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Evaluation of Spermatic Cord Ligation as an Alternative Method of Castration in Dogs

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ABSTRACT

The study evaluated open spermatic cord ligation as an alternative method of castration in dogs. Three Nigerian indigenous puppies were randomly selected (identified as SL1, SL2 and SL3) and used for the study. They underwent the same surgical procedure. Blood samples of the experimental puppies were taken daily for 3 days, pre-surgery. Additional blood sampling was at 24 hrs post-surgery, and, subsequently at 48 hrs interval for 3, 2, and 1 week(s) in SL1, SL2, SL3 respectively. Sera samples were harvested from the blood samples. At weeks 3, 2 and 1, the testicles of the puppies were harvested for macroscopic and microscopic evaluations. The puppies were physically examined daily throughout the study period. Serum testosterone concentration of the puppies was determined using ELISA kit. The pre-surgical values ranged from 0.1ng/mL-0.5ng/mL before the surgery and declined to 0ng/mL 24 hrs post-surgery. Gross appearance of the testicles showed atrophy of the testicles. Testicular histopathology showed distortion of germ and supporting cells due to ischaemic necrosis, which was more evident in SL1 followed by SL2, and then SL3. It was concluded from this study that the procedure was effective, minimally invasive, simple and fast.

Key words: Spermatic cord ligation, Castration, Evaluations, Dogs

INTRODUCTION

Dog owners present their pets for neutering due to several reasons among which is overpopulation, a serious problem in many developing countries including Nigeria despite local efforts to control population growth (Ortega- Pacheco, 2006). In these developing countries, free roaming dogs are sources of ecological and social problems because they attack other animals and people, they cause road accidents, frighten the public and contaminate the environment with urine and faeces (Ortega- Pacheco, 2006), which could serve as vehicles for disease transmission, since most of these dogs may not have medical records. In addition to the above-mentioned problems, free roaming dogs are source of serious public health hazards by transmission of diseases through their saliva, feces and urine to humans and other animal species (Slauson and Cooper, 2002).

Moreover, the beneficial effect of castrating male dogs, apart from decrease in the birth of unwanted litters, is the reduction in the testosterone levels which in turn minimizes the risk of developing prostatic disease and the avoidance of undesirable male behaviors such as roaming, aggregation, mating and urine marking (Neilson *et al*., 1997; Kim *et al*., 2006). In situ spermatic cord ligation (pinhole castration) has been described as a novel minimally invasive technique for calf and kid sterilization (Ponvijay, 2007; Okwee-Acai *et al*., 2008) and stray dogs (Baba *et al.,* 2013).

Some researchers reported that, the pediatric spay offer added advantage of the procedure being easier, faster and less expensive than in adult animals with shorter surgery times, shorter anesthetic episode, less incidence of peri-operative complication, fast anesthetic recovery and shorter healing time than in the adult (Buhby and Griffin, 2011).

There is growing concern about surgical castration which is associated with complications such as wound dehiscence, haemorrhage, infections and scrotal swellings (Abd El- Wahed *et al*., 2014). These necessitates the need for development of alternative methods of castration which would be simple, cheap, fast, effective with little or no complication and acceptable to the pet owners (Höglund *et al*., 2014; Ajadi and Gazal, 2016). In this regard, the present study evaluated open spermatic cord ligation as an alternative to conventional method of open castration in dogs,

MATERIALS AND METHODS

Study area

The research was conducted at the Small Animal Surgery unit of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria ($11^0$10'N, 07038'E), located in the Northern Guinea Savannah Zone of Nigeria.

Experimental Animals and Management

Three male Nigerian Indigenous puppies weighing between 3-5 kg and aged three months served as experimental subjects. They were housed in the kernel of the Veterinary Teaching Hospital of Ahmadu Bello University, Zaria, where they were acclimatized for two weeks prior to the commencement of the research. During the acclimatization period, the puppies were examined clinically, and samples (blood, feces) taken for laboratories analyses (and treated accordingly). They were fed with maize, beans, blood meal and rice, cooked with palm oil and salt. Water was provided *ad libitum*.

Experimental Design

The three puppies were randomly selected, identified (Table 1) and underwent similar Open spermatic cord ligation (2 points ligation at 1cm interval) and blood (sera) samples collected as in (Table 1)

Pre-surgical Considerations

Blood samples were collected from each of the puppies three days prior to surgery, in sample bottles containing ethylene-diamine tetraacetate (EDTA) for haemogram, to ascertain the fitness of the puppies for the procedure. The puppies were withheld from food and water for a period of 12 and 6 hours respectively. In preparation for surgery, the ventral pelvic region (from the prepuce extending to the perineum including the medial aspect of the thigh, 10cm on either side of the ventral midline) of each puppy was shaved and scrubbed-clean with soap and water; and the skin aseptically prepared for surgery using 0.05% chlorhexidine gluconate.

Anaesthesia

Each puppy was pre-medicated using Atropine sulphate (AMOPIN_Yanzhou Xierkangtai Pharma. Co., Ltd, China) at 0.05mg/Kg and Chlorpromazine hydrochloride (Clomazine-Maxheal Pharmaceuticals, Karnal, India) at 4mg/Kg intravenously. Ketamine hydrochloride (ketamine-Rotex Medica Trittau, Germany) at a dosage of 11mg/Kg intramuscularly was used for both induction and maintenance anaesthesia. In addition, 2% lignocaine hydrochloride solution (Pauco-Kwality Pharmaceuticals PVT. Ltd, India) was infiltrated locally (linear infiltration) on either side of the proposed line of incision (pre- scrotal) at 0.4ml/cm. Each puppy was placed on dorsal recumbency with the fore- and hind-limbs extended and restrained on the surgical table. The surgical site was prepared aseptically using povidone iodine solution and draped in triangular pattern. Aseptic conditions were strictly adhered to in all cases and throughout the procedures.

Surgical Techniques /Procedure

The testicles were accessed via pre-scrotal mid-ventral skin incision as described by Hassan and Hassan (2003). The skin incision made was 1 cm length, which was 1cm away from scrotal raphae (Figure 1). The testicles were popped out singly through the incision and exteriorized completely along with their spermatic cords (Figure 2). An Incision (1 cm) was made over the parietal vaginal tunic covering the spermatic cord to separate the parietal vagina tunics from the spermatic vascular supply and ductus deferens. Two-clamp technique (1 cm in between) was performed on exposed the spermatic vasculature and ductus deferens (in situ) using two circumferential ligatures with chromic catgut size 2/0 for each testicle (Figure 3). The testicles were returned to their normal anatomical location. A subcuticular closure of the pre-scrotal incision using Chromic catgut size 2/0, was applied to appose the skin incision.

Table 1: Sampling period pre- and post-surgery of the puppies involved

Dog identity	Sampling
SL1	Sera was collected daily for 3 days pre-surgery and for days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 post-surgery for testosterone assay. The testes were harvested at day 21 post-surgery for gross and histologic evaluation.
SL2	Sera was collected daily for 3 days pre-surgery and for days 1, 3, 5, 7, 9, 11, 13 post-surgery for testosterone assay. The testes were harvested at day 14 post-surgery for gross and histologic evaluation.
SL3	Sera was collected daily for 3 days pre-surgery and for days 1, 3, 5, 7 post surgery for testosterone assay. The testes were harvested at day 7 post-surgery for gross and histologic evaluation.

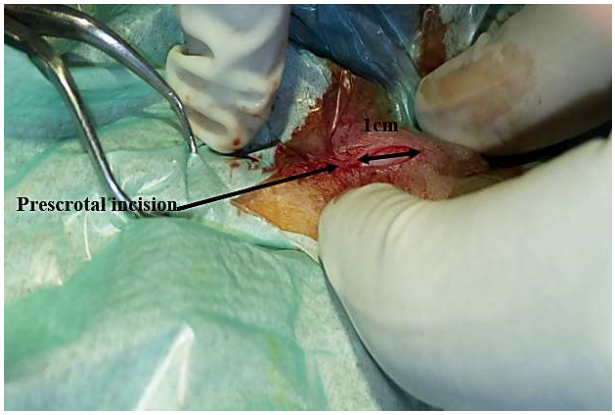

Figure 1: Pre-scrotal incision 1 cm away from the scrotal rapha.

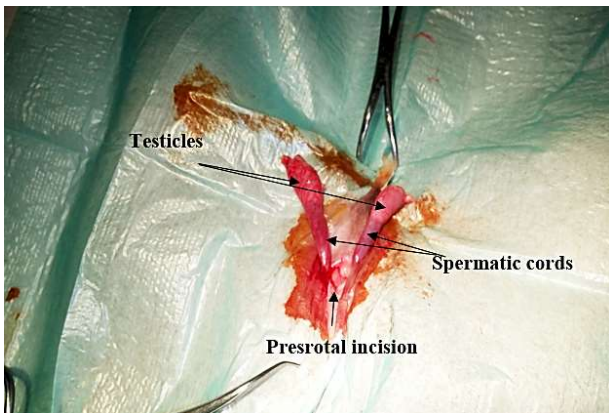

Figure 2: Exteriorized testicles along with their spermatic cords through the pre-scrotal incision.

Post-surgical Care and Evaluation

An Elizabethan collar was applied and attached to the collar belt (improvised) on each of the puppies to prevent interference with the surgical site. Water and food were provided immediately after recovery from anaesthesia. The puppies were monitored daily post-surgery by physical examination(with vital parameters) and all findings were recorded. The surgical site was cleaned daily with mild antiseptic (chlorhexidine) solution. Pain was managed by administering paracetamol (25 mg/kg) intramuscularly for 3 days.

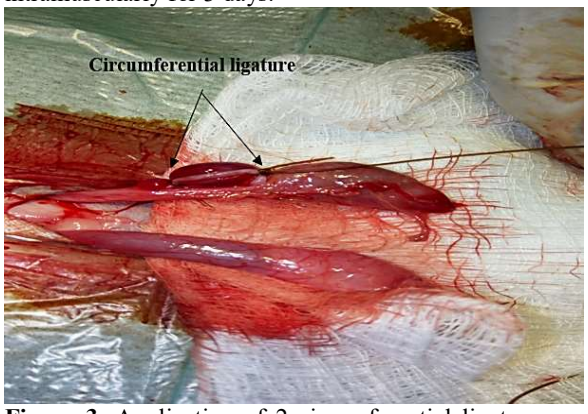

Figure 3: Application of 2 circumferential ligatures on the exposed spermatic vessels and Vas deferens

Collection of blood sample

Blood sample (2ml) collected through cephalic venipuncture was used to harvest serum. The blood was aseptically collected from each of the puppies in plain sample bottles. Serum was harvested from each of the blood 24hrs after its collection. For each of the puppies three sera samples were collected daily for 3 days prior surgical procedure to obtain baseline serum testosterone. The sera were also collected 24hrs post operatively and continued at 48hrs interval to 1, 2 and 3 weeks for SL3, SL2 and SL1 respectively. The sera samples harvested in plain bottles were stored at -20^{0}C and later used for testosterone assay using an enzyme-linked immuno-sorbent assay (ELISA) according to manufacturer's instruction**.**

Testicular Harvest for Histologic Examination

The testicles from each puppy were surgically harvested at the end of the week one, two and three, post-spermatic cord ligation for SL3, SL2 and SL1 respectively, using conventional method of castration and fixed in 10% buffered formalin solution for routine histological evaluation.

Ethics Committee Approval

The study protocol was approved by the Faculty of Veterinary Medicine Animal Care and Use Committee, Ahmadu Bello University, Zaria. (FVM/ABU2017/08).

RESULTS

Clinical Evaluation

The surgical procedure (skin incision to closure) took 12.0 minutes averagely to be performed and the puppies responded well to the procedure. Physiological parameters were used to assess surgical stress. Apparently increased activity and recovery from stress was observed on third day after surgery**.**

Physical Examination of the Puppies

The daily vital parameters of the puppies were measured and recorded, which fluctuate within the reference value (Temperature 38.5-39.4^{0}C, pulse rate 90-120 b/m, respiratory rate 15-30c/m) throughout the period of observation. No post-surgical complication was observed throughout the period of study.

Testosterone Assay

The serum testosterone concentration of the puppies ranges from 0.1ng/mL – 0.5ng/mL pre-surgery. Complete cessation of testosterone production was demonstrated 24 hours post spermatic cord ligation in all the puppies throughout the period of study (Figure 4).

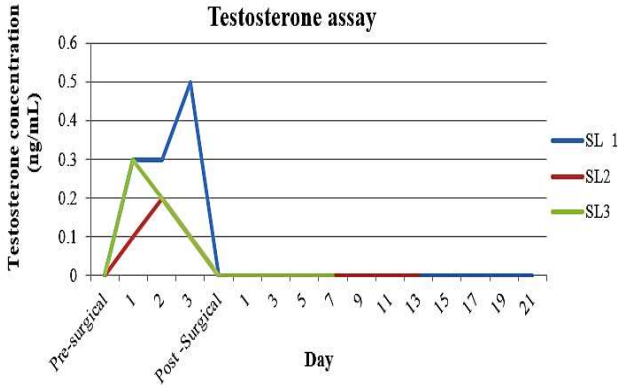

Figure 4: Graph showingtestosterone concentration pre- and post-surgery

Testicular Examination

Grossly, all ligated testes were atrophied which was more prominent in SL1, followed by SL2 and the least in SL3 as shown in Figure 5. Histopathological examination of the preserved testicles revealed testicular degeneration and atrophy. As shown in Figures 6, 7 and 8, there was distortion of germ and supporting cells due to ischaemic necrosis because of spermatic cord ligation which was more evident in SL1.

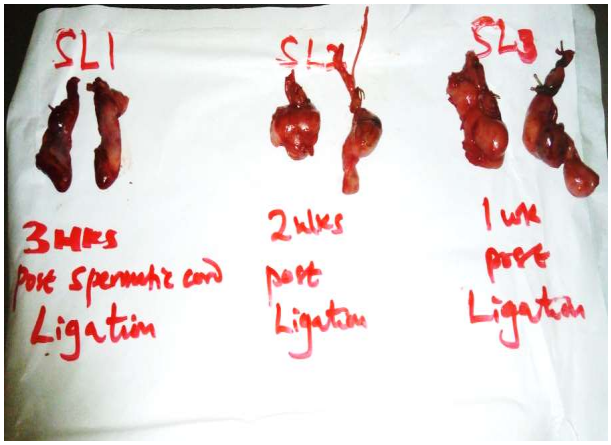

Figure 5: Photograph showing atrophied testicles harvested at day 21, 14 and 7 post-surgery respectively. The testicular size decreased in the following order: SL1<SL2<SL3.

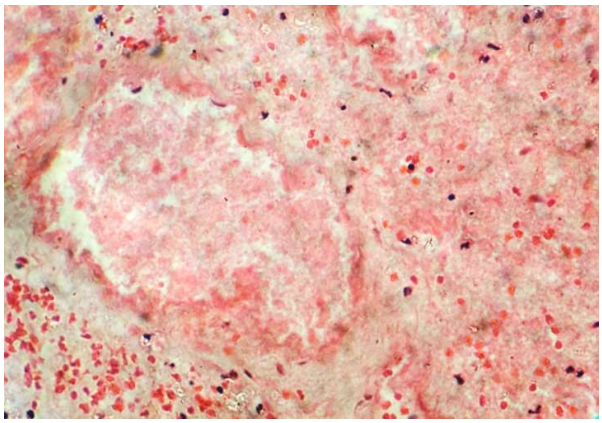

Figure 6: Photomicrograph of the testis showing extensive fibrosis of the testicular tissues in SL1, Haematoxylin and Eosin x 400

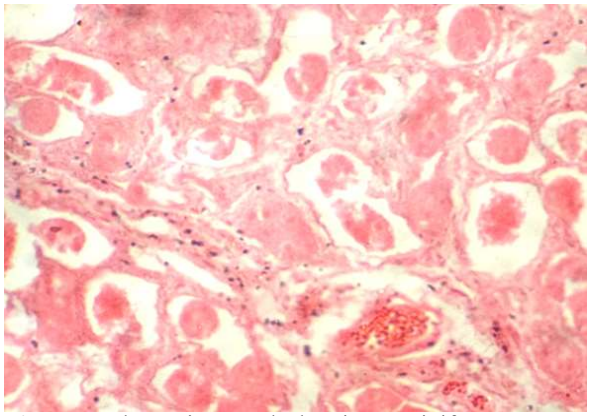

Figure 7: Photomicrograph showing seminiferous tubules with necrotic junks in the center in SL2, Haematoxylin and Eosin x 100

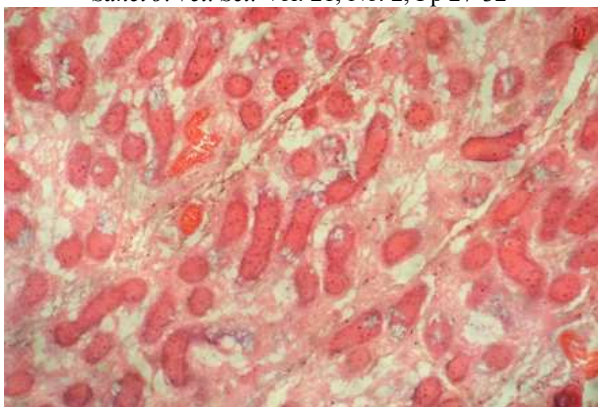

Figure 8: Photomicrograph showing necrotic and haemorrhagic seminiferous tubulesin SL3, Haematoxylin and Eosin x 100

DISCUSSION

The surgical procedure (skin incision to closure) took 12.0 minutes averagely to be performed. An average of 14 minutes was reported by Okwee-Acai *et al*. (2012) in their studies of *In situ* spermatic cord ligation in puppies. However, an average of 1-1.5minutes was reported by (Ponvijay, 2007; Fazili *et al*., 2009; Baba *et al*., 2013) in their studies of percutaneous spermatic cord ligation in calves, rams and adult dogs respectively. The differences in time taken could be because of variations in species, age and method of spermatic cord ligation used. Compared to conventional standard orchidectomy, the mean time required may take thrice longer than this procedure (Okwee-Acai *et al*., 2012). This supports the widely held concern that surgical castration is tedious; requiring a lot of skills/experience and is time consuming (Jana *et al.*, 2005). Hence, the present study appeared faster and could be employed in large castration campaigns, especially for stray dogs.

The findings of the present study indicated that all ligated testes were atrophied. Similar findings were documented by Ponvijay (2007) in bull calves and Okwee-Acai *et al.* (2008) in goats. Testicular atrophy observed in this study is typical of conditions that lead to degeneration or dysfunction of the testes. This supports the observation of Awal *et al*. (2004).

The evidence of coagulative necrosis observed in the present study resulting from acute ischemia is responsible for irreversible testicular dysfunction. This corroborates the findings of Bergh *et al*. (2001) in adult rats. Again, similar finding in rats showed that acute testicular ischemia for as little as five hours is sufficient to produce this effect (Turner and Brown, 1993; Bergh *et al*., 2001).

The necrosis of the germinal epithelium and supporting cells along with interstitial fibrosis noticed in the testicular tissue are valid indications suggestive of a successful castration as was similarly reported by Abu-Ahmed *et al.* (2012) in their study of comparative evaluation of three *In situ* castration techniques for sterilizing donkeys. Again, degenerative changes with accumulation of necrotic material within the lumen of seminiferous tubules was also observed by Baba *et al.*

(2013) in dogs.Inanother study in dogs, 12 days of vascular occlusion resulted in seminiferous tubules replaced by an 'amorphous mass' and had no basement membrane (Dixit, 1977).

The findings on the level of testosterone post-surgery in this study agrees with the observation of Polisca *et al.* (2013), who reported that decrease or absence of testosterone levels in male dogs were observed in neutered, hypothyroidism, sertoli cell tumors and with exogenous use of deslorelin.

Conclusion

This study demonstrated that the procedure is minimally invasive, simple, fast and effective as oppose the conventional open castration in dogs which is associated with various complication such as excessive haemorrhage, wound dehiscence and infection, surgical adhesion and scrotal swelling. In addition, complete cessation of testosterone production 24 hrs post spermatic cord ligation and ischemic necrosis of the germ and supporting cells are valid indicators suggestive of a successful castration.

Conflict of Interest

The authors have no conflict of interest to declare.

Author's Contribution:

YFL and MST contributed to the designing, analysis of data, drafting and final approval of the work. BA, SRU and IML contributed to collection of blood samples, analysis of data drafting and final approval of the work.

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