Surgical Sterilization of the African Giant Pouched Rats

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SUMMARY

Surgical sterilization of rodents is increasing as a result of increased biomedical research centers which employ rodents. In order for them to perform their duties effectively, the rodents have to be sterile to avoid interference from estrus, pregnancy and lactation. Other reasons include control of breeding, treatment of diseases or conditions such as tumors cysts, pyometra, pyometritis, and endometritis. In this study three surgical procedures were performed to African Giant Pouched Rats; 6 females were ovariectomized, 6 females were ovariohysterectomized, and 6 males were Orchidectomized. All eighteen (18) animals in the study recovered well and have returned to APOPO to continue with the training on smelling and detecting landmines and tuberculosis in human sputum samples without any difficulties. Complications from these surgical procedures are rare but comprise anesthetic overdose, dehydration, hypothermia, pulmonary hypostatic congestion, hemorrhage, eviscerations, wound dehiscence, and infections. However, many of these complications can be prevented by careful physical examination and selection of health animals fit for the intended surgical procedure, as well as adherence to all principles of surgery. This study has demonstrated the three surgical procedures for African Giant pouched rats as being useful to veterinarians in public/private practice and/or in biomedical research facilities.

Keywords: Surgical sterilization, ovariectomy, ovariohysterectomy, orchidectomy, African giant pouched rats.

INTRODUCTION

As the rodents continue to become popular pets (Wenger, 2012) and their increased uses in biomedical research laboratories, as also recently rats are used to detect tuberculosis in sputum samples (Poling *et al*., 2010; Mahoney *et al*., 2013), there is a need to ensure their availability as well as control of their behavioral characteristics. Recently, rats have been trained to detect landmines (Poling *et al*., 2011). In order to exploit the full usefulness of these animals, they must be spayed or neutered to reduce their aggressive behavior. Surgical sterilization is the oldest and safest method employed for neutering and spaying (Stubbs, 1995). Pre-operative assessments with intra-operative and postoperative monitoring are critically important to the success of the surgery. The surgical procedures performed include ovariectomy and ovariohysterectomy for females; and orchidectomy (castration) for males.

Surgical sterilization is a procedure that when done leave a male or female animal unable to reproduce (MacKenzie, 2010). In female animals the procedure is called "spaying" which involve opening into the abdomen (laparotomy) followed by surgical removal of both ovaries, uterine tubes and the uterine body including a part of the cervix (overiohysterectomy). Tubal ligation (close of fallopian tubes) and removal of ovaries (ovariectomy) in females can be done to attain sterilization too. In males the procedure is known as

castration that is the removal of both testicles (orchidectomy), and deferentectomy where the ductus deferens is cut and closed (Looney *et al*., 2008).

The main indications for surgical sterilization of animals are to control breeding, and genetic diseases, as well as prevention and/or treatment of reproductive diseases (Richardson and Flecknell, 2006; MacKenzie, 2010; Capello, 2011). The most common encountered reproductive diseases and/or conditions are tumors, cysts, pyometra, endometritis, and metritis. Castration for instance prevents and/or treats a number of testicular diseases (MacKenzie, 2010) and testosterone-enhanced medical conditions. Testosterone is responsible for many male-animal behavioral traits that some owners find challenging for example mounting, roaming, aggression, inter-male aggression, urine marking, territorial marking, dominance, and leg cocking (Jenkins, 2000). Castration, by removing the source of testosterone, may help to resolve and modify the male animal behavior. Recently, there is increased demand to sterilize animals used in biomedical research in order to ensure that animals are available for duty without interference from physiological process such as estrus, pregnancy, and lactation (Olfert *et al*., 1993; Sharp and Villano, 2012). Moreover, with the attractiveness of rodents and rabbits as pets, more and more veterinarians are expected to perform elective surgical procedures for sterilization to reduce the aggression of these animals.

The surgical procedures are done while the patients are under general anesthesia or sedation and local analgesia can be opted in males. The anesthetic protocol depends on a number of factors including animal species, availability of anesthetic agents and equipment (Hoogstraten-Miller and Brown, 2008). The main objective of this study was to demonstrate appropriate surgical procedures for sterilizing male and female rats. In this study African giant pouched rats were used as model animals.

MATERIALS AND METHODS

Animals and experimental design

Eighteen adult rats (12 females and 6 males) weighing between 600 and 900 g were used in this study. Rats were obtained from SUA-APOPO Rodent Research Project. The present study was approved by Ethical Committee for Animal Studies of Sokoine University of Agriculture. Prior to surgery, the animals received clinical examination, blood testing (complete blood count and serum biochemistry profile) and examination of the abdomen using ultrasonography, to assess the condition of the reproductive tract and ensure the rats were fit for surgery. The surgical procedures performed included ovariectomy (n = 6), ovariohysterectomy (n = 6) and castration (n = 6).

Preparation of the animal prior to surgical procedure

Proceeding to operation, animals were fed a small amount of food 12 hours before operation and only allowed to access water freely. Body weights were measured and used for anaesthetics dose calculations. The rats were sedated by intramuscular injection of 5.0 mg/kg xylazine hydrochloride. Surgical site was aseptically prepared by shaving the hairs around the surgical sites, cleaned and then disinfected with chlorhexidine gluconate, 70% alcohol and finally painted with 2.5% iodine tincture. The animals were anesthetized with intramuscular injection of 50.0 mg/kg

ketamine hydrochloride as previously described by Hall and Clarke, (1991).

Ovariectomy Procedure

Laparotomy procedure was performed through ventro-midline abdominal approach using surgical blades number 24 (GMH, IMP. Exp. Corp., China). The incision started 1 cm below umbilicus, running towards the anterior border of the pubis. Two centimeters incision was made starting with the skin through subcutaneous tissues; then using rat-tooth forceps, the linea alba was grasped in the middle and tented up followed by a stab incision using surgical blade to the linea alba and the peritoneum. Finally, a pair of scissors i.e. blunt on one side was used to extend the incision.

The reproductive organ (uterine horns and uterus) were located using spaying hook; one of the uterine horns was then followed cranially up to the ovary, which is located in the fat-filled ovarian bursa. The ovary was identified, grasped and two hemostatic forceps were placed. The ovary was sub-exteriorized from the abdomen to facilitate placement of a ligature as close as possible to the root of the pedicle to ensure hemostasis of the ovarian artery.

The catgut suture 2/0 was used to ligate the ovarian pedicle as close as possible to the lumbar wall. Another ligature was placed around the cranial portion of the uterus including the uterine vessels. The clamps were then placed between the two ligatures and the pedicle was sectioned between the two to remove ovary and oviduct. The efficiency of the hemostasis was checked once the ovarian pedicle has been sectioned and when satisfied that there was no bleeding the ovarian pedicle was released. The second horn was located and the corresponding ovarian bursa grasped with hemostatic forceps and the same procedure was repeated as in the first ovary, and then stumps were returned to the abdominal cavity before closure of the abdomen.

The incisions were closed by simple continuous sutures using surgical gut 3/0 (Vista Care Co., Ltd, Province, P.R. China) for the peritoneum, linea alba and subcutaneous tissues while the skin was closed by silk 3/0 (Wuxi Medical Instrument Factory, Jiangsu, China) interrupted horizontal mattress. After recovery from anesthesia, the rats were taken back to their respective cages and wound healing progress was monitored daily for 2 weeks.

Ovariohysterectomy Surgical Procedure

Laparotomy procedure was performed through ventro-midline abdominal approach using surgical blades number 24 (GMH, IMP. Exp. Corp., China). The incision started 1 cm below umbilicus, running towards the anterior border of the pubis. Three centimeters incision was made to go through the skin and subcutaneous tissues;, then using rat-tooth forceps, the linea alba was grasped in the middle and tented up followed by stab incision using surgical blade to the linea alba and the peritoneum. A pair of scissors i.e. blunt on one side was finally used to extend the incision.

The reproductive organs (uterine horns and uterus) were located using spaying hook; one of the uterine horns was then followed cranially up to the ovary, which is located in the fat-filled ovarian bursa. The ovary was identified, grasped and two hemostatic forceps were placed. The ovary was sub-exteriorized from the abdomen to facilitate

placement of a ligature as close as possible to the root of the pedicle to ensure hemostasis of the ovarian artery.

The catgut suture material was used to ligate the ovarian pedicle as close as possible to the lumbar wall. A clamp was then placed between this ligature and the ovary, and the pedicle was sectioned between the two. The ovarian pedicle was held throughout this procedure with Allis forceps. The efficiency of hemostasis was checked once the ovarian pedicle has been sectioned and when satisfied that there was no bleeding the ovarian pedicle was released. The second horn was located and the corresponding ovarian bursa grasped with hemostatic forceps and the same procedure was repeated as in the first ovary. Finally, the two uterine horns were followed caudally until the cervix was located, followed by ligation of the body of cervix together with uterine arteries and veins. Then the cervix was sectioned and removed together with the ovaries and uterus. Hemostasis was checked and all the ligated stumps were returned to the abdominal cavity before closure of the abdomen.

The incisions were closed with simple continuous sutures using surgical gut 3/0 (Vista Care Co., Ltd, Province, P.R. China) for the peritoneum, linea alba and subcutaneous tissues while silk 3/0 (Wuxi Medical Instrument Factory, Jiangsu, China) was used to close the skin by interrupted horizontal mattress pattern. After recovery from anesthesia, the rats were taken back to their respective cages where wound healing progress was monitored daily for 2 weeks.

Orchidectomy Surgical Procedure

The surgical procedure involved making an incision of 2 cm into the skin just ahead of the animal's scrotal sac, on the scrotal raphe by using surgical blades number 24 (GMH, IMP. Exp. Corp., China). Caution was taken to ensure that the urethra is not accidentally cut into during this incision. The testicle and tunica vaginalis was elevated through the incision in the skin. Both testicles were removed through the single incision. The first testicle was pushed forwards towards the incision in the animal's skin. The fat surrounding the testis and tunica vaginalis was trimmed away and the testis (enclosed within the thick, capsule-like tunica vaginalis) was lifted through the incision in the animal's skin. The tunica vaginalis was incised and the testicle exposed. The testicular blood vessels and sperm ducts were ligated using surgical gut 3/0. Two ligatures (one circumferential and another one trans-fixation) were placed on each of the spermatic cord for security reason. The ligatures were tied tightly to ensure that the blood vessels supplying the testes were fully occluded. Then the testicle (testis and epididymis) was severed above the level of the ligatures and discarded. The same was repeated for the opposite testicle. The tunics were sutured by simple continuous suture using surgical gut 3/0 to avoid intestinal evisceration and peritonitis. Thereafter, subcutaneous tissues was sutured using surgical gut 3/0 while the skin incision was closed with interrupted horizontal mattress by using silk 3/0 suture.

Post-operative care

Wounds in all animals were sprayed with oxytetracycline wound spray once after the procedure. After surgery, the rats were housed individually in polyurethane boxes provided with clean and dry bedding sets made paper towel for extra comfort and warmth for a period of one week in order to avoid hypothermia and to prevent possible contamination. The animals were

housed individually for a period of one week to allow recovery and then re-grouped in their home cages. Animals were monitored for wound healing progress by checking the wound status daily for a maximum of two weeks.

Evaluation of wound healing

To evaluate wound healing, duration of healing, absolute and normalized length area of the wound were used. The maximum length area was measured on the second day after surgery. Thereafter, this measurement was carried out every two days until full healing occurred. The healing percentage or the normalized values were calculated by dividing the maximum length of the wounds by that measured on the second day after surgery. The duration of wound healing was the time taken for full contraction of the wound. Wound healing percentage was calculated using the equation [(L2-L1)/L2 x 100], where L2 and L1 are the maximum wound lengths on the second and any other day, respectively.

Data analysis

Results are given as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by Bonferroni's test did the comparisons between different groups. In all cases, a probability error of less than 0.05 was selected as the criterion for statistical significance.

RESULTS

The results show that there was no procedure-related death reported in the study. After operation, there was no significant difference in the rat's body weight between groups. Table 1 summarizes the effects of ovariectomy, ovariohysterectomy and orchidectomy on body weight in all groups. There was no significant difference in decreased body weight of animals after surgery in all three groups.

Table 1. Comparison of different surgical procedures of African Giant Pouched Rats

Groups	Operation	Body weight (grams)	Surgical duration (minutes)	Wound healing time (days)
Group A (n=6)	Orchidectomy	801.5 ± 0.6	5.55 ± 0.12	5.50 ± 0.50
Group B (n=6)	Ovariectomy	792.9 ± 0.8	15.52 ± 0.31	6.50 ± 0.50
Group C (n=6)	Ovariohysterectomy	802.1 ± 0.7	19.65 ± 0.86	7.00 ± 0.80

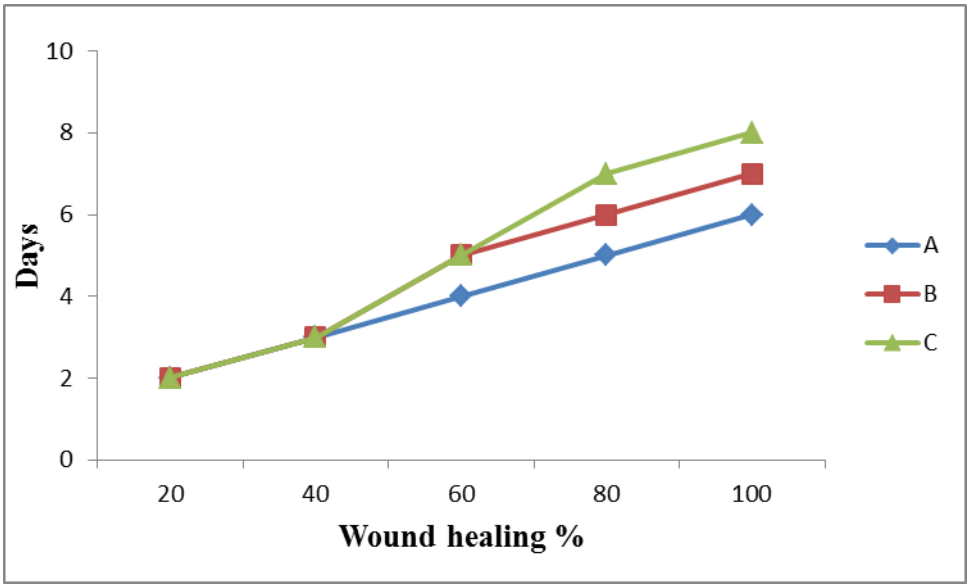

Figure 1. Wound healing percentage per day in groups A, B, and C.

Surgery duration

The surgery duration for orchidectomy (5.55 ± 0.11 min) was significantly less than that for ovariectomy (15.52 ± 0.30 min, $P < 0.02$) and ovariohysterectomy (19.65 ± 0.86 min, $P < 0.001$), as shown in Table 1. Also the duration of ovariectomy procedure was significantly lower when compared with ovariohysterectomy ($P < 0.05$).

Wound healing time

The wound healing time for orchidectomy, ovariectomy and ovariohysterectomy groups were 5.5 ± 0.50, 6.5 ± 0.50 and 7.0 ± 0.82 days, respectively (Table 1). The wound healing time was significantly shorter ($P < 0.05$) in male animals than in female animals. However, we observed no significant difference ($P > 0.06$) in the wound healing time between ovariectomy and ovariohysterectomy groups. The distributions of wound length and healing percentage per day showed significant variation between groups A and C.

DISCUSSION

All animals involved in the present study recovered well and returned to their normal duties without any difficulty. Ovariectomy and ovariohysterectomy were successfully performed in the female rats. In these animals, ovaries are associated with the caudal pole of the kidneys and are implanted in fats. The suspensory ligament of the ovary is adequately long to allow easy exteriorization of the ovary, fallopian tubes and uterine horns. In the adult animals the ovaries appear as a mass of follicles. The rats have uterine horns that open directly into a single cervix connecting the uterus and vagina. A single artery and vein run along the entire length of the medial side of each ovary and uterine horn (Olson and Bruce, 1986).

Male rats were castrated successfully. Rats have very large testes relative to their body size. The testes descend into the scrotal sac within the first three weeks after birth. The inguinal canals of rodents including rats remain open throughout life and the testes

pass freely from the scrotal sac into the abdominal cavity through the vaginal process because of a functional cremaster muscle (Olson and Bruce, 1986; Capello, 2011). Through such a huge inguinal canal, one might expect that hernias and intestinal strangulations would be common in rodents but this is not the case. There is a bulky mass of fat that is associated with the epididymis and it is frequently found within the inguinal canal.

Complications from these surgical procedures are rare but comprise anesthetic overdose, dehydration, hypothermia, pulmonary hypostatic congestion, hemorrhage, eviscerations, wound dehiscence, suture reaction or sinus formation, and infections (Brown, 2000; Richardson and Flecknell, 2006; Sontas *et al*, 2007). However, many of these complications can be prevented by careful physical examination and selection of healthy and fit animals for the intended surgical procedure.

The most common complication is anesthetic overdose, which can be circumvented by using the safest anesthetics, minimal required dosage, and efficient monitoring of anesthetized patients. In this study, animals were fasted in preparation for the procedures. This is important because animals that receive anaesthetic agents such as xylazine may vomit if they have a full stomach and this could lead to potentially fatal complications (Kohn *et al*., 1997). The rat could choke on the vomited food particles or inhale them into its lungs resulting in severe bronchoconstriction (reaction of the airways towards irritant food particles, which results in them spasming and narrowing down such that the animal cannot breath normally) and even bacterial or chemical pneumonia (Sumitra *et al*., 2004).

Rats can easily become hypothermic, thus, it is necessary that care should be taken to provide supplementary heat such as using the blankets, hot water bottles, and heating pads to warm the animals during recovery from anesthesia (Longley, 2008). If the recovery is extended, dehydration can be counteracted by intra-peritoneal or subcutaneous injections of normal saline or lactated Ringer's solution. It is also, advised that recovering animals be turned every ten to fifteen minutes to stimulate respiration and reduce chances of pulmonary hypostatic congestion (Fish *et al*., 2011). Hemorrhage is frequently associated with accidental injury to the visceral organs such as liver, and spleen during surgical operation. Eviscerations and wound dehiscence are regularly connected to suture removal by the patient or cage mates; and this can be prevented by applying tissue adhesive on incision. In this study however, the wounds were not covered.

The suture reaction or sinus formation is very rare but sometimes occurs when a sensitive body reacts to a certain types of suture material applied during closure of incision. This may result in a draining wound and even pus formation; it may require further operation to remove the suture material. Wound infections are best controlled by performing aseptic surgical procedures. In this study, the ventral approach meant that the wounds were in an almost constant direct contact with the beddings, which could cause more frequent contamination and wound breakdowns. Housing the animals individually for the first week post surgery helped to minimize the contamination. No wound showed signs of infection. In cases where wounds are infected, it is recommended to carefully use antibiotics. If aseptic procedures are performed, as was done in these animals,

antibiotics are not needed, as some of them are toxic to rats (Luft *et al*., 1978).

Despite complications been reported in some studies, in our study we did not encounter serious complications. A number of factors including good nutrition, good hygiene in cages where animals were kept individually, and ambient temperature (28ºC - 32ºC) during the study attributed to the enhanced wound healing.

The surgical procedures to sterilize animals are the oldest methods that are relatively safe, simple and rapidly carried out. These procedures have been done in other animal species such as dogs and cats for many years with minimal complications especially when carried out aseptically and by a qualified veterinarian. Therefore, this study demonstrated the surgical procedures (ovariectomy, ovariohysterectomy and orchidectomy) for sterilization of rats that are very useful to veterinarians in public/private practice and/or in biomedical research facilities.

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