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Evaluation of the Effectiveness of Uterine Hook Application in Large Breed Canine Ovariohysterectomy: Physiological Study

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Abstract

Objective- The aim of this study was to compare the duration of surgery as well as some physiological stress indicators in large breed dogs undergoing open ovariohysterectomy with or without use of uterine hook.
Design- Experimental study
Animals- Fourteen healthy dogs underwent open OHE with (N=7) or without use of uterine hook (N = 7).
Procedures- Physiological measurements as well as serum glucose and cortisol concentrations were measured 30 minutes before the surgery, and at 2, 6 and 24 hours postoperatively. CRP concentrations were measured as well. Values at each time point were compared between the two groups.
Results- Our results indicated significantly lower serum glucose at 6 and 24 hours after surgery in the hook group compared to other group. Serum cortisol concentrations didn't show significant difference. Significantly lower serum CRP concentrations were detected in hook group at 24 hours after OHE. The average temperature, heart rate and breathing at any time between the two study groups were not significantly different.
Conclusions and Clinical Relevance- Amount of blood sugar and serum CRP level in OHE with hook group was lower, which may be indicator of decreased surgical stress. Ovariohysterectomy with hook has a series of advantages and disadvantages. It can be concluded that using uterine hook to exteriorize the horns of uterus during OHE may lead to shorter abdominal incision and result in fewer trauma and surgical stress after operation, if uterine hook is applied by an experienced and skilled surgeon.
Keywords- Uterine hook**,** Ovariohysterectomy, Stress, Dog.

Introduction

Elective sterilization of female dogs and cats is one of the most common procedures in companion animal practice and is considered by private veterinary practitioners as one of the most important skills necessary for new graduate veterinarians.[1-2]Surgical removal of the female reproductive organs is called ovariohysterectomy (OHE) or spaying. The operation removes both ovaries, the uterine horns and the body of the uterus. Potential benefits of sterilization are population control, inhibition of diseases related to reproductive tract, and prevention of undesirable behaviors associated with hormonal cycling.[2-3] It has been mentioned that many canine mammary tumors are hormone-dependent, and most can be controlled if spaying is performed at an early age.[4]

Many surgical techniques of OHE and their benefits and complications in dogs and cats have been widely presented and discussed for decades.[5-7] Each technique, even routine surgeries, offers advantages and disadvantages to both the patient and surgeon. Complications may result from inappropriate techniques, and efforts should be made to follow good surgical and aseptic standards to avoid these complications.[8] Less invasive techniques may cause lower surgical stress, reduce the time of hospitalization and result in a shorter recovery time and fewer complications when compared to traditional OHE.[4,9,10] Analysis of the surgical stress response can be used for evaluation of surgical techniques as well as comparisons of different methods and surgical instruments. Physiologic parameters and hematological biomarkers are measured commonly for evaluation of stress response.[11]

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Cortisol is a commonly used parameter for measuring the responses of the hypothalamic-pituitary-adrenal axis to environmental alterations[12,13], anesthesia[14], and surgery[14,15] in both humans and animals[17,16-18].
Cortisol concentration increases in response to surgical stress.[19] The serum cortisol concentration appears to be a good measure of surgical stress in the canine model.[20]
In dogs, one of the main acute phase proteins (APP) secreted by the trauma of the surgery is C-reactive protein (CRP).[21,22] Plasma concentration of CRP is a sensitive and reliable method to measure the amount of trauma and evaluate the inflammatory state during postoperative period.[21,22]
The aim of this study was comparison of duration of surgery, surgical complications and postoperative stress through changes in physiological parameters as well as serum concentration of cortisol, glucose and CRP, in large dogs undergoing open ovariohysterectomy with or without using uterine hook (less invasive technique) in a randomized, prospective clinical trial.

Materials and method

Animal model

14 healthy mixed-breed female dogs, weighing 17 to 40 kg (mean, 28.5 kg; median, 29.5 kg) with 1 to 6 years old (mean, 2 years; median, 2 years) maintaining in a local animal shelter, were used for the study. Physical evaluation and preoperative CBC and serum biochemical profile were performed and found to be within reference ranges in all dogs. Animals were randomly divided into 2 groups of open surgery with the use of the uterine hook (group 2) or without it (group 1). All the dogs had free access to water, but food was withdrawed 12 hours before surgery. The protocol of this study was approved by institutional animal research committee.

Surgical preparation

Each dog was given a subcutaneous injection of Tramadol (2 mg/kg of body weight) for analgesia before anesthesia. Acepromazine (1%, Alfasan, Woerden, The Netherlands, 0.1 mg/kg) and ketamine hydrochloride (10%, Alfasan, Woerden, The Netherlands, 10 mg/kg) were intramuscularly injected in a syringe as combination for sedation.
A venous catheter was placed in the right cephalic vein and administration of 10 mL/kg/hour of normal saline was started. Prophylactic antibiotic (Cefazolin, Jaber Ebne Hayyan Pharmaceutical Company, Saveh, Iran, 25 mg/kg) and a combination of diazepam (Zepadic®, 10mg/2ml, Caspian Tamin Pharmaceutical Co., Iran, 0.27 mg/kg) and Ketamine (5.5 mg/kg) were administered to induce general anesthesia. Anesthesia was maintained using intravenous ketamine (5mg/kg) according to standard clinical practice.

The animals were positioned in dorsal recumbency and cranial abdomen was prepared for aseptic surgery. All surgeries were performed by the same surgeon. Surgery time was calculated from beginning of incision to placing of the last skin suture of the procedure.

Open surgery without using the uterine hook (group 1)

On average, a 10.5 cm ventral midline incision was made from 1 cm caudal to the umbilicus toward the pubis through the skin, subcutaneous tissue, and linea alba. Left uterine horn was first exteriorized with index finger and the left ovary was identified. Then, using three forceps technique, the left and right ovarian arterio-venous complex were ligated and transected, respectively. A figure eight suture was placed on the uterine body distal to the cervix and the uterus was resected. The abdominal wall, subcutaneous tissue and skin layers were sutured routinely.

Open surgery using the uterine hook (group 2)

Method was similar to Group 1 except that ventral midline incision was on average 3.8 cm and exteriorization of the horn of uterus was performed by a uterine hook.

Monitoring and Laboratory testing

Values for heart rate, respiratory rate, and rectal temperature were obtained 30 minutes prior to the procedure, 2 hours, 6 hours and 24 hours after OHE.Blood samples were collected from the cephalic vein of all bitches at the mentioned time points. Blood was collected into microtubes containing EDTA. Serum was prepared by 10-min centrifugation (3000–4000 × g) of full blood, frozen at -20°C and stored. The following serum determinations were made: (1) CRP levels using the ELISA method and a commercial CRP kit (Tridelta, Kildare, Ireland); (2) cortisol levels using the competitive ELISA method and a commercial kit (DiaPlus Inc., San Francisco, USA); and (3)Glucose was assayed by an enzymatic (glucose oxidase) colorimetric method (Pars Azmoon®, Tehran, Iran).

Statistical analysis

All statistical analyses were performed using statistical software SPSS (version 22). Data are presented as mean (±SD). Surgical time and amount of blood loss as well as physiological parameters and blood glucose and cortisol concentrations obtained at various times were compared between treatment groups with an independent-samples t test. For all comparisons, $p \leq 0.05$ was considered statistically significant.

Results

Average surgery time for group 1 was 45.8 minutes (range 36 to 55 minutes) and for hook group was 54 minutes (range 41 to 66 minutes). There was no significant difference in surgical time between the two surgery groups (p> 0.05).

No significant differences in heart rate, respiratory rate and rectal temperature were found between the two surgery groups (p> 0.05), (Table 1).

Table 1. Heart rate, Respiratory rate and rectal temperature in dogs undergoing routine open ovariohysterectomy (group 1) and open ovariohysterectomy with use of a uterine hook (less invasive technique) (group 2). Data presented as mean ± SD.

	Time / Group	Pre anesthesia	30 min pre-operation	2 h after operation	6 h after operation	24 h after operation
Heart rate	Group 1	97.1 ± 10.5	113.1 ± 21.5	123.4 ± 29.8	90.8 ± 28.1	119.4 ± 60.1
	Group 2	126.3 ± 34.7	161.1 ± 22.5	150.8 ± 22.2	114.3 ± 23.6	128 ± 24.9
Respiratory rate	Group 1	37.7 ± 14.9	30.8 ± 11	34.3 ± 18.5	31.4 ± 8.4	65.7 ± 43.9
	Group 2	40.6 ± 19.1	20 ± 4.6	28.6 ± 13.1	26.8 ± 8.2	46.6 ± 26
Rectal temperature	Group 1	38.3 ± 0.4	38.5 ± 0.5	37.1 ± 1.4	38.2 ± 1	38.9 ± 0.2
	Group 2	38.5 ± 0.2	38.5 ± 0.5	36.4 ± 1.2	38.5 ± 0.7	38.7 ± 0.3

The average blood sugar between the two groups, half an hour before operation and 2 hours after the operation had no significant difference, but at 6 and 24 hours postoperatively its level in hook group was significantly lower (p< 0.05), (Fig.1). There was no significant difference in serum cortisol concentrations between two groups at any time points (p> 0.05), (Fig.2)

In both groups, we observed significantly higher serum CRP concentrations at 6 and 24 hours after OHE compared to physiological values 30 minutes before surgery. Figure3 shows the blood CRP concentrations determined in the two groups of animals. The mean preoperative CRP concentration for hook group was 12.42 (±5.63) mg/l. This value significantly increased to 94 (±21.24) mg/l and 275.32 (±32.7) mg/l, 6 and 24 hours after surgery respectively (p< 0.05). In the other group, CRP concentrations rose from a preoperative value of 11.15 (±7.5) mg/l to 81.95(±21.54) mg/l after 6 hours and to 332.75(±46.75) mg/l after 24 hours (p< 0.05).

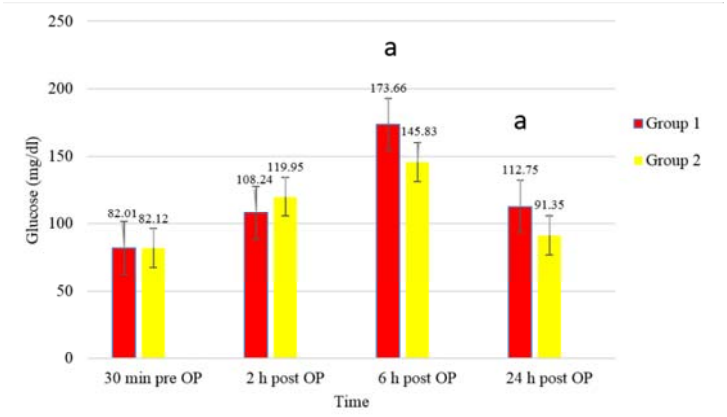

Figure 1. Serum glucose concentrations (mg/dl) (Mean±SD) in healthy dogs undergoing routine open ovariohysterectomy (group 1) and open ovariohysterectomy with use of a uterine hook (less invasive technique) (group 2). (a) Statistically significant differences compared to the other group at the same time point (p< 0.05).

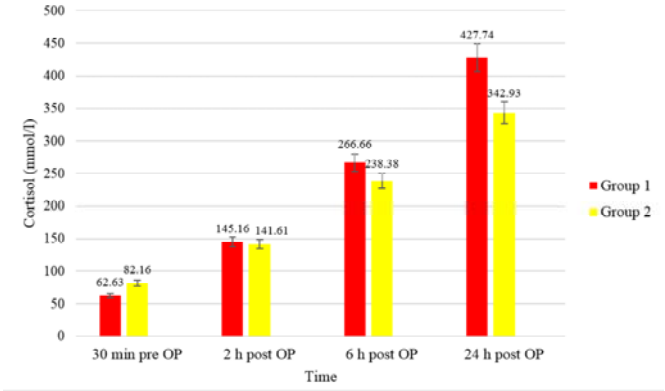

Figure 2. Serum cortisol concentrations (mmol/l) (Mean±SD) in dogs undergoing routine open ovariohysterectomy (group 1) and open ovariohysterectomy with use of a uterine hook (less invasive technique) (group 2). No statistically significant differences were observed between two groups at the same time point ($p < 0.05$).

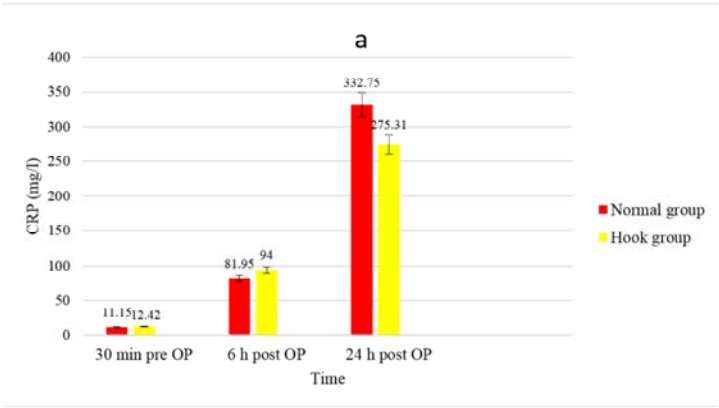

Figure 3. Serum C-reactive protein (CRP) concentration (Mean±SD) in bitches throughout the experiments (mg/l). Group I: healthy bitches undergoing routine open ovariohysterectomy. Group II: healthy bitches sterilized with shorter incision using a uterine hook. (a) Statistically significant difference compared to the other group at the same time point ($p < 0.05$).

Discussion

inaccessible bleeding, minimal access to uterine bifurcation and less handling possibility have impeded the widespread use of hook by most veterinarians in Iran. The main concept in a successful surgery is to make smaller incisions. It is clear that short incisions can be closed much more rapidly than long incisions. Ovariohysterectomy with hook requires shorter incision; but the question is whether shorter incision would significantly decrease post-operative inflammatory reactions and stress or not. In a surgical patient, the stress reaction is considered detrimental for wound healing. Surgery time is important and affects the stress of surgery as well.[23]This study was performed to compare postoperative stress and surgery time of dogs undergoing OHE with and without use of the uterine hook. Compared to conventional midline OHE, minimally invasive techniques like laparoscopy are believed to cause less surgical stress, reduce duration of

Despite the enthusiasm for using hook during OHE in dogs, some difficulties including concern about hospitalization, improved cosmesis, and result in a shorter recovery time.[4, 9,10,24,25] However, Very limited data are available to exactly evaluate the rate of complications for these techniques.[26,27]In a study of 34 dogs, laparoscopic OHE has been compared with traditional technique in terms of surgical time, complications and pain scores.[4] In that study laparoscopic OHE was performed successfully, but surgical times and complication rates were higher than those of traditional OHE. The authors noted, however, that equipment cost and the necessity for more than one surgeon may limit the technique's usefulness in small animal practice.

Changes in heart rate, blood pressure, body temperature, respiration and plasma cortisol concentrations have been used to identify pain and stress responses in humans.[28] Physiological signs and measurement of endocrine responses have also been used to evaluate the

mammalian response to stressful events and to evaluate the effectiveness of analgesics following painful procedures.[29-31]The results of our study showed that the mean rectal temperature, heart rate and respiratory rate in any of the time between the 2 groups were not significantly different. Although the procedure is stressful for dogs with behavioral changes in the24hours aftersurgery,[32]but the study of Bernard Hansen et al. (1997) about the analgesic effect of the oxymorphone on the dogs were examined at OHE showed that the physiological parameters are not appropriate and sensitive to the stress response in dogs, while plasma cortisol concentration index is more appropriate.[28] Another study by Conzemius et al. (1997) on dogs and the other one by Smith et al. (1999) on cats had been shown that temperature, heart rate and respiratory rate don't significantly change after surgery and this parameters didn't measure pain after surgery.[33] In a study comparing three methods of OHE, open surgery, laparoscopy and natural orifice transluminal endoscopic surgery (NOTES), no significant difference was seen on physiological parameters between studied groups.[34]This may be because the physiological signs are not sensitive indicators of a stress response to ovariohysterectomy in dogs and our results are in line with findings of other studies.

In this study, the amount of glucose and cortisol, as indicators of stress, have been compared between the two groups. The blood glucose and concentration of plasma cortisol, which is part of the neuroendocrine response to surgical stress, are appropriate criteria for measuring surgical stress and postoperative pain in OHE surgery.[9,33] In our study, the average amount of cortisol in 6 and 24 hours after surgery in hook group was lower than the other group, but this difference was not significant. In previous studies, significant increase of cortisol concentrations have been seen after OHE in cats and dogs.[19,33,35]A relationship between cortisol and increased postoperative complications has been noted in humans.[36]Cortisol stimulates gluconeogenesis by liver resulting in increased blood glucose.[37]Our results indicated significantly lower serum glucose at 6 and 24 hours after surgery in the hook group compared to other group. The decreased amount of glucose in the hook group may be attributed to lower stress and tissue damage due to shorter incision in hook group. Cortisol possesses complex metabolic effects on carbohydrate, fat and protein. It promotes protein breakdown and gluconeogenesis in the liver. Cellular use of glucose is inhibited, so that blood glucose concentrations are increased.[38] This results correspond with the results of this study because cortisol levels after surgery at all studied time point shave been trending upward. It is noteworthy that the amount of glucose in 2 groups, 6 hours after surgery has been in an increasing direction, but the rate dropped in 24 hours.

The magnitude of metabolic response to surgery measured by serum glucose and cortisol concentrations indicates the amount of stress due to magnitude of surgical trauma. The highest levels of cortisol is in 4 to 6 hours after surgery.[20, 28, 38]In this study, hook group showed lower cortisol levels compared to other group at 6 and 24 hours after surgery, however this difference was not significant, which it can be due to less invasiveness of the procedure and lower surgical trauma in hook group compared to other group.

Freeman et al. (2010), comparing three methods of open surgery, laparoscopy and NOTES, reported increase in the amount of cortisol and glucose in all groups.[34]These results correspond with the results of the present study. Freeman et al. did not report significant differences in mean serum glucose concentrations in any of the groups, but they noted higher cortisol concentrations in the NOTES animals. They explained this increase as a result of pneumoperitoneum due to higher intra-abdominal pressures,[20] longer operating times,[39]or differences in thermal injury. Cortisol is the major stress hormone released by the adrenal gland. Cortisol concentration increases in response to surgical stress and pain. Longer duration of surgery can increase the amount of cortisol.[33] In the present study cortisol concentration in the hook group was lower than the other group. It may be related to method of procedure as less invasive technique. The serum cortisol concentration is a good measure of surgical stress in the canine model.[20] Lower serum cortisol after OHE using hook compared with the other group may indicate a lesser degree, or quicker resolution of surgical stress in the former. If significant reduction of the duration of the surgery could be achieved by hook, it is expected to decrease cortisol levels to a significant level.

Serum APP concentrations reflect the severity of inflammation and this marker enables the early detection and restoration of abnormal homeostasis, particularly when clinical symptoms are absent.[40] Indeed, the determination of APP concentrations in humans and animals has proved extremely useful in postoperative monitoring.[41-44] It has been shown that plasma concentration of CRP is a sensitive and reliable method to measure the degree of surgical trauma and evaluate the early postoperative complications.[22] The CRP concentrations can be used to assess the inflammatory state during the post-ovariohysterectomy period. In both groups of dogs, we observed higher serum CRP concentrations 6 and 24 hours after surgery (Fig. 3). The increase in serum CRP concentrations has been attributed to an increased production of pro-inflammatory cytokines due to the tissue discontinuity and local inflammation provoked by surgical trauma.[43,45,46] Despite longer surgery time for hook group in this study, CRP level was significantly lower at 24 hours after surgery. The main challenge extending the time of surgery in hook group was finding uterine horn by the hook. Skill in using uterine hook can be improved during the time and practice. The other

problem to handle uterus was ligating uterine arteries, because cervix often is not accessible via a short incision immediately caudal to umbilicus, especially in large breed dogs. Pukacz et al. described a method of minimally invasive surgery for canine OHE to resolve this problem, which is performed via two midline portals. In this method, the uterine horn is pulled backwards from a second midline incision, just cranial to the pubic bone, until the cervix becomes visible.[5]This method seems to be effective to get uterine body ligated, but total length of incisions would be long affecting stress of surgery. One long-term study examined the effects of ovariectomy and OHE in 135 bitches that had undergone neutering during 8–11 years.[47] In this research, results indicated that removing the uterus during routine neutering of healthy bitches was not required, while the option of ovariectomy could be considered. However, cystic endometrial hyperplasia-pyometra complex may occur in case of progestin influence. But, this concern was not supported by the results of another report on ovariectomy in 72 bitches, in which no cases of pyometra were identified during the 6–10 years follow-up period.[48]Thus, uterine arteries may be ligated as far from cervix as they are able to exteriorize from small abdominal incision without fear of pyometra.

Because of the shorter incision using the hook, fewer traumas occur and risk of wound dehiscence and following incisional hernia decreases. This less invasive procedure is more simple compared to other methods such as laparoscopy and does not require expensive equipment.

It can be concluded that using uterine hook to exteriorize the horns of uterus during OHE may lead to shorter abdominal incision and result in fewer trauma and surgical stress after operation, if uterine hook is applied by an experienced and skilled surgeon.

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چکیده

ارزیابی تاثیر استفاده از قلاب رحمی در برداشت رحم و تخمدان سگهای نژاد بزرگ: مطالعه فیزیولوژیکی

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هدف- مقایسه و ارزیابی پارامترهای فیزیولوژیکی نشان دهنده‌ی واکنش التهابی و استرس پس از جراحی برداشت رحم و تخمدان با قلاب و بدون آن در سگ‌های بزرگ جثه

طرح- مطالعهٔ تجربی

حیوانات- تعداد ۷ قلاده سگ تحت عمل جراحی با قلاب قرار گرفت و ۷ قلاده سگ بدون قلاب جراحی شد.

روش کار- تعداد ضربان قلب و تنفس، دمای مقعدی، گلوکز و کورتیزول سرم، ۳۰ دقیقه قبل از جراحی، ۲، ۶ و ۲۴ ساعت پس از جراحی اندازه گیری شد و میزان هر کدام بین ۲ گروه مقایسه شد. میزان CRP سرم نیز بین دو گروه مقایسه گردید.

نتایج- نتایج حاصل از مطالعه نشان داد که میزان گلوکز سرم در گروه قلاب در ساعات ۶ و ۲۴ پس از جراحی به طور معنی‌داری پایین تر از گروه بدون قلاب بود. میزان CRP سرم ۲۴ ساعت بعد از جراحی در گروه جراحی با قلاب به طور معنی داری پایین تر بود. تفاوت معنی داری در پارامترهای فیزیولوژیکی و میزان کورتیزول سرم بین ۲ گروه مشاهده نشد.

نتیجه‌گیری و کاربرد بالینی - در گروه قلاب به دلیل برش کوتاه‌تر، میزان گلوکز خون حیوانات که شاخصی از میزان استرس می‌باشد کاهش یافته بود. انجام جراحی برداشت رحم و تخمدان با استفاده از قلاب دارای یک سری مزایا و معایب می‌باشد، ولی استفاده از آن برای پیدا کردن شاخ رحم توسط یک جراح متبحر منجر به برش کوتاهتری شده و در نتیجه آسیب و استرس کمتری بعد از جراحی حادث می-شود.

کلمات کلیدی- قلاب رحمی، برداشت رحم و تخمدان، استرس، سگ.