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Research Article

Intratesticular Injection of Calcium Chloride Is a Useful Alternative for Neutering the Male Dog

Bikash Puri [1], Manoj Kumar Shah[2*], Bablu Thakur[2], Bharat Regmi[2], Ishwor Dhakal[2]

[1]Institute of Agriculture and Animal Science, Kathmandu, Nepal
[2]Department of Surgery and Pharmacology, Agriculture and Forestry University, Chitwan, Nepal

Abstract

Nepal is a rabies endemic country where stray and community dogs are the main reservoir of this life threatening diseases. The orchidectomy is performed to control the male dog population, which is supposed to be very painful and invasive thereby not rational in context of animal welfare. Therefore present study was undertaken to study the efficacy of single intratesticular injection of calcium chloride for neutering of the male dog. Twelve apparently healthy male dogs were randomly allocated into 3 groups (n=4). Group-I received single bilateral intratesticular injection of 20% calcium chloride together with 1% lignocaine-HCl at dose determined accordingly with testicular width. Similarly, Group-II received a single bilateral intratesticular injection of sterile normal saline containing 1% lignocaine-HCl. However, the orchidectomy was performed following the standard surgical protocol in the dogs of Group-III. Histopathologically, the calcium chloride injected testicular section demonstrated dissolution of the germ cell association, atrophied seminiferous tubules and washing out of the germ cells from the tubules. In some area, there was coagulative necrosis of the seminiferous epithelium and interstitial spaces as well as degenerated and coagulated germ cells in combination with fibrous tissue. Intertubular edema, fibrosis, hemorrhage were also evident. Some necrotic cells showed desquamation or even calcification. Intertubular vessels were severely congested. There was complete loss of tubular architecture without any distinct boundary between the tubular and extra tubular compartment in some region of testis. The intratesticular injection of 20% calcium chloride did not show any apparent pain, stress, metabolic toxicity and untoward side effect. Histomorphological findings revealed the uneven distribution of damage and inconsistent affect on seminiferous tubules. Therefore, intratesticular injection of calcium chloride could be useful alternative for neutering the male dog.

Keywords: Calcium chloride; intratesticular; neutering; histopathological

Introduction

Nepal is a rabies endemic country where stray and community dogs are the main reservoir (Bhandari, 2005). In a survey conducted in Kathmandu valley, the capital city of Nepal, human to dog population ratio was found to be 1:4.7 and the stray dog density was 2,930 per km^2 (Kato *et al*., 2003). Gongal and Rai (2001) reported that 70-80% of the total dog population are stray and 90% of these stray dogs are found in the city areas of Nepal. Similarly, more than

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[2]*Corresponding author
Manoj Kumar Shah,
Department of Surgery and Pharmacology, Agriculture and Forestry University (AFU), Chitwan, Nepal
Email: mkshah@afu.edu.np / drmanojnp@gmail.com

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90% i.e. approximately 150 human rabies deaths each year in Nepal are the result of bites by unvaccinated dog (WHO, 2005). The incidence of dog bite is estimated as 8 per 1000 people (Bhandari, 2005). The population of free roaming dog in the Nepalese community is huge and has negative impact on the environmental hygiene and zoonoses, compromising public health and animal welfare.

The orchidectomy is a common surgical procedure results in reduction of dog population, and also individuals susceptible to rabies (Soto *et al*., 2007). It can also alter the behavior of male dog limiting their mobility and aggressiveness which may directly facilitate the spread of rabies (WHO, 2005). Though, surgical orchidectomy is relatively simple procedure requiring general anesthesia, surgical instruments, aseptic laboratory facilities, adequate recovery time and advance training of veterinary surgeon. This method is supposed to be very painful and invasive thereby not rational in context of animal welfare (Immegart and Threlfall, 2000). Furthermore, the cost of surgical method of neutering is also high. Therefore, the chemical neutering could be the appropriate and low cost alternatives to be used in a wide range of canine population, especially in poor countries where rabies is endemic (Soto *et al*., 2007). Several researches investigated chemical and hormonal alternatives to be implemented by practioners. The chemical is injected into testis and/or epididymides of dog to assure them as an effective (Joechle, 2004). An ideal chemo-neutering agent is yet to be validated. Therefore, the present study was undertaken to determine the effects of a single intratesticular injection of 20% calcium chloride ($CaCl_2$) dihydrate with 1% lignocaine-HCl in male dogs as an alternative of orchidectomy surgery.

Materials and Methods

Experimental Animals

Twelve stray- and owned-male dogs (N=12) admitted to Veterinary Teaching Hospital, Rampur, aged 15.33±4.65 months and weighing 18.16±5.28 kg were used in this study. The dogs were maintained without medication, considered apparently healthy and were free from any painful conditions. All protocols were approved by the ethics committee of Agriculture and Forestry University, Rampur, Nepal.

Study Design

All dogs were thoroughly examined to ascertain their health status. A total of 12 apparently healthy male dogs were randomly allocated into SAL, $CaCl_2$ and ORCH groups each consisting of 4 dogs. The dogs were sedated with Xylazine-HCl at dose rate of 2 mg kg^{-1} intramuscularly (IM). The scrotal regions of dogs were cleaned with soap and water followed by scrubbing with chlorhexidine. The dogs of SAL group received a single bilateral intratesticular injection of sterile normal saline containing 1% lignocaine-HCl. A single dose of solution containing 20% $CaCl_2$ mixed in 1% lignocaine-HCl was injected into the testicles bilaterally in the dogs of ORCH group. Each intratesticular injection was performed using a sterile tuberculin syringe with 5/8-inch 28-gauge needle directed from the carniodoral portion of testis beside epididymis without anesthesia, placing the dog in dorsal recumbency, at dosage in accordance with the testicular width (Table 1). Care was taken to prevent the leakage of solution from injection site.

However, the orchidectomies were performed in dogs of ORCH group following the standard surgical protocol. Surgery was performed on early hours of the day. Food, but not water was withheld for 12 hours prior to surgery. All dogs were clinically examined prior to premedication. Atropine Sulphate (0.05 mg kg^{-1} IM) was administered in the lumbar epaxial muscles. After intravenous catheter had been positioned into the cephalic vein, the Xylazine-Ketamine-HCl combination (1:2 v/v) a rate of 2 ml per 10 kg was injected intravenously (IV) to effect. Dogs were prepared for surgery by clipping the hair from the inguinal area and cleansing the skin with povidone-iodine scrub solution followed by painting with sprit. The surgical site was draped, and bilateral orchidectomies were performed using standard technique. The incision was closed intradermally using vicryl 2-0 to avoid the need for skin suture removal after healing. The scheme of the experiment has been summarized in Fig. 1.

Table 1: Total volume (ml) for of $CaCl_2$- and normal saline solution intratesticular injection according to testicular size (mm)

Testicular size (mm)	Chemical volume (ml)
12-17	0.3
18-23	0.7
24-27	1.0
27 or more	1.5

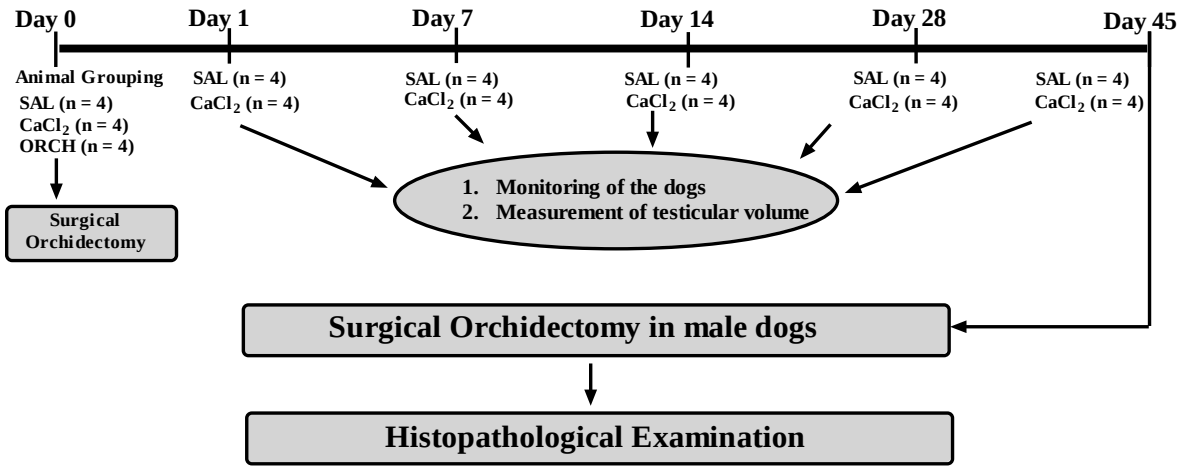

Fig. 1: Scheme of the experiment

Monitoring of the Dogs

Dogs that received intratesticular injection of $CaCl_2$ and normal saline mixed in 1% lignocaine-HCl were released immediately after injection (Fig. 2A). However, orchidectomized dogs were housed in kennel and handed to their owners on next day of surgery. No any measures were undertaken to confine the dog or to prevent licking and inflicting of the incisions or injection sites. Routine clinical examination was carried out on 1,7,14, 28 and 45 days after chemical and surgical orchidectomy. Parameters like body weight, general attitude, appetite, ability to walk, temperature, heart rate, respiration rate, scrotal changes (pain, swelling and dermatitis), etc were evaluated twice a day up to 3 days and thereafter weekly until the end of the study.

Collection of Testis

Both testes were collected after 45 days of intratesticular injection by performing orchidectomy surgery as described previously.

Measurement of Testicular Volume

Length and width of the right and left testicles were measured using vernier calipers (Fig. 2B), and the volume of each testis was quantified by multiplying the length (cm) by width (cm^2) by 0.524. Total testicular volume (cm^3) was calculated by adding the volume of the right and left testis as described in an earlier study (Jana and Samata, 2011).

Histopathological Examination

Entire testis was fixed in Bovin's fixative and embedded in paraffin wax for histopathological studies. A section of 5-µm thick was cut from the middle portion of each testis, stained with hematoxylin-eosin and examined under light microscopy at 20× magnifications. The structure of seminiferous tubules and interstitial spaces in testis was observed microscopically.

Statistical Analysis

Data are expressed as the mean ± SD. Statistical analysis was performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance followed by Student's *t*-test was used to compare the changes in testicular volume of the $CaCl_2$ and saline-injected dogs. A difference was accepted as significant if *P* was less than 0.05.

Results

Every dog survived in a state of good health throughout the experiment. Routine clinical examination was performed for 45 days after chemical neutering and surgical orchidectomy. The dogs of Group-I and II did not show any remarkable changes in body weight, general attitude, appetite, gait, temperature, heart rate, respiration rate as compared to dogs of Group-III. None of the $CaCl_2$-treated dog exhibit severe complication except for a slight increase of firmness of a testis on palpation. Testicular swelling was evident in every treated dog by 24 hrs following injection. Testicular swelling was greatest in treated animal at 72 hrs but gradually start to subside after 4^{th} weeks (Fig. 2D). In two dogs, there were edema and swelling of the scrotum (Fig. 2C).

However, there was no change in behavior, appetite, mobility and other clinical parameters in $CaCl_2$-treated dog but surgically castrated dogs were depressed and showed lack of appetite for the first 2 days. On recovered testes there was decrease in the volume of the testes as well as suppuration at the site of chemical deposition.

Gross Morphological Changes in Testes

Grossly, the saline-injected testis showed no apparent morphological changes (Fig. 3A-B). However, $CaCl_2$-injected testis showed atrophy and suppuration in the testicular parenchyma (Fig. 3C-D).

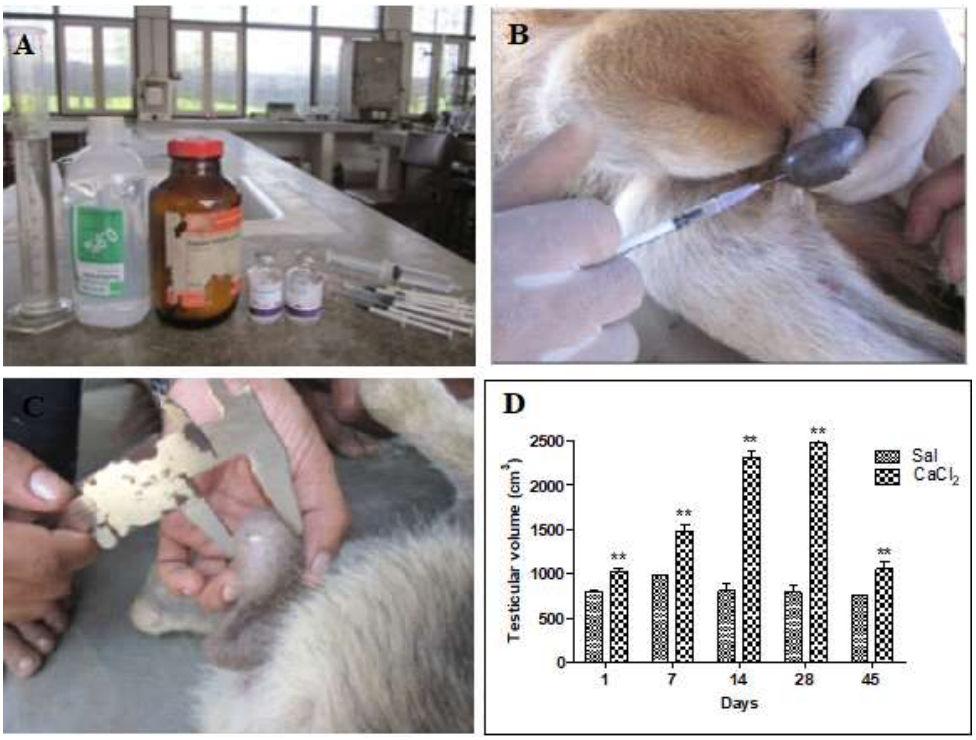

Fig 2: Preparation of 20% $CaCl_2$ solution (A); Intratesticular injection of calcium chloride (B); Measuring the testicular volume (C) and Change in the testicular measurement (D)

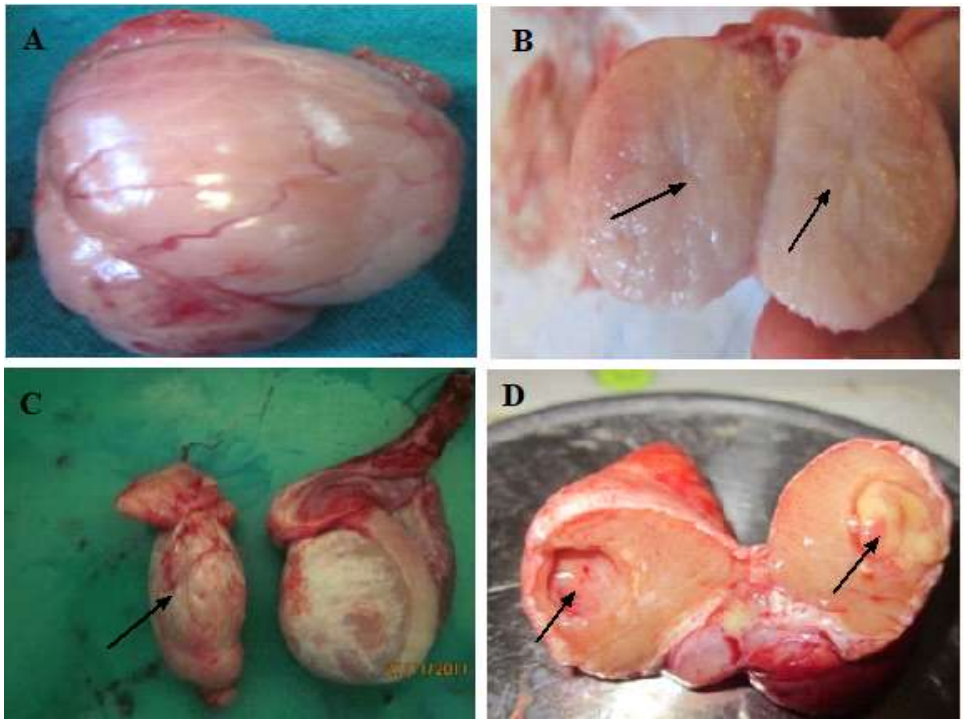

Fig. 3: Morphological changes in saline and calcium chloride injected testes of dogs. Gross appearance of saline injected testes **(A)**; Cross section of saline injected testis (**B)**; Reduced testicular volume in calcium chloride injected group **(C)** and Suppuration of testicular parenchyma **(D)**

Histopathological Changes in Testes

Normal saline injected testis revealed distinct interstitial spaces containing normal amount of connective tissue and interstitial leydig cells (Fig. 4A). However, $CaCl_2$-injected testis demonstrated dissolution of the germ cell association and atrophy in seminiferous tubules of some area and washing out of the germ cells from the tubules (Fig. 4B). The induced damage was not distributed evenly and tubules were affected inconsistently. In some area there was coagulative necrosis in the seminiferous epithelium and the interstitial spaces as well as presence of degenerated and coagulated germ cells in combination with fibrous tissue in tubular and interstitial spaces.

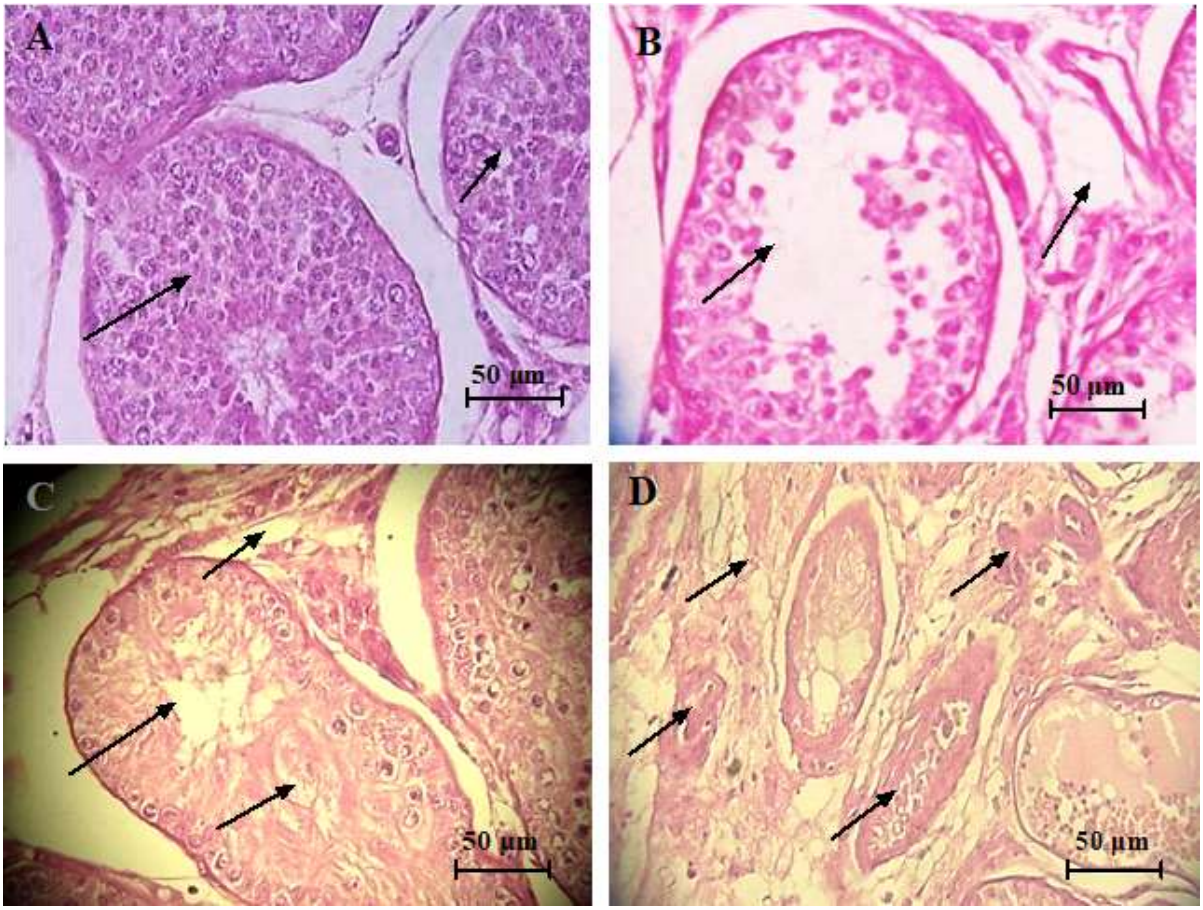

Fig. 4: Histopathological appearance of saline and calcium chloride solution injected testicles of mature male dogs after 45 days. Normal arrangement of germ cells in seminiferous tubules with distinct interstitial spaces of saline injected testis **(A)**; Washing out of the germ cells from the tubules of calcium chloride injected testes **(B)**; Eosinophilic infiltration in the seminiferous tubules together with intratubular edema and fibrosis in the calcium chloride injected testes **(C)** and Dissolution of the germ cell association and atrophy in seminiferous tubules and eosinophilic infiltration in the tubules and congestion of major vessels in calcium chloride injected testes **(D)**.

Intertubular edema, fibrosis, eosinophilic infiltration and hemorrhage were also evident (Fig. 4C). Some of the necrotic cells showed desquamation or even calcification. Intertubular vessels were severely congested. In some area there was complete loss of tubular architecture without any distinct boundary between the tubular and extra tubular compartment (Fig. 4D). Similarly, some inflammatory cells were also present in interstitial spaces.

Discussion

A variety of chemical sterilant has been developed for injection into the testis and/or epididymides of dogs, which were either safe but not effective, or vice versa (Joechle, 2004). Surgical orchidectomy is the most stressful and painful moment to the animals. However, the use of intratesticular injection of $CaCl_2$ is simple, quick and well accepted by the dogs and rarely shows signs of pain. This may be due to afferent nerve ending associated with pain sensation being located only on the scrotal skin and in the capsule of testes rather than within the testicular and epididymal parenchyma (Jana and Samata, 2011). Moreover 1% lignocaine-HCl has also been used to subside the pain.

Histological findings revealed dissolution of germ cells association and washing out of germ cells from the seminiferous tubules with distinct tubular compartment with respect to extra tubular compartment. This effect was consistent with findings in cat, using 5% $CaCl_2$ solution containing 1% lignocaine-HCl following 60 days after intratesticular injection (Jana and Samata, 2011). In some place there was complete loss of tubular architecture. Similar finding was reported in cat after infiltration of 20% $CaCl_2$ following 60 days after intratesticular injection (Jana and Samata, 2011). However, there was coagulative necrosis in the seminiferous epithelium and interstitial space as well as presence of degenerated and coagulated germ cells at other place as reported in cat (Jana and Samata, 2011). These histopathological changes were more severe in areas closest to the site of injection; this could be related to necrotizing property of $CaCl_2$ (McGinnis *et al*., 1999) but less severe morphological changes distal to the site of injection leading to uneven distribution of damage and inconsistent affect on tubules. Under this condition some viable testicular tissue remains or regenerate and may continue secretion of testosterone, although at lower level (NADA, 2003).

The lack of complete testosterone suppression following treatment with calcium chloride can be considered either a positive or a negative feature. One purpose of bilateral orchidectomy is to suppress undesirable aggressive and sexual behaviors and medical conditions associated with testosterone production. However, next reasons noted by many dog owners for opposing bilateral orchidectomies are anthropomorphic empathy regarding emasculation and a fear that desired behaviors such as protection and decreased hunting instinct (Blackshaw *et al*., 1994; Soto *et al*., 2005). Thus, preservation of testosterone production and presence of testes following castration may be viewed as desirable by these owners. Presence of inflammatory cells in the interstitial space after $CaCl_2$-injection may be due to testicular degeneration that releases chemotactic factor responsible for ingression of inflammatory cells. Similarly, there was swelling of testes in $CaCl_2$-treated dogs after 24 hrs that reached maximum within 15 days and thereafter gradually subsided as reported in other animals (Tepsumethanon *et al*., 2005; Soto *et al*., 2007; Jana and Samata, 2011). Injection of irritant chemicals often leads, swelling of testes resulting from an inflammatory reaction (Okwee *et al*., 2008). In some testes there was formation of abscess which may be due to failure of full absorption of injected calcium chloride, thereby causing excessive irritation and abscess formation (Al-Ismail *et al*., 2002; Okwee *et al*., 2008).

Conclusions

The histomorphological findings revealed uneven distribution of damage and inconsistent affect on tubules due to slow damaging effect of $CaCl_2$. Additional studies are necessary with a larger animal population and for a longer observation period to verify sterility and absence of fertility recovery.

Acknowledgements

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Competing Interests

The authors declare that they have no competing interests.

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