (a) and at site of needle insertion (b)

3.4. Evaluation of cortisol

After 24 hours of surgery, mean cortisol levelswas higher in the pinhole castration (8.2 ± 0.96 ug ⁄ dL) than the prescrotal castration (6.12 ± 1.09ug ⁄ dL). The mean cortisol levels in all treated dogs had reduced by the day 5 th; but it remained relativevely higher in the pinholegroup (7.79 ± 1.41 ug ⁄ dL) than the prescrotal group (5.22± 0.71 ug ⁄ dL). Cortisol levels in two techniques were returned to normal after 10 days from surgery, table (2).

2.4. Scrotal Dimensions following Unilateral Pinhole Castration:

As showen in Table 3,mean scrotal dimensions values (length and width) ,in dogs of unilateral pinhole castration, significantly higher on day 5 when compared to the values recorded at days 0, 7, 16, 23 and 28. The maximal mean of scrotal dimensions was recorded on day 5 after ligation (4.43 ± 0.35 X 2.90 ± 0.21 cm) and the minimal was recoded on day 28 (3.20 ± 0.92 X 2.27 ± 0.18 cm).

Table (2): Showing Mean ± SD values of serum cortisol concentrations (ug ⁄ dL) of dogs underwent prescrotal and pinhole castration.

Time interval	**Serum cortisol (ug ⁄ dL)**	
	Prescrotal castration	**Pinhole castration**
	Mean±SE	Mean±SE
Day 0	4.52±0.61a	4.82±0.63a
After 24 hours	6.12±1.09a	8.22±0.96a
5 days	5.22±0.71a	7.79±1.41a
10 days	4.49±0.90a	4.91±1.17a

a,b,c,d, Mean values in the same raw with different superscript letters are significantly different from each other (P < .05) (N = 3).

Table (3): Values (cm) of scrotal dimensions (length and width) in dogs underwent unilateral pinhole castration

Day	*Length*	*Width*
	Mean±SE	Mean±SE
0	4.00±0.46a	2.27±0.18a
5	4.43±0.35a	2.90±0.21a
7	4.10±0.38a	2.60±0.31a
16	3.90±0.56a	2.57±0.35a
23	3.57±0.83a	2.43±0.45a
28	3.20±0.92a	2.33±0.60a

a,b,c,d, Mean values in the same colum with different superscript letters are significantly different from each other ($P < .05$) (N=3).

2.5. Histopathological Findings following Pinhole Castration

The macroscopic examination revealed that the testis with pinhole ligation was atrophied with less dimension when compared with the non ligated testis (Fig.4).

The pattern of lesions recorded in testes of all castrated animals was similar. The majority of the seminiferous tubules showed degeneration and necrosis with acute ischemic coagulative necrosis of the germinal epithelium, with indistinct cellular borders and complete absence of nuclei. Thereby, massive destruction of the cellular tissue was seen with complete hyalinization of the seminiferous tubules with no signs of spermatogenesis (Fig. 5). Moreover, the interstitial tissue replaced by a thick layer of fibrous tissue with necrosis of Leydig cells (Fig. 6).

Undesirable testicular finding can be described as edematous changes in the interstitial tissue with or without infiltration with neutrophils (suppurative inflammation) beside the hyalinized seminiferous tubules (Figs. 7 & 8), this also appeared clearly by macroscopic appearance (Fig. 9).

Concerning the spermatic cord examination, the manifestations of revascularization can't be detected in any of the examined tissues.

Fig 4: Left testicle underwent atrophy at 28th day in abcess unilateral pinhole technique as compared to right non ligated one

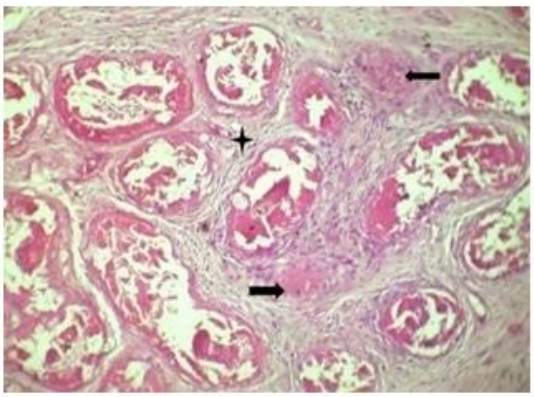

Fig.5: A photomicrograph showing completely degenerated seminiferous tubules filled with hyalinized materials with some tubules began to disappear completely leaving ghost tubule **(arrows)**, notice the thickened fibrotic interstitial tissue **(astrikes)**.***H&E. X100.***

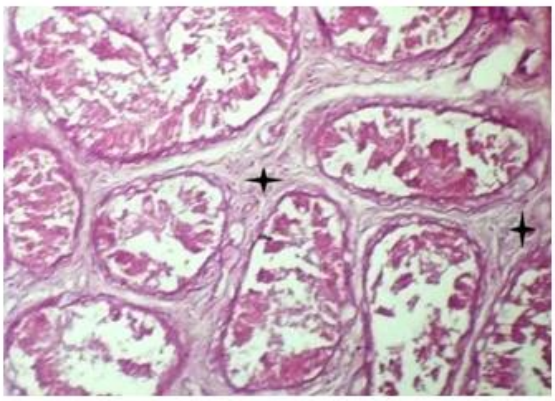

Fig.6: A photomicrograph showing acute ischemic coagulative necrosis and hyalinization of the germinal epithelium,with the interstitial tissue replaced by a thick layer of fibrous tissue with necrosis of Leydig cells **(astrikes)**. ***H&E. X250.***

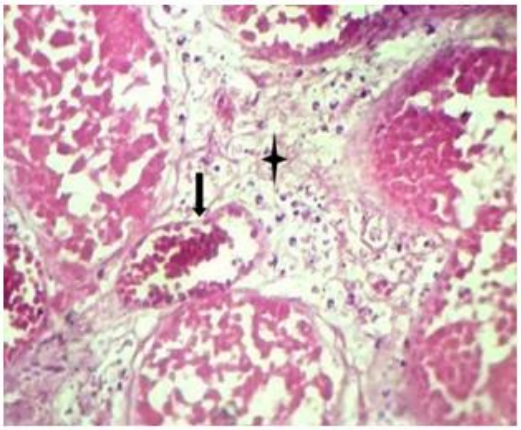

Fig.7: A photomicrograph showing interstitial edema with infiltration of neutrophils **(asterisk)** and congested intertubular blood vessels **(arrow)**, beside the hyalinized seminiferous tubules. ***H&E. X400.***

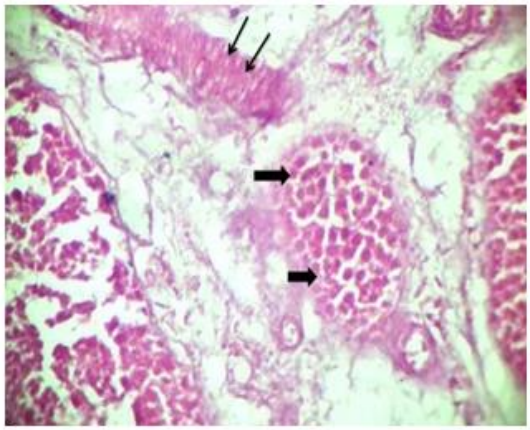

Fig.8: A photomicrograph showing interstitial edema without inflammatory cells infiltration, beside a coagulative necrosis of the germ cells and damaged tubular basement membrane **(thick arrows)** or completely atrophied tubules **(thin arrows)**. ***H&E. X400.***

Fig 9: The testicular tissue had necrotized and formation in pinhole technique

4. DISSCUSION

In the recent years, more efforts to identify reliable methods of pharmacologic and chemical sterilization for dogs, but surgical methods have remained the mainstay (Howe, 2006). The main purpose of this study was to compare between prescrotal and pinhole castration technique in male dogs which are the most common performed surgical techniques in veterinary practice.

In this study a closed prescrotal technique that was more easily performed, not requiring a lot of experience and was not time consuming this agreed with (Hedlund, 2007 and Booth, 2003) who reported that the prescrotal technique was the most common, simpler to perform and the partietal vaginal tunic had not been opened, there by minimizing risk of peritoneal contamination via the communication between the abdomen, while Okwee-Acai et al. (2012) stated that the standard surgical castration procedure (prescrotal) is tedious, requiring a lot of experience and is time consuming.

The pinhole casration used for induction of complete ischemic necrosis of the testis in dogs (Baba et al., 2013). The spermatic cord was easily manipulated within the scrotal skin so can be identify by palpation within the scrotal neck did not pose any problem (Nickel et al., 1973). Fazili et al., (2009) and Baba et al. (2013) also used silk due to its low cost and easy availability. Meanwhile, Ponvijay (2007) and Okwee - Acai et al. (2012) have used cat gut with hypodermic needle to pass easily. This technique demand more professtional skills to identify the site of spermatic cord for efficient ligation around it. In the present study, the results of post operative wound healing in all cases of prescrotal castration were almost closed, dry, clean and take short time for healing. These results were quite similar with (Misk and Seleim, 1991; Gizawiny et al., 2004 and Okwee -Acai at al., 2012). While, the most of cases of post-operative wound healing in pinhole castration either bilateral or unilateral had healing score (+) which refers to poor healing with inflamed, swollen and wet wounds with blood or pus oozing at site of needle penetration, this was because of sepsis due to introduction of infection into a scrotal cavity Searle et al. (1999) and Adin (2011). On the other hand, a clean surgical prescrotal incision healed very fast by primary intention (Harari, 2004). Another explanation to this also was that all tissue injury, including that from surgery, may cause pain with activity of dog lead to licking of wound which predispose the animal to infection (Booth, 2003 and Gizawiny et al., 2004) and induced stress responses that lead to inflammatory mediators cause delayed healing (Hellyer et al., 2007; Adin, 2011).Injection of antibiotics and anti-inflammatory drugs post spermatic cord ligation were not used in this study to effect on cortisol estimation and to reduce the cost of castration in dogs (Baba et al. 2013).

The present study revealed that there was no serious complications following prescrotal castration such as scrotal bruising, abscess, granuloma, urinary in continence and torn pedicles which may lead to ablation of scrotum after castration but these complications observed in studies of Booth (2003); Hedlund (2007); Tobias (2010) and Adin (2011).

In the present study, pinhole castration needed xylazine, ketamine, hypodermic needle, and a suture strand. Meanwhile, in cases of prescrotal technique required xylazine, ketamine, antiseptics, antibiotic, dressings and sutures (catgut and silk), making the cost of prescrotal castication three times higher than the pinhole technique that agrees with Ponvijay (2007); Fazili et al. (2009); Okwee-Acai et al. (2012) and Baba et al. (2013). In this study, we also concerned the effort of labour which was higher in pinhole technique than prescrotal technique due to long post-operative managemental care in pinhole castration (28 days), versus 10 days in prescrotal technique .

Pain is a normal response to tissue damage and inflammation which occurs with tissue injury or surgery (Anil et al., 2002 and Hellyer et al., 2007). Pain-induced stress responses, mediated by the endocrine system, which characterized

by the increase of cortisol (Hellyer et al., 2007). So assay of cortisol concentration has been used as an indicator of stress and pain in dogs (Okwee-Acai et al., 2012 and Azab, 2012). The mean serum cortisol concentrations in this study is higher at 24 hours and 5 days after castration in the bilateral pinhole cases compared with the prescrotal cases. These results don't agree with Okwee-Acai et al. (2012) found that in dogs mean cortisol levels at 5^{th} day in prescrotal technique was higher than pinhole technique but by 10^{th} day cortisol reduced more in prescrotal than pinhole technique mean cortisol levels for prescrotal were returned to normal after 20 days and faster than pinhole technique. Meanwhile in goats, showed elevation in plasma cortisol values in all cases of pinhole castration by day 7 when compared with surgical castration (Okwee-Acai et al., 2008) which was agree with this study. These results might be attributed to the pain inflicted at the time of surgery and inflammation following surgery due to trauma is major inducers of cortisol secretion (Kehlet, 1991).

In the present study, the mean testicular length and width values of some cases of unilateral pinhole at day 5 were highest due to initial scrotal swelling because of testicular edema (Ponvijay, 2007), and then subsided after 16 to 28 days, this indicated atrophy of the testicular tissue (Fazili et al., 2009). Okwee-Acai et al. (2008) also reported maximum swelling in the ligated caprine testis on day 2, then subsided at the end of one month observation period. Baba et al. (2013) stated that the mean values of testicular length and breadth on day 3 were highest and the values on day 7, 14 and 21 were significantly higher when compared to the value of day 28 which was significantly lower than that of day 0.

The histopathological finding of the testicles from the dogs subjected to the pinhole technique revealed testicular degeneration and atrophy, which is a typical condition that leads to testicular dysfunction (Awal et al., 2004). Moreover, there were marked interstitial fibrosis, degenerative and necrotic changes of the germinal epithelium, Sertoli and Leydig cells. These results are agreed with Smith (1955) who showed that dogs spermatogenesis ceases and the Sertoli and the Leydig cells also get damaged in some testicles after 4 hour of testicular ischemia induction, while 10 hour or more of sustained ischemia results in elimination of all Leydig cells and replacement of the testicular elements by fibrous connective tissue. Another detectable lesions of the testicles with spermatic cord ligation were acute ischemic coagulative necrosis of germinal epithelium and hyalinization of the seminiferous tubules, This finding has been similarly reported in previous studies of pinhole castration in bull calves, goats and dog (Ponvijay, 2007; Okwee-Acai et al., 2008; Fazili et al., 2009; Okwee-Acai et al., 2009 and Baba et al., 2013) ,this coagulative necrosis resulting from acute ischemia and it is responsible for irreversible testicular dysfunction (Bergh et al., 2001). Furthermore, this result is in complete agreement with Dixit (1977) who demonstrated that after 12 days of vascular occlusion in dogs, seminiferous tubules were replaced by an 'amorphous mass' and had no basement membrane. Another undesirable finding was detected namely interstitial edema and suppurative inflammation could be owing to infected unsuccessful spermatic cord ligation, this result was agreed with Abu-Ahmed et al (2012).

From the results of this study, it was concluded that, compared to prescrotal castration technique (closed method), the pinhole technique is a cheap and noninvasive method but it was more stressful, need more professional experience and failed to induce necrosis of the testis of dogs in situ. The prescrotal castration technique proved advantageous than pinhole technique in dogs due to they are less painful, effective and simple for male dog sterilization.

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