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Original Article

# Evaluation of Two Different Ovariohysterectomy Approaches on Alpha-1-Acid Glycoprotein (AGP) Level in Cats

Amir Allahdin[1]*, Abutorab Tabatabaei-Naeini[1], Amin Bigham-Sadegh[1], Khatereh Safavi-Naeini[2]

*[1] Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. [2] Department of Pharmacology, Faculty of Medicine, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran.*

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**ABSTRACT**

This study aimed to compare acute phase proteins in cats undergoing ovariohysterectomy (OHE) by median celiotomy or left flank laparotomy approach. Nineteen native intact queens were used. Cats were randomly divided into four groups for midline (Test, n=5 and control, n=5) and left flank (Test, n=5 and control, n=4) ovariohysterectomy. Blood samples were taken immediately before surgery, 24, 48, 72 hours, and 7 days after surgery for assessment of Alpha-1-acid glycoprotein (AGP), total protein, and albumin values. According to the results, standard and left flank OHE approaches increased AGP levels, but no significant difference was observed compared to the control group ($p > 0.05$). No significant difference was observed on serum total protein and albumin levels using standard and left flank OHE approaches compared to the control group ($p > 0.05$). The results suggested two surgical procedures had the same effect on acute-phase proteins in ovariohysterectomized cats.

## Introduction

Cat population have been increasing excessively and sterilization of female cats is topic of interest for veterinary surgeons. With increasing advancements and demands for mass sterilization, there is a growing deal for newer techniques which are easy to perform and precise with higher clinic-surgical feasibility.[1] The management of unowned domestic cat populations is a global problem for individual cat welfare.[2] One of the most frequent procedures performed in veterinary medicine is surgical sterilization of the cats.[3] Several surgical sterilization techniques have been described such as traditional midline ovariohysterectomy (OHE), lateral flank OHE, castration, ovariectomy, vasectomy, laparoscopic OHE and ovariectomy.[4] However, method of contraception and subsequent diseases is a problem in pet.[3] For instance, Coe *et al*. compared flank and midline approaches to OHE of the cats and revealed greater incidence of discharges via the flank than midline approach.[5] Sterilization of female cats can be carried out by removing the ovaries and uterus

* Correspondence to: Amir Allahdin, Department of Clinical Sciences, School of veterinary Medicine, Shiraz University, Shiraz, Iran.
E-mail: dr.amir.allahdin@gmail.com

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altogether (OHE) or just by removing the ovaries (ovariectomy). Conventional surgery is generally high-risk and inconvenience intervention due to surgical traumatism with the risk of infectious contamination, postsurgical pain and acute phase proteins as an outcome of visceral manipulation. So, it is better to use minimally invasive procedures.[6]

There is developing interest on role of the acute phase proteins (APPs) in animals.[7] APPs have key role in regulation of the immune response, inflammation and protection against infection. The same individual APP, like a sword have both pro- and anti-inflammatory effect, with a delicate balance between the two functions. The APPs play a positive role in the innate host defense mechanisms. However, increases in APPs also have been described in chronic inflammation.[6] They have deniable pharmacokinetic implications in clinical therapy because alterations in the plasma levels of APPs during inflammation can alter the free plasma concentration of drugs.[8] The APPs assist to heal and re-establish tissue function and limit tissue damage when the body is affected by trauma, infection, stress, surgery, neoplasia or inflammation.[9] Subsequently, APPs response may add to the underlying tissue damage which accompanies the disease and further complications such as protein deposition and reactive amyloidosis.[7] Thus, the goal of the current study was to analyze impact of flank and midline OHE approaches on acute phase proteins in cats.

## Materials and Methods

### *Animals*

This study was affirmed by the Shiraz University Animal care and Use Committee. Nineteen adult queens from domestic short hair breeds, weighing between 3.3 ± 0.3 kg, with mean age of 9 ± 3 months were used in the study. All of cats were presented with no history of illness for routine physical examination, CBC, vaginal cytology and ultrasonographic evaluation of the reproductive tract two weeks before surgery. In addition, cats were evaluated for immediate medical attention on daily basis. At the end of the study, the entire cats were adopted-out to private homes.

The animals were randomly allotted into four groups (n = 19). Experimental groups were standard midline group (midline), control standard midline approach (con midline), left flank celiotomy (flank), and control left flank celiotomy (con flank). The presence of control groups is necessary to minimize the role of surgical manipulations, inflammation caused by absorbable sutures and other independent variables in the fluctuation of acute phase proteins (Alpha-1-acid glycoprotein) levels in cats that underwent surgery in comparison with each of the experimental study groups. Therefore, in the control groups, all the cases were treated and performed exactly the same as the study groups, except for sterilization in cats. In addition, all cats in the control group were neutered and adopted-out at the end of the study.

### *Pre-Operative Preparations*

Cats were kept in fasting for 12 hours of water and food before the surgical procedure.[10] Surgical area for flank and ventral midline approaches was prepared by clipping the hair and removing dust and grease by applying chlorhexidine solution. Then incision site was aseptically prepared by application of povidone–iodine solution and ethyl alcohol 70% (Zakaria Jahrom Co.)

All surgical procedures were performed under anesthesia by acepromazine maleate 2% (0.02 mg/kg, (Alfasan Co., Woerden, Netherlands) and ketamine hydrochloride 5% (15 mg/kg, Rotexmedica, Trittau, Germany) by intramuscular injection (IM) as sedation. Once lateral recumbency was achieved, a 22-gauge catheter was inserted into the cephalic vein using aseptic technique.

For induction, ketamine hydrochloride 5% (10 mg/kg) concurrent with midazolam hydrochloride (0.2 mg/kg, Irandarou, Iran) was injected intravenously (IV). A ketamine bolus (0.5 mg/kg) was administered intravenously if there were signs of an inadequate depth of anesthesia. Additional boluses given as required to prolong anesthesia (30%–40% of the initial dose). Anaesthetic depth was assessed by monitoring trends in heart and respiratory rate, eye position, jaw tone, response to surgical stimulation and palpebral reflex. During surgery and anaesthesia a 10 ml/kg/h of 0.9% sodium chloride solution (Darou Pakhsh, Tehran, Iran) was administered through IV line. Active heating was performed using an electric heat pad placed beneath the bedding on which the cat was positioned.

### *Standard Midline Approach*

The cats were placed in dorsal recumbency. The length of incision was 2cm in all cases, the skin was incised and subcutaneous fat were dissected bluntly in the incision site between the umbilicus and the pubis, for exposing the *linea alba*. A midline incision was done on the *linea alba* directly and parietal peritoneum to

enter the peritoneal cavity. The procedure was then identical to that described for the flank approach. The uterus was exposed and grasped with atraumatic forceps by repelling the intestines cranially and the bladder caudally. On completion of the OHE the incision in the abdominal wall was closed with 2-0 Polyglactin 910 (Vicryl) in a simple continuous pattern. The subcutaneous fat was closed with 2-0 Vicryl suture material. A subcuticular/intradermal continuous suture of 2-0 Vicryl was used to oppose the skin edges and no skin sutures were inserted.[5]

### *Left Flank Celiotomy Approach*

For feral cat OHE, the lateral flank approach has been suggested as a satisfactory approach, in which postoperative monitoring and assessment may be limited.[11] The cat was positioned in right lateral recumbency and its legs were extended caudally and secured with tape. The incision position was determined using visualizing an equilateral triangle with vertices at the greater trochanter, the wing of the ilium and the center point of the incision.[12] The length of incision was 2cm in all cases. In Left flank celiotomy the routinely used incision include the skin, subcutaneous fat, external aponeurosis, internal and transverse abdominal obliques and peritoneum in a dorsal to ventral direction to enter the peritoneal cavity. The uterus was identified and exteriorized using spay hook. Proximal to the ovary a window was made in the broad ligament/mesovarium and the ovarian pedicle was double clamped with Halstead mosquito hemostats. The pedicle was ligated immediately beneath the hemostats with 2-0 Vicryl suture material. The pedicle was sectioned between the clamps and the stump was grasped with rat-toothed forceps for inspection after the removal of the hemostat; after meticulously observing that there was no bleeding the pedicle was released. The uterine body was exteriorized and an encircling ligature was placed cranial to the cervix without clamping. After ligature placement, uterine body was triple clamped and a further ligature was tied into the crush of the lowest clamp. The uterine body was sectioned among the second and third clamps and the stump of cervix was inspected for hemorrhage. The inner and outer muscle layers were sutured together with 2-0 Vicryl suture material in a simple continuous pattern. Simple continuous suture pattern was applied to close the subcutaneous tissue with 2-0 Vicryl suture material. To oppose the skin edges a subcuticular/intradermal continuous suture of 2-0 Poliglecaprone 25 (Monocryl) was used to insure the skin closure, and no skin sutures were embedded.[5]

### *Post-Operative Preparations*

Tramadol (Texidol 50 mg, Tehranchemie Co., Iran) at a dosage level of 2 mg/kg were injected intravenously (once) immediately after surgery.[13,14] Sterile fixed dressing (TGMED, Tadbir Gostar Darman Iranian Co., Iran) was applied at the incision site after suturing. Antibiotics were given to all cats to avoid secondary infections.[15] Cefazolin (Cefazolin-Exir, Exir Pharmaceutical Co., Iran) at a dose of 20 mg/kg IV at time of surgery followed by a second dose of 20 mg/kg SC 6 hours later.[13]

### *Blood Samples*

Blood samples for serum protein concentrations were collected from the cats by jugular venipuncture by use of 1-inch, 21-gauge needles and placed in tubes before the surgical procedures (0 h) and at 24, 48, 72 hours, and 7 days later. Two milliliters of each collected blood sample was placed in a vial containing 10% EDTA for performance of a CBC. A portion of the blood was centrifuged at 1000× G for 10 min and the plasma stored at -20° C.[16] Then serum albumin and total protein were measured using colorimetric assay kit (Pars Azmoon Inc., Tehran, Iran) (BT-1500, Biotechnica, Italy) and Biuret method; (Pars Azmoon commercial kits, Tehran, Iran) and biochemical auto analyzer (BT-1500, Biotechnica, Italy), respectively. Alpha 1-acid glycoprotein (AGP) levels were measured using ELISA commercial kits (Zellbio GmbH, Germany) according to the manufacturer's instructions.

### *Statistical Analysis*

Data were analyzed for repeated measurements by two-way analysis of variance (ANOVA) and is presented as the mean ± standard error of mean (SEM). For treatments found to have an effect according to the ANOVA, mean values were compared with Tukey's test. $p < 0.05$ were considered to indicate significant differences between the treatments.

## Results

Evaluation of different OHE approaches on acute phase protein in cats is shown in Figures 1-3. According to the Figure 1, despite standard and left flank OHE approaches increased AGP levels, but no significant difference observed compared to control group ($p >$

0.05). As seen in Figure 2, no significant difference observed in serum total protein using standard and left flank OHE approaches compared to control group ($p$ > 0.05). Based on the Figure 3, standard and left flank OHE approaches had no significant difference on serum albumin compared to control group ($p$ > 0.05).

## Discussion

Cat population have been expanding excessively and new sterilization programs developed for control their population. Therefore, minimally invasive methods have been employed to decrease discomfort, pain and APPs. Tissue injury after surgery can change acute phase proteins. The acute phase response is a nonspecific inflammatory reaction which happens following tissue injury.[17] The response leads to changes in APPs, which some of them decrease (known as negative APPs) while proteins such as albumin or transferrin, C-reactive protein, Serum Amyloid A (SAA), haptoglobin, AGP and ceruloplasmin increase (positive APPs).[18] Measurement of the acute phase proteins is a potentially useful clinical tool in veterinary medicine, but further studies are required to assess their responses in different pathological processes according to the species.[9] According to the results, standard and left flank OHE approaches increased AGP levels, but no significant difference observed compared to control group. No significant difference observed on serum total protein using standard and left flank OHE approaches compared to control group. Standard and left flank OHE approaches decreased fasting blood sugar (FBS) levels, but no significant difference observed compared to control group.

In as study about discomfort of cat after the operation, no difference reported between cats undergoing OHE by a midline or flank approach.[5] Another study, reported Wound inflammation in cats following OHE was low in cats undergoing midline OHE compared to the flank OHE.[2] Also, no significant difference observed among cats undergoing OHE by a midline or flank approach after 7 days.[5] Inclusion of a subcutaneous closure affects the postoperative wound swelling. In this regard, subcutaneous closure suggested for midline OHE.[19] However, higher pain scores and wound tenderness was reported for cats using flank OHE while higher incidence of postoperative wound swelling and discomfort in cat with midline OHE.[20] However, because of limitation of the current study, we were not able to determine pain or discomfort of the cats. In this regard, Fazio *et al.* reveled cortisol concentrations and haematological variables, body weight and behavior are not significantly affected following OHE in cat and dogs.[21] The use of APPs has not been widespread in veterinary practice. This is most likely due to few commercial veterinary assay kits. Also, routinely this tests are using for feline infectious peritonitis.[7]

Generally, SAA, haptoglobin, AGP and ceruloplasmin concentrations increase in sick and/or older cats compared to healthy cats. This highlights the capability of acute phase proteins as diagnostic markers in cats, yet in addition accentuates that the signalment of the cat should be contemplated. Because of the variable response of different APPs to tissue damage, measuring multiple APPs are more useful than assessing a single protein. In cat, SAA, AGP and haptoglobin are recognized as acute phase reactants,[7] which in the current study we determined AGP levels in the OHE cat.

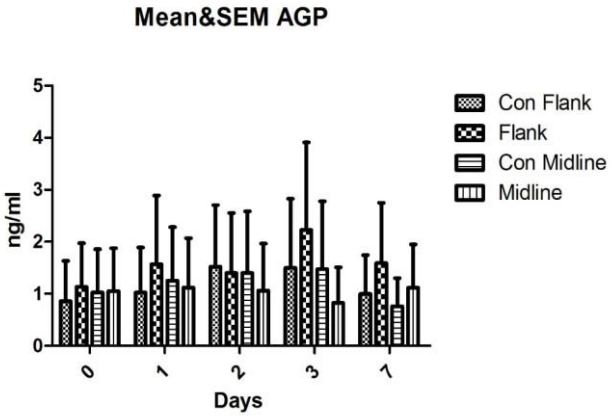


***Figure 1***. Effect of standard and left flank ovariohysterectomy approaches on serum alpha 1-acid glycoprotein (AGP) in cats. Data is presented as the mean ± SEM.

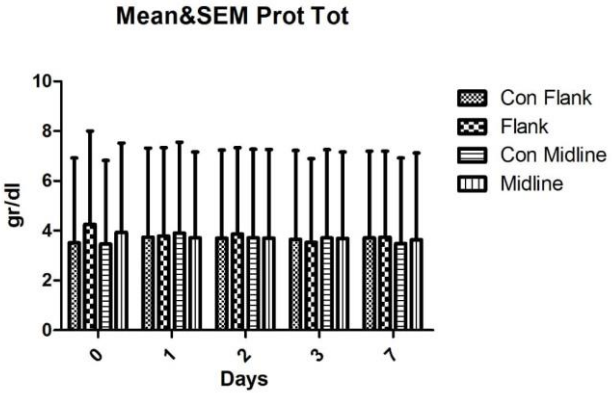


***Figure 2.*** Effect of standard and left flank ovariohysterectomy approaches on serum total protein in cats. Data is presented as the mean ± SEM.

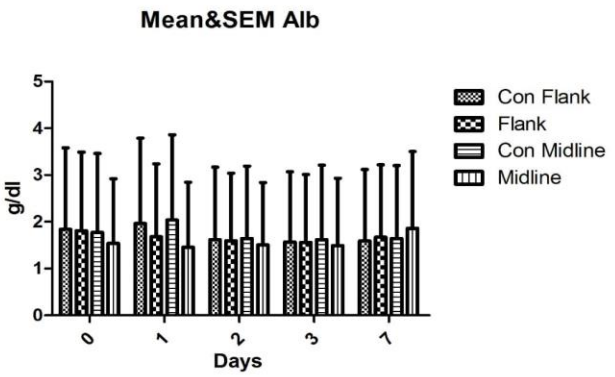


***Figure 3.*** Effect of standard and left flank ovariohysterectomy approaches on albumin in cats. Data is presented as the mean ± SEM.

The surgical texts suggested the flank approach has several expected complications, including the likelihood that the entire uterine body may be difficult to remove, recovery of a dropped ovarian pedicle might be hard, and that it could be hard to expose the opposite ovary and uterine bifurcation.[5] However, existing of the opposite ovary and uterine horn was difficult from the flank approach as well as discoloration or darkening of oriental cats' fur are the disadvantages for this approach. However, findings of the current study in agreement with Coe *et al.* revealed no difference observed in the flank or midline approaches.[5] We think further researches with larger sample size needs to determine effects of the APPs in flank or midline approaches.

The results suggested two surgical procedures had the same effect on acute phase proteins in ovariohysterectomized cats.

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## Conflict of Interest

There is no conflict of interest to declare.

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