Comparison of Ovariohysterectomy, Ovarian Electrocautery, and Ovarian Blood Supply Ligation for Elective Sterilization of Bitches

Mohamed I. Khalifa, Moustafa M. Kassem, Mahmoud H. El-Kammar, Howida H. Abu-Ahmed
Department of surgery, Faculty of Veterinary Medicine, Alexandria University, Egypt.

ABSTRACT

The purpose of the present study was to investigate and evaluate ovariohysterectomy, electrocautery of ovaries, and ligation of ovarian blood supply as an alternative option for sterilization of bitches. The study was conducted on 12 adult mongrel bitches. Animals were equally divided into 3 groups; after laparotomy the following procedures were performed: group 1 - Ovariohysterectomy: ovaries and uterine horns were excised using the standard method; group 2 - electrocautery of ovaries: ovaries were thermally treated using forceps of electrocautery device to induce damage of ovarian tissue by deep heating; group 3 - Ligation of ovarian blood supply: the ovarian pedicle was ligated using a circumferential ligature, animals in this group underwent laparotomy one month after ligation to examine the status of the reproductive tract. Blood samples were collected pre- and post-operative to measure level of estrogen and progesterone as indicator of ovarian function, and C-reactive protein as inflammatory marker. Results showed that duration of surgery was statistically equal in electrocautery and ligation groups recorded at 17.0 ± 0.41 and 15.7 ± 0.48 minutes respectively. Ovariohysterectomy required longer time (24 ± 0.71 minutes).

Levels of estrogen and progesterone significantly declined over the period of 1-2 weeks after ovariohysterectomy and electrocautery, and continued in declining until it reached lowest levels at 3 weeks after the procedure. In the ovarian blood ligation group, estrogen and progesterone levels showed a temporal decline at weeks 1-2; however it increased again by the third week after surgery. Gross examination of ovaries in the 3rd group showed the presence of large growing follicles and edema in ovarian bursa. CRP increased significantly at 24 and 48 hours in all groups as compared to pre-operative stage. Although it declined in the ovariohysterectomy and ligation groups by week 2, CRP stayed significantly high in electrocautery group after 2 weeks of the procedure compared to pre-operative value and also compared to values in the other groups. Conclusion: findings indicate that ovariohysterectomy and electrocautery of ovaries are equally effective as a method for sterilization of bitches; electrocautery required shorter time and was less in handling of internal viscera, but its applicability is dependent on the availability of the device. Ligation of ovarian blood supply did not result in elimination of ovarian activity evidenced by high estrogen and progesterone levels and presence of follicular structures on ovaries.

1. INTRODUCTION:
Overpopulation of stray dogs is a public health issue that mandates cooperation of veterinary and public health institutions to resolve this problem. Surgical sterilization of female dogs is more humane than euthanizing stray animals, therefore some rescue organizations participate in a "catch and release" program. The catch and release program is sometimes known as TNR (Trap-Neuter-Return). The program targets homeless pets, which are captured, spayed and released in an attempt to control overpopulation of stray animals (Levy et al., 2014). Surgical sterilization of bitches is performed by either ovariohysterectomy or ovarioectomy (Kirpensteijn, 2008). Elective sterilization is also indicated for healthy bitches that are kept as house companions; the procedure is performed to prevent estrous cycle and its associated undesirable behaviors. Also, house pets are sterilized to prevent uterine infections and other pathologies such as uterine and mammary gland tumors as well as dystocia during parturition. It is occasionally undertaken as a treatment
for a behavioral problem such as aggression (Bencharif et al., 2010). Despite the obvious preference for ovariohysterectomy in the United States and Canada, ovarioectomy appears to have become the standard procedure of care in many European countries (van Goethem et al., 2006). Technically, ovarioectomy is less time-consuming than ovariohysterectomy because the broad ligaments are not disrupted, and the uterine horns and stump are left intact. From a practical perspective, both procedures result in elimination of ovarian steroid hormones and sexual behavior (Le Roux, 1983; Van der Walt et al., 1983) and also a decline in reproductive affections dependent on these hormones (Nickel, 1998). In the previous years, laparoscopic ovariohysterectomy and ovarioectomy was introduced as alternative to standard open surgery. The laparoscopic procedure provide the advantage of being less invasive with good visualization of the genitourinary tract, less tissue trauma, and reduced postoperative pain, recovery time, and risk of infection and other complications compared to traditional ovariohysterectomy (Davidson et al., 2004; Hancock et al., 2005). Disadvantages of laparoscopy include equipment cost, potential morbidity related to bleeding and injury to the viscera, the need for specific training and anesthetic protocols, equipment limitations usually preventing large-scale use in neutering programs, and difficulty moving laparoscopic techniques into field or mobile applications (Dupre and Fiorbianco, 2008). Finding alternatives to ovariohysterectomy and ovarioectomy is an objective of several studies. For example, experimental occlusion of ovarian blood supply in rats was performed by Murakami et al. (2014); authors in this study ligated ovarian vessels with titanium clips and nylon thread and compared the results with ovarioectomized group. Ligation resulted in ovarian atresia at 60 days after surgery and histological examination showed that ovarian tissue was replaced by dense fibrous connective tissue (Murakami et al., 2014). Similar approach was conducted previously in cows by placing a latex ring around the ovarian pedicle (Meirelles et al., 2007), and recently in bitch by ligation of ovarian pedicle (Mogheiseh et al., 2016). The procedure should lead to failure of follicular growth and ovarian degeneration, thus the animal loses the ability to reproduce. While efforts are maintained to control numbers of stray dogs, yet this serious problem still persist. Therefore, the aim of the current study was to evaluate ovariohysterectomy, electrocautery of ovaries, and ligation of ovarian blood supply as an alternative option for sterilization of bitches. The objective is to define the most effective method that eliminate steroid hormones and produce least post-operative complications.

2. MATERIALS AND METHODS:

2.1. Animals:
The present study was conducted on 12 adult mongrel bitches, their age ranged between 2 to 4 years. Animals were subjected to thorough clinical examinations, pulse rate palpation, body temperature, and they were dewormed 10 days before the start of the study using Drontal Plus® (Praziquantel/ Pyrantel pamoate/ Febantel, Bayer) at a dose of 1 tablet/10 kg bwt. Animals were also treated for external parasites using Dicтомax® (Doramictin, zoetis) at a dose of 1 cm/50kg bwt by s.c injection. All procedures used in the study were approved by the Institutional Animal Care And Use Committee (IACUC) Alexandria University.

2.2. Experimental design:
12 sexually mature bitches were equally divided into 3 groups;
Group 1: Ovariohysterectomy
Group 2: Electrocautery of ovaries
Group 3: Ligation of ovarian blood supply

Animals were allowed for fasting at least 8hrs before surgery. They were sedated and anesthetized using xylazine HCl (Xylaject®, Adwia pharma) at dose of 2 mg/kg, followed by ketamine hydrochloride (Ketamine®, Sigmatech Pharma) at dose of 5 mg/kg by i.m injection, in addition to Atropine sulphate (Atropine®, Adwia pharma) at dose of 0.01mg/kg. The three surgical procedures were performed through laparotomy; in group 1: ovaries and uterine horns were excised using the standard method of open ovariohysterectomy (Bencharif et al., 2010); in group 2: both ovaries were visually identified and located, then each ovary was thermally treated by applying electrode of electrocautery device to induce damage of ovarian tissue by deep heating; in group 3: ovaries were exposed, then blood supply of both ovaries were ligated at the ovarian pedicle using a circumferential ligature using vecryl as suturing material. After ligation, ovaries were returned to its place and abdominal wall was closed; animals in this group underwent laparotomy one month after the procedure to examine the status of the reproductive tract. Closure of abdominal wall was performed using simple continuous suture pattern to close linea alba and muscle layer (Knots were tied securely and tension was...
repeatedly checked during suturing by gapping the incision). Subcutaneous tissue was sutured, followed by skin closure using simple interrupted suture by silk, and then the skin wound was covered with sterile gauze covering for protection.

2.3. Sample collection and measured parameters:
For each animal the following parameters were recorded; 1- Duration of surgery was estimated and it was measured as the time from the start of the skin incision until closure of the abdominal wall was completed. 2- Post operative pain signs: after surgery signs of pain were observed by monitoring movement of the animal (normal, agitated, recumbent), and reaction to palpation of surgical site (no response, react with vocalizing, attempt to bite). 3- Blood samples: blood samples were collected by aspiration form cephalic vein; serum was separated after centrifugation for 15 min at 1000x g and stored at -20˚C for later assay. C-reactive protein as an inflammatory marker was measured at the following time points: pre-operative, 24hrs, 48hrs, and 2 weeks after surgery. Estrogen and progesterone as indicators of ovarian activity were measured at the following time points: preoperative, 1, 2, and 3 weeks after surgery.

2.4. Statistical analysis:
The data of the present study was analyzed with SAS (Statistical Analysis System software version 9.1, SAS Institute Inc., Cary, NC, USA). Repeated measures analysis of variance was used to analyze variables with multiple measurements per dog using the MIXED Procedure of SAS. Estrogen and progesterone were transformed to log2 prior to analysis. However, duration of surgery was compared with one-way ANOVA. Tukey’s test was used as a post-hoc. Probability less than or equal to 0.05 was considered statistically significant Data are expressed as mean ± standard error of the mean.

3. RESULTS:
No significant surgical complications occurred during and after surgery, also no significance in pain signs was found among groups.

3.1. Duration of surgery:
Table (1) shows results on duration of surgery among groups. Standard ovario-hysterectomy lasted about 24 ± 0.71 minutes which was significantly longer than other groups (P<0.05). Electrocautery and ligation showed comparable values recorded at 17.0± 0.41and 15.7± 0.48 minutes respectively.

3.2. Hormonal profile:
Estrogen level:
Table (2) shows changes of estrogen level before and after surgery. Estrogen was significantly declined (p<0.05) 1 week after ovariohysterectomy to 3.98± 0.96 Pg/ml (compared to 24.2 ± 5.58 Pg/ml before surgery) and continued in declining until it reached to the lowest levels 3 weeks after the procedure. Same pattern of declining was also observed in electrocautery, as estrogen showed lowest level by week 3 after the procedure. On the other hand, after ligation of ovarian blood supply estrogen declined over the 1st week and the 2nd week of surgery to 11.5 ± 4.26 and 9.10 ± 3.66 Pg/ml respectively, but showed an increase after 3 weeks to similar level that was recorded at the pre-operative stage. Statistical analysis showed that no significance was found in estrogen level between ovariohysterectomy and electrocautery at week 3 after surgery. However, estrogen level was significantly higher in ligation group compared to the other methods (P<0.01) at the time point.

Table 1: Duration of surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariohysterectomy</td>
<td>24.0 ± 0.71 a</td>
</tr>
<tr>
<td>Electrocautery of ovaries</td>
<td>17.0 ± 0.41 b</td>
</tr>
<tr>
<td>Ligation of ovarian blood supply</td>
<td>15.7 ± 0.48 b</td>
</tr>
</tbody>
</table>

- Means without a common superscript letter differ significantly (P<0.05).

Table 2: Estrogen level (Pg/ml) before and after surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Time post-operative</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 w</td>
<td>2 w</td>
<td>3 w</td>
</tr>
<tr>
<td></td>
<td>Pre-operative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovariohysterectomy</td>
<td>24.2 ± 5.58</td>
<td>3.98 ± 0.96”</td>
<td>1.25 ± 0.31”</td>
<td>0.50 ± 0.00”b</td>
</tr>
<tr>
<td>Electrocautery of ovaries</td>
<td>16.8 ± 7.06</td>
<td>1.88 ± 0.65”</td>
<td>0.81 ± 0.18”</td>
<td>0.55 ± 0.05”b</td>
</tr>
<tr>
<td>Ligation of ovarian blood supply</td>
<td>20.1 ± 8.45</td>
<td>11.5 ± 4.26”</td>
<td>9.10 ± 3.66</td>
<td>13.1 ± 4.81a</td>
</tr>
</tbody>
</table>

- Values are means ± standard error of mean.
- (P<0.05) and ” (P<0.01) differ significantly compared to the pre-operative mean.
- Means without a common superscript letter in the last column differ significantly (P<0.01).
3.3. Progesterone level:
Table (3) shows changes in progesterone level before and after surgery. After ovariohysterectomy and electrocautery progesterone declined significantly to reach the lowest level by week 3 after surgery that was recorded at 0.90 ± 0.25 and 0.79 ± 0.18 ng/ml respectively. In ligation method: progesterone showed significant decrease 2 weeks after the procedure (5.01 ± 0.40 ng/ml) as compared to the pre-operation stage (14.6 ± 2.12 ng/ml) but stayed at equal level after 3 weeks of surgery that was recorded at 6.41± 2.49 ng/ml. Statistical analysis showed that no significance was found in progesterone level between ovariohysterectomy and electrocautery at week 3 after surgery. However, progesterone was significantly higher in ligation group compared to the other methods (P<0.01) at the time point.

3.4. Inflammatory marker (C-reactive protein):
As shown in table 4, CRP increased significantly at 24 and 48 hours in all groups as compared to pre-operative stage (P<0.001). After 2 weeks of the surgical procedure, CRP in ovariohysterectomy and ligation groups declined to normal values (similar to pre-operative stage) that were recorded as 0.71 ± 0.08 mg/dl and 0.97 ± 0.15 mg/dl respectively. On the other hand, CRP stayed significantly high in electrocautery group after 2 weeks of the procedure compared to the pre-operative value and was recorded at 2.18 ± 0.09 mg/dl. Statistical analysis among groups by the end of the experiment showed that CRP in electrocautery group was significantly higher than ovariohysterectomy and ligation groups (P<0.01).

3.5. Gross examination of ovaries and uterus in ligation group:
After one month of ovarian blood supply ligation, the bitches were sedated then ovaries and uterine horns were excised via laparotomy. Gross examination showed that ovaries carry large follicles (outlined arrow) and accumulation of fluids in the ovarian bursa (white arrow). Uterine horns were edematous (black arrows) with thickened uterine wall.

Table 3: Progesterone (ng/ml) level before and after surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Time post-operative</th>
<th>Pre-operative</th>
<th>1 w</th>
<th>2 w</th>
<th>3 w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariohysterectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.4 ± 4.47</td>
<td>16.3 ± 2.52**</td>
<td>4.00 ± 0.64***</td>
<td>0.90 ± 0.25***b</td>
</tr>
<tr>
<td>Electrocautery of ovaries</td>
<td></td>
<td>22.1 ± 5.00</td>
<td>12.7 ± 2.38**</td>
<td>4.73 ± 0.99**</td>
<td>0.79 ± 0.18***b</td>
</tr>
<tr>
<td>Ligation of ovarian blood supply</td>
<td></td>
<td>14.6 ± 2.15</td>
<td>9.38 ± 2.32</td>
<td>5.01 ± 0.40*</td>
<td>6.41 ± 2.49**u</td>
</tr>
</tbody>
</table>

- Values are means ± standard error of mean.
- **(P<0.01), *** (P<0.001) differ significantly compared to the pre-operative mean.
- Means without a common superscript letter in the last column differ significantly (P<0.01).

Table 4: CRP (mg/dl) level before and after surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Time post-operative</th>
<th>Pre-operative</th>
<th>24 h</th>
<th>48 h</th>
<th>2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariohysterectomy</td>
<td></td>
<td>0.61 ± 0.03</td>
<td>1.78 ± 0.16***</td>
<td>1.95 ± 0.06***</td>
<td>0.71 ± 0.08b</td>
</tr>
<tr>
<td>Electrocautery of ovaries</td>
<td></td>
<td>0.63 ± 0.03</td>
<td>1.86 ± 0.07***</td>
<td>2.36 ± 0.15***</td>
<td>2.18 ± 0.09***a</td>
</tr>
<tr>
<td>Ligation of ovarian blood supply</td>
<td></td>
<td>0.63 ± 0.02</td>
<td>1.73 ± 0.12***</td>
<td>1.96 ± 0.05***</td>
<td>0.97 ± 0.15**b</td>
</tr>
</tbody>
</table>

- Values are means ± standard error of mean.
- *(P<0.05), **(P<0.01), and *** (P<0.001) differ significantly compared to the pre-operative mean.
- Means without a common superscript letter in the last column differ significantly (P<0.01).
4. DISCUSSION

The presented study aimed at evaluating electrocautery of ovaries, and ligation of ovarian blood supply in comparison to ovariohysterectomy. The objective was to define the most effective method that produces significant reduction in sex hormones with minimal post-operative complications. Evaluation was based on comparing duration of surgery, level of estrogen and progesterone as indicator of ovarian function, and C-reactive protein as inflammatory marker among the three methods. The results showed that duration of surgery in ovariohysterectomy was longer than the other two methods; this finding is expected because of the extra time needed for ligations to excise the uterus. The mean duration recorded for ovariohysterectomy was $24 \pm 0.71$ minutes. In comparison to other studies, a shorter duration of about 17.5 minutes by Tallant and others (Tallant et al., 2016), similarly Devitt and colleagues reported about 18 ± 3.9 minutes for the same procedure (Devitt et al., 2005). On the other hand, Davidson et al. (2004) and Hancock et al. (2005) reported longer time of about 69 minutes and 31.7 minutes respectively. Duration of surgery is greatly dependent on the surgeon experience; therefore it is by practice the veterinarian can attain shorter time to perform the procedure. The mean duration recorded for the other 2 methods were $17.0 \pm 0.41$ minutes for electrocautery and $15.7 \pm 0.48$ minutes for ligation of ovarian blood supply.

Ovariohysterectomy is being the preferred approach in the United States; this preference is most likely based on the concept that future uterine pathology is prevented by removing the uterus if compared to ovariectomy where the uterus is left in place. However, a comparative study showed that no significant differences between the 2 techniques for the incidence of long-term postoperative urogenital problems, such as endometritis, pyometra, and urinary incontinence (van Goethem et al., 2006).

In the current study, electrocautery of the ovarian tissue was made through laparotomy and in-situ cauterization of the ovaries while uterus was kept intact. Cauterization is a medical practice of burning a part of a body to avoid bleeding, remove an undesired growth, or minimize other potential medical harm, such as infections when antibiotics are unavailable. Ovarian cauterization for treatment of polycystic ovarian disease in women was previously described by several studies (Gadir et al., 1992; Kahyaoglu et al., 2008). Electrocautery of ovaries seemed a possible alternative to standard ovariectomy in bitch with anticipated equal duration for completion of the procedure. Mean duration recorded for electrocautery was $17.0 \pm 0.41$ minutes; similar time of about 15.4 minutes was reported for ovariectomy by Tallant and others (Tallant et al., 2016). Though, in another study the procedure...
extended up to 35 minutes (Peeters and Kirpensteijn, 2011). Beside the surgeon experience, the difference in duration of the procedure between studies could be related to body weight of the animal as positive correlation was found between the two variables (Peeters and Kirpensteijn, 2011). Electrocautery of ovarian tissue as a mean of gonadectomy is advantageous when compared to standard ovarioectomy that is because the lack of hemorrhage. Results showed that estrogen declined significantly after 1 week of ovariohysterectomy and electrocautery of ovarian tissues. This decrease continued to lowest values at week 3 after the procedure; these findings are in agreement with previous studies (Le Roux, 1983; Nickel, 1998; Van der Walt et al., 1983) in ovariectomized bitches. After ligation of ovarian supply, levels of estrogen decreased by week 1 and 2 of surgery, however it started to rise again by the third week. Large follicles and edematous uterine horns were noticed in those animals after the procedure by 1 month. These observations implicate that the ligation procedure did not eliminate ovarian activity. It also indicates that ovaries were able to compensate the shortage of blood supply after ligation by other means such as developing new branches of blood vessels. These results are similar to observations of Mogheiseh et al (2016) as the authors stated that ultrasonographic examination showed large follicles and corpus luteum on ligated ovaries in bitches. Moreover, ligated ovaries responded by progesterone secretion upon stimulation with eCG (Mogheiseh et al., 2016). Similar approach was conducted in cows (Meirelles et al., 2007), however cows suffered some side effects such as edema, peritonitis, abscesses, and suture dehiscence. The reason for different output of the procedure in bitch might be due to differences in anatomy of ovarian blood supply, more studies are needed to clarify these results.

Progesterone followed a similar pattern of decline in bitches underwent ovariohysterectomy and electrocautery to low levels by 3 weeks after surgery. Similar results were reported previously in ovariectomized bitches (Le Roux and van der Walt, 1977). These levels of low progesterone also correspond to observed values in anestrous bitches (Concannon et al., 1975). On the other hand, after the ligation procedure progesterone did not drop to low levels as observed in the other groups at week 3 after the procedure, this finding confirms functioning ovaries. To evaluate the inflammatory reaction of animals to surgery C-reactive protein was measured pre-and post-operative. Results showed that CRP increased significantly at 24 and 48 hours in all groups as compared to pre-operative levels; this observation is expected due to the immunological reaction of the surgical trauma and increased production of pro-inflammatory cytokines (Conner et al., 1988). Our finding is in agreement with Dabrowski and others, as they stated that CRP and other acute phase proteins such as serum amyloid A component (SAA) can be used to assess the inflammatory state during the post-ovariohysterectomy period (Dabrowski et al., 2007). CRP in ovariohysterectomy and ligation groups declined to normal values by 2 weeks after the procedure, which means that inflammation is subsiding with healing of the incision. On the other hand, CRP stayed significantly high in electrocautery group after 2 weeks of the procedure, indicating a persisting inflammatory process that might signify adhesions with surrounding tissues. This finding implies that electrocautery is more stressful to the animal than ovariohysterectomy and ligation of the ovarian supply. It is noteworthy to mention that CRP levels in bitch subjected to ovariohysterectomy are affected by surgeon experience (Michelsen et al., 2012).

5. Conclusion

From the findings of the study it can be concluded that electrocautery can be a potential alternative to ovariohysterectomy. Electrocautery required shorter time about 17 minutes with less handling of internal viscera; however its use is reliant on the availability of the device. Ligation of ovarian blood supply did not result in elimination of ovarian activity evidenced by high estrogen and progesterone levels and presence of ovarian follicles on ovaries upon gross examination.

6. REFERENCES


