

CLINICAL EVALUATION OF LAPAROSCOPIC STERILIZATION TECHNIQUES IN FEMALE RHESUS MACAQUES (MACACA MULATTA)

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ABSTRACT: Clinical study on laparoscopic evaluation of two different techniques of sterilization was carried out in 60 healthy female Rhesus macaques divided into two equal groups viz., ovariectomy (Group I; n=30), tubectomy (group II; n=30). After anesthesia, animals were placed in Trendelenburg's position on a tilted surgical table. Two pre-umbilical ports were created for insertion of forceps and laparoscope. The monopolar electrocautery was utilized for cutting and electrocoagulation. Surgical time differed between groups, there was shorter surgical time for the laparoscopic tubectomy (4.0 ± 0.8 min) as compared to ovariectomy (7.0 ± 0.6 min). There were also lesser bleeding and inflammatory changes in the laparoscopic tubectomy. Laparoscopic procedure was performed under xylazine-ketamine anesthesia at a 10 mmHg pressure gradient of CO₂. Both the methods of laparoscopic sterilization in female Rhesus macaques were found to be effective but laparoscopic tubectomy was preferred for routine clinical utility.

Key Words: laparoscopic sterilization, pneumoperitoneum, ovariectomy, tubectomy, Rhesus macaque (*Macaca mulatta*)

AVALIAÇÃO CLÍNICA DE TÉCNICAS DE ESTERILIZAÇÃO LAPAROSCÓPICA EM FÊMEAS DE MACACOS RHESUS (MACACA MULATTA)

RESUMO: Estudo clínico sobre a avaliação laparoscópica de duas técnicas diferentes de esterilização realizadas em 60 fêmeas saudáveis de macacos Rhesus divididos em dois grupos iguais: ovariectomia. (Grupo I, n = 30), tubectomia (grupo II, n = 30). Após anestesia, os animais foram colocados em posição de Trendelenburg sobre uma mesa inclinada cirúrgica. Duas aberturas pré-umbilicais foram criadas para a inserção de uma pinça e laparoscópio. O eletrocautério monopolar foi utilizado para o corte e eletrocoagulação. O tempo cirúrgico diferiu entre os grupos, houve menor tempo cirúrgico para a tubectomia laparoscópica ($4,0 \pm 0,8$ min) em relação à ovariectomia ($7,0 \pm 0,6$ min). Havia também menor sangramento e alterações inflamatórias na tubectomia laparoscópica. O procedimento laparoscópico foi realizado sob anestesia xilazina-cetamina em um gradiente de pressão de 10 mmHg de CO₂. Ambos os métodos de esterilização laparoscópica em macacos Rhesus fêmeas foram eficazes, mas a tubectomia laparoscópica foi preferida para utilidade clínica de rotina.

Palavras-chave: esterilização laparoscópica, pneumoperitônio, ovariectomia, tubectomia, macaco Rhesus (*Macaca mulatta*)

INTRODUCTION

With increasing advancement in Veterinary Sciences, the newer techniques and technology are also being adopted to increase the efficiency as well as efficacy. Various techniques for female sterilization were carried out in female Rhesus macaques throughout the world viz. ovariectomy (Andrew *et al.*, 2006) and tubal ligation (Gupta *et al.*, 2001). Among the various modern surgical methods, laparoscopy is now held as one of the most potent and promising aid for both its diagnostic and therapeutic use. Thus, it explores a new modality to intervene between the intra-abdominal organs in an easier and less stressful way for therapeutic necessities. It involves minimal invasiveness (keyhole surgery) with maximum visibility, shorter surgical time, decreased postoperative discomfort and pain, less incidence of infection, uncomplicated healing with minimal scarring, and minimal surgical morbidity, fast recovery and minimal loss of blood (Wildt *et al.*, 1977 ; Maiti *et al.*, 2001). Thus it provides some distinct advantages over conventional laparotomy. In a large scale animal birth control program, the conventional methods of sterilization require a long period between capture and release, due to the time taken for the surgical wounds to heal. In this aspect, keyhole surgery (laparoscopic surgery) has the potential to revolutionize the entire program, as it creates only a very small surgical wound, which usually needs very less postoperative care or regular dressings.

The present study was undertaken to compare two different sterilization techniques in female Rhesus macaques to find out the more effective and faster method in a population control program.

MATERIAL AND METHODS

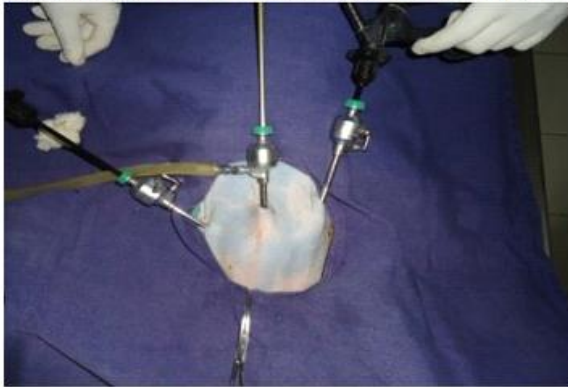
A total of 60 clinically healthy female Rhesus macaques with mean (\pm SEM) weight of 6.4 ± 1.6 kg, were used after obtaining approval of the Ethical Committee of the Wild Life Wing, Government of Himachal Pradesh, India. The free range Rhesus macaques were captured from different locations of Shivalik Hills of Himachal Pradesh in India, as a part of population control program for Rhesus macaques. The animals were transported to Monkey Sterilization Centre as per approved procedures and providing adequate feed and water on the way during transportation from the long distances. The animals were given rest for one day before surgery to relieve them from transportation stress.

The animals were divided into two groups of equal size (30 female Rhesus macaques in each group); Group I ovariectomy; Group II tubectomy. Animals were kept off fed for 15-hours and water withheld for 8 hours before the surgery. The animals were transferred to a squeeze cage and were anesthetized by using xylazine and ketamine combination ; ketamine 10 mg/kg (100mg/ml) and xylazine 2mg/kg body weight (100mg/ml) (Troy Laboratories Pty Ltd, Australia). Anesthesia was maintained with ketamine only. Surgical anesthesia was judged to be present if there was an absence of response to cornea reflex and compression of the pelvic limb nail roots. The blood samples were collected for the hematological examination.

Clipping and shaving of the area from post xiphoid up to pubis was done. Asepsis was achieved by scrubbing with 70 % alcohol. The animals were positioned in dorsal recumbency on a tilted surgical table in Trendelenburg's position. The animals were securely attached with straps to the surgical table. An incision of 5 mm was made on

pre-umbilical midline area on the abdominal cavity. A Veress needle connected to CO₂ gas insufflator was carefully inserted through this incision. After insertion of the Veress needle insufflation of the abdominal cavity was achieved by CO₂ gas at the rate of 1 L/min with a pressure gradient of 10 mmHg. After induction of pneumoperitoneum the Veress needle was removed and two pre-umbilical paramedian lateral ports were created at 4-5 cm left and right side from the midline port. A 5 mm safety trocar and cannula was inserted through the first port into the abdominal cavity (Fig. 1).

Fig.1 Insert the telescope, forceps and scissors with pointed lateral

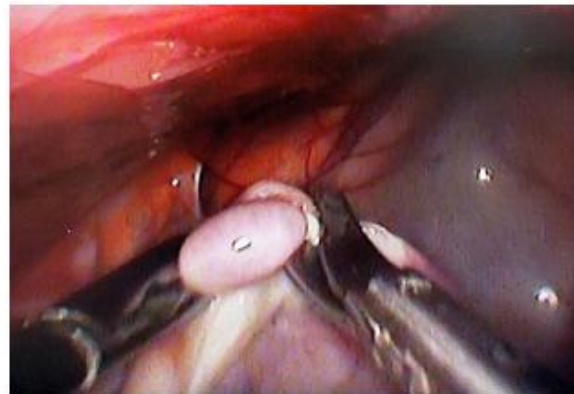


Trocar was taken out and a rigid-type telescope (30 degrees, 5 mm in diameter, Frontline Co., Germany) connected to a light source (40 W, halogen lamp) was introduced through the cannula. A monopolar cautery forceps was introduced through the left paramedian port and Grasping forceps inserted through the right paramedian port. The intraperitoneal organs were clearly visualized. The urinary bladder was identified first and then uterus and other structures were thoroughly visualized. The ovaries and fallopian tubes were clearly visualized.

In group I, ovariectomy was achieved by grasping the ovarian suspensory ligament and gentle traction was applied to lift the left ovary (Fig. 2) into the visual field. Electro cautery was

used to transversely dissect the mesovarium and resected ovary was gently grasped by a grasper forceps and extracted out through the cannula. The remaining tissue, ovarian pedicles, arteries and veins were checked for bleeding. The right ovary was also removed in the same manner.

Fig2. Ovariectomy: cutting and coagulation of the ovarian



In group II , the trocars were introduced in the same manner as described in group I. After clear identification of the Fallopian tube, it was elevated with the help of grasper to reveal the vascular supply. The cutting scissor was introduced through lateral cannula and gentle traction was maintained on the elevated section of tube and approximately 3 cm of Fallopian tube was excised (Fig.3) and resected portion was scooped out through the cannula. Both ends of the oviduct were examined for bleeding and cauterization and coagulation of the open ends of the Fallopian tubes were achieved. The same procedure was repeated on the opposite Fallopian tube.

The laparoscope along with camera was pulled out and surgical wounds were sutured with 3-0 Vicryl suture (Ethicon® India). Injections of enrofloxacin 10% w/v (Fortivir®, Virbac Animal Health Ltd. India) @ 7.5 mg/kg of body weight and ketoprofen 2 mg/kg of body weight, i/m, was routinely used for three days. Animals were released after surgical wound healing on 5th day postsurgery.

Fig3. Cutting and coagulation of the fallopian tube



All the methods were evaluated for the duration of surgery, haematology, hemostatic competency and physiological parameters ; respiration rate, heart rate, and rectal temperature., Heart rate was recorded with a stethoscope and rectal temperature was taken with a digital thermometer. Respiration rate was counted by observing thoracic wall movements.

RESULTS AND DISCUSSION

Anesthesia was performed successfully by administering xylazine (2mg/kg body weight) and ketamine (10mg/kg body weight). The induction of anesthesia was generally smooth with good muscle relaxation throughout the surgical procedure. The induction time was observed to be 2.20 ± 0.12 min. Duration of anesthesia was found to be 72 ± 0.24 min, recovery time was 38 ± 0.08 min. The mean surgery time for laparoscopic sterilization statistically differed, 7 ± 0.6 min, and 4 ± 0.8 min ovariectomy was in case of tubectomy were observed, respectively, although laparoscopic surgical time varies with the surgeon's skill and experience in the techniques. All the animals were examined at resected sites for hemorrhage after procedure completion. After thorough examination, the telescope was taken out. Intraabdominal CO₂ gas was allowed to escape through the cannula.

The hematological examination revealed acute rise in leucocyte count after 8 hours of surgery in both groups but this increase was significantly ($P < 0.05$) higher in the laparoscopic ovariectomy procedure as compared to laparoscopic tubectomy. This increase in leucocyte count was in confirmation to the post-surgical acute inflammatory changes (Sastry, 1983).

The respiratory rate decreased from 28.16 ± 0.31 (immediately after induction) to 22.01 ± 0.38 (before recovery). The decrease in respiration rate could be due to the effect of xylazine to potentiate the respiratory depressive effect of ketamine (Haskins *et al.*, 1986). The combination of ketamine and xylazine anaesthesia may produce hypothermia (Paddleford, 1999). The rectal temperature reduced following induction with xylazine-ketamine. The decline in body temperature agreed with Bush *et al.* (1977) when ketamine was used in non-human primates. The mean heart rate decreased significantly ($P < 0.05$) after xylazine-ketamine administration. The major side effects of α_2 -adrenoreceptor agonists on the cardiovascular system may have contributed to the decreased heart rate in this anesthetic protocol (Hall *et al.*, 2001; Kul *et al.*, 2000).

Laparoscopy is an endoscopic procedure that bridges the gap between clinical evaluation and surgical exploration. The laparoscopic surgical techniques in both human and veterinary medicine have grown tremendously. Laparoscopy requires a minor surgical intervention and provides the only available practical means of making repeated direct observation of abdominal viscera (Maiti *et al.*, 2008). The advantages of surgical laparoscopy over the conventional open surgical exploratory laparotomy include faster patient recovery because of smaller surgical sites, improved visualization of abdominal organs, lower postoperative

morbidity with lower infection rates, and less postoperative pain and stress (Rothuizen, 1985).

Induction as well as recovery from general anesthesia was smooth and uneventful in all animals. Smooth induction could be attributed to the effect of ketamine that altered the reactivity of the central nervous system to various sensory impulses without blocking sensory input at spinal or brain stem levels. The muscle relaxation could be attributed to the effect of xylazine mediated through central nervous system (Wright, 1982).

Proper fasting prior to surgery emptied the intestine and urinary bladder and thereby facilitated proper visualization of the utero-ovarian structures, ovarian blood vessels, and broad ligaments (Wildt *et al.*, 1981; Thiele *et al.*, 1993). Electrocautery with a 60 W monopolar current revealed a good hemostatic measure in bilateral tubectomy. Rodgerson *et al.* (2001) also observed that monopolar electrocautery alone was sufficient for effective hemostasis in equine mesovarium cauterization. However, before final withdrawal of the telescope, all of the animals were closely observed at the resected site and no further complications were observed.

A 5 mm 30 degree oblique angle telescope was found to be suitable for visualization and to perform surgical procedures. The remaining port (5 mm) was at the umbilical site for insertion of the telescope, as reported by Wildt *et al.* (1985). The urinary bladder was visualized first with the introduction of the telescope into the abdominal cavity; it was identified by its characteristic tortuous structures of blood vessels. Most clinicians have reported the use of a 5-mm telescope during ovariectomy in dogs (Dharmaceelan *et al.*, 2000; Wildt *et al.*, 1981).

A minute complication encountered in this study was accidental touch and thereby minor damage to peritoneum during electro-cauterization in two or three animals. However, this minor trauma did not affect the animal during or after surgery. Malm *et al.* (2004) reported splenic lesions in 3 out of 30 animals during ovariectomy. Minor complications like splenic laceration and vaginal discharge were observed by Davidson *et al.* (2004) in 9 out of 16 laparoscopic ovariectomy patients. Thus, this study appeared to have fewer laparoscopic complications in comparison with other studies. The early recovery from ketamine anesthesia was attributed to ketamine induced catecholamine, which enhanced the basal metabolism leading to faster elimination of ketamine (Luna *et al.*, 1997). The animals appeared quite alert and responsive. All of these observations reflected the early recovery of the animals after laparoscopic sterilization operation. No postoperative complications like emphysema, portal herniation, peritonitis, ascites, or wound abscess were recorded in any animals of both groups.

More bleeding, longer surgical time as well as more inflammatory changes were observed in laparoscopic ovariectomy as compared to tubectomy. The laparoscopic tubectomy by electrocautery in Rhesus macaques provided optimum hemostasis and effective removal of utero-ovarian structures.

The results of this study show that both methods were found to be safe and can be used for sterilization of female Rhesus macaques but laparoscopic tubectomy may be preferred over laparoscopic ovariectomy. After achieving the ovariectomy and tubectomy successfully, all female rhesus

macaques were set free in the free range from where they were captured.

The effect of ovariectomy or tubectomy has been studied by various workers on the ovariectomized macaques, and showed a decrease in the sexual receptivity behavior or also called as clutching reaction in which the female rhesus macaques shows the activity like turning round, looking backwards, lipsmacking, clutching and biting behaviour of the female rhesus monkey that occurs during the ejaculatory mount of the male (Doris *et al.*, 1968). Ovariectomy in Rhesus females results in decrease in sexual invitations as female receptivity declined (Richard *et al.*, 1970). In human females also there has been seen a decrease in sexual desire (Dennerstein *et al.*, 1977), while the replacement of estrogen level lead to increase in female sexual activity (Zehr *et al.*, 1998).

The effect of ovariectomy in humans as well as non human primates also affects the cognitive functions. The studies on non human primates showed that estrogen levels affects the cognitive functions both in young and aged monkeys (Lacreuse *et al.*, 2006). In a study ovariectomized cynomolgus monkeys were deficits in a measure of visual attention (Voytko *et al.*, 1996) while postmenopausal rhesus macaques showed delayed response task compared with age-matched premenopausal females (Roberts *et al.*, 1997). The declining in estrogen level after ovariectomy or menopause also found to cause impairing of verbal memory in human females (Sherwin *et al.*, 1996). There is need of behavioural study on the free range female Rhesus macaques. The present study was done on the clinical evaluation of laparoscopic sterilization techniques was found an effective methods of sterilization in female Rhesus macaques (*Macaca mulatta*).

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