The effect of pre-emptive incisional bupivacaine block on postoperative pain after coeliotomy in dogs

A Knowledge Summary by

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KNOWLEDGE SUMMARY

PICO question
Is there an effect on analgesia following coeliotomy in dogs after a block with local anaesthetics?

Clinical bottom line

Category of research question
Treatment

The number and type of study designs reviewed
All the available evidence for this PICO question comes from clinical trials and one experimental/pharmacology study. All the studies were randomised; 5/7 were blinded clinical studies and 4/7 studies were prospective

Strength of evidence
Moderate

Outcomes reported
It is not clear if the use of a local anaesthetic including bupivacaine or lidocaine as incisional blocks minimises the postoperative pain especially in the first 24 hours, as the results are not statistically significant between the groups

Conclusion
In conclusion, bupivacaine or lidocaine can minimise the postoperative pain but more clinical trials are needed

How to apply this evidence in practice
The application of evidence into practice should take into account multiple factors, not limited to: individual clinical expertise, patient’s circumstances and owners’ values, country, location or clinic where you work, the individual case in front of you, the availability of therapies and resources.

Knowledge Summaries are a resource to help reinforce or inform decision making. They do not override the responsibility or judgement of the practitioner to do what is best for the animal in their care.

The evidence
All the available evidence for this PICO question comes from clinical trials and one experimental/pharmacology study. All the studies were randomised; 5/7 were blinded clinical studies and 4/7 studies were prospective. The strength of the evidence is not the strongest one as there is no meta-analysis research about this subject, however, it is moderate as most of the included trials were randomised clinical studies.
### Summary of the evidence

#### Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CMPS</td>
<td>Composite Measure Pain Scale</td>
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<tr>
<td>DIVAS</td>
<td>Dynamic and interactive visual analogue scale</td>
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<tr>
<td>EtCO₂</td>
<td>End-tidal carbon dioxide concentration</td>
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<tr>
<td>FPIA</td>
<td>Fluorescence polarisation immunoassay technology</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>IM</td>
<td>Intramuscularly</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenously</td>
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<tr>
<td>MWTs</td>
<td>Mechanical wound thresholds</td>
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<td>NRS</td>
<td>Numeric rating scale</td>
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<tr>
<td>RR</td>
<td>Respiratory rate</td>
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<tr>
<td>SC</td>
<td>Subcutaneously</td>
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<tr>
<td>SpO₂</td>
<td>Oxygen saturation</td>
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<td>UMPS</td>
<td>University of Melbourne Pain Scale</td>
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<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
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#### Wilson et al. (2004)

<table>
<thead>
<tr>
<th>Population:</th>
<th>Various breeds of healthy dogs participating in surgical training exercises</th>
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<tr>
<td>Sample size:</td>
<td>Six dogs</td>
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</table>
| Intervention details: | • Dogs (n=6) were premedicated with: Acepromazine at 0.1 ± 0.07 mg/kg IM, butorphanol at 0.3 ± 0.1 mg/kg IM  
• Induction with thiopental at 11.5 ± 2.7 mg/kg IV  
• Maintenance with halothane or isoflurane  
• The dose of lidocaine hydrochloride (20 mg/ml) administered intraperitoneally was 8.8 mg/kg. The solution was diluted with an equal volume of isotonic saline resulting in a volume of 0.88 mL/kg. This diluted solution was placed into the peritoneal cavity just prior to closure of the incision in the body wall.  
• 2 mg/kg of 2% lidocaine hydrochloride with epinephrine was placed in the wound after the closure of the abdominal wall. |
| Study design: | Pharmacological experimental study, non-blinded, non-controlled |
| Outcome studied: | • Dogs were observed for signs of toxicity (seizures, nausea) at each of the sampling periods and again 18 h after the drug administration.  
• Venous blood was collected through a catheter aseptically placed in the cephalic or lateral saphenous vein.  
• The baseline blood sample was collected once the dog was anesthetised and in 5, 10, 15, 20, and 30 mins and 1, 2, 3, 4 and 5 hrs after the intraperitoneal (IP) injection.  
• The lidocaine assay utilised FPIA and a commercially available reagent kit. |
Main findings: (relevant to PICO question):

- No adverse consequences or signs of toxicity were noted during the postoperative period in these dogs.
- Detectable concentrations of lidocaine were found in all dogs in the sample taken 5 mins after administration.
- Time to peak serum concentration was 0.37 ± 0.26 hr.
- Peak serum concentration was 1.45 ± 0.36 μg/mL.
- A rapid decrease in serum concentration was shown with an elimination half-life of 1.17 ± 0.11 hr.

Limitations:

- Small sample size.
- Non-controlled, non-blinded study.
- No details about which dogs receive halothane and which received isoflurane for the maintenance of anaesthesia.
- Anaesthetic values were not mentioned in the results.

### Carpenter at al. (2004)

<table>
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<tr>
<th>Population:</th>
<th>Client-owned dogs or from local humane societies and rescue groups presented to Veterinary Hospital for routine ovariohysterectomy. They were determined to be healthy by physical examination. Dogs were excluded if they were less than 1 year of age, weighed less than 4.5 kg, or were having other procedures performed in addition to ovariohysterectomy.</th>
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</thead>
</table>
| Sample size: | 30 dogs
13 mixed-breed dogs, 17 purebred dogs |
| Intervention details: | 10 dogs in saline group (SAL), 10 dogs in lidocaine group (LID) and 10 dogs in bupivacaine group (BUP).
- Premedication with 0.02 mg/kg acepromazine IM and 0.22 mg/kg butorphanol IM.
- Induction with thiopental sodium at 13.2 mg/kg IV
- Maintenance of anaesthesia with isoflurane in oxygen.
- Intravenous fluids at a rate of 11.0 mL/kg/hr.
- Before closure of the linea alba following the procedure, 10 dogs received 0.88 mL/kg 0.9% saline, 10 dogs received 8.8 mg/kg 2% lidocaine with epinephrine diluted to an equivalent volume with saline and 10 dogs received 4.4 mg/kg 0.75% bupivacaine diluted to an equivalent volume with saline in their IP space at the cranial site of the incision.
- Prior to closure of the skin, the SAL dogs received 2.0 mL of 0.9% saline, the LID dogs received 2.0 mL of 2% lidocaine with epinephrine and the BUP dogs received 2.0 mL of 0.75% bupivacaine as a splash on linea alba.
- General anaesthesia was maintained until skin closure.
- The dogs were placed in sternal recumbency for 10 minutes after the recovery from anaesthesia.
- Dogs with a pain score greater than 50 mm on the VAS-pain at any time were given 0.22 mg/kg butorphanol IV or IM. Dogs that were extremely agitated or they did not respond to butorphanol were given additional doses of butorphanol and/or acepromazine as needed. |
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<th>Study design:</th>
<th>Prospective, randomised, controlled, blinded clinical trial</th>
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| **Outcome studied:** | • Pre- and post-procedural pain scoring was performed using the CMPS and VAS by one observer.  
• Pain scores at baseline, 0.5, 1, 2, 3, 4, 6, 8 and 18 hrs post extubation.  
• The scorer first observed the dog quietly for 1 min outside the cage. Then, he rattled the cage door and entered the cage saying hello.  
• RR was measured at the first minute of observation.  
• Behaviour and HR were recorded.  
• Palpation of the incision was performed by handler gently three times.  
• Position, activity and vocalisation were recorded during quiet observation.  
• Position change and behaviour were recorded during the opening of the cage door.  
• A single number from the CMPS was recorded at each time period. VAS scores were recorded by placing a cross on a 100 mm line between “no pain” and “worst imaginable pain” for pain scores (VAS-pain) and between “no sedation” and “very sedate” for sedation scores (VAS-sedation).  
• All treated dogs were scored throughout the surgery.  
• The length of the incision was measured upon completion of surgery.  
• Observation for signs of local anaesthetic toxicity (sedation, nausea, seizures). |
| **Main findings:** (relevant to PICO question): | • 7/10 dogs in SAL group, 4/10 in LID group and 2/10 in BUP group were given supplemental analgesia (significantly more dogs in SAL group than in BUP group).  
• 2/7 dogs in SAL group were given two doses of analgesics.  
• Dogs in LID group were given supplemental analgesia at 0.5, 1, 2 and 3 hrs after extubation. Two out of four dogs in LID group were given two doses of supplemental analgesia.  
• In BUP group dogs were given supplemental analgesia 1 hr after extubation and only with butorphanol.  
• None of the dogs in BUP group were given supplemental analgesia more than once.  
• None of the dogs received supplemental analgesics after 3 hrs post-extubation.  
• VAS-pain scores: a mean of 52 ± 24 at 0.5 hrs to 2 ± 3 at 18 hrs for the SAL group, a mean of 40 ± 19 at 0.5 hrs to 1 ± 3 at 18 hrs for LID group and 27 ± 4 at 0.5 hrs to 2 ± 1 at 18 hrs for the BUP group.  
• No significant difference among groups between VAS-pain scores at times 0.5 and 18 hrs.  
• Dogs in BUP group had statistically significant lower pain scores than dogs in SAL group.  
• At 0.5 hrs: BUP group had significantly lower pain scores than LID and SAL scores. |
CMPS scores: a mean of 7.4 ± 3.9 at 0.5 hrs to 0.1 ± 0.3 at 18 hrs for the SAL group, 5.0 ± 2.3 at 0.5 hrs to 0.2 ± 0.4 at 18 hrs for the LID group and 3.5 ± 2.1 at 0.5 hrs to 0.7 ± 0.7 at 18 hrs for the BUP group.

Significantly lower CMPS score at 0.5 hrs in BUP group than in SAL group.

VAS-sedation scores: a mean of 83 ± 7 at 0.5 hrs to 2 ± 2 at 18 hrs for the SAL group, 82 ± 9 to 2 ± 3 for the LID group and 73 ± 14 to 1 ± 1 for BUP group.

BUP dogs were significantly less sedated than the SAL dogs.

Limitations:
- Pain scores were subjective, but the observer was well trained.
- The exact p-values for the statistically significant differences were not displayed in the results.

Savvas et al. (2008)

Population: Dogs that were presented for midline celiotomy. Dogs that were in the American Society of Anesthesiologists (ASA) physical status classification system >3, were excluded from the study.

Sample size: 60 dogs (35 females, 25 males)

Intervention details:
- Four groups (15 dogs per group randomly assigned):
  1. bupivacaine preoperatively (B-pre)
  2. bupivacaine postoperatively (B-post)
  3. normal saline preoperatively (NS-pre)
  4. normal saline postoperatively (NS-post)
- All animals were fasted for 8 hrs before anaesthesia.
- Premedication with acepromazine 0.05 mg/kg IM and meperidine 3 mg/kg IM.
- Induction with thiopentone 6–8 mg/kg IV.
- Maintenance of anaesthesia with isoflurane in oxygen.
- Before the start of the incision, 0.8 mL/kg bupivacaine 0.25% or normal saline in the B-pre or NS-pre respectively, was injected SC and IM at the incision site.
- Fentanyl at 2 μg/kg IV and morphine at 0.1–0.3 mg/kg IM were given when the pain score was above 5.
- Dogs were monitored from induction to discontinuation of anaesthesia for variables including systolic, diastolic and mean arterial pressure, electrocardiogram, oxygen saturation, inspired and EtCO₂, isoflurane concentration, oxygen percentage and RR.
- Any dogs that required additional analgesia were excluded from the study.

Study design: Blinded, placebo-controlled, randomised clinical trial

Outcome studied: Pain and sedation were scored at 1, 2, 3, 4, 5, 6, 16 to 20 and 24 hrs after surgery. Pain was assessed based on a numerical scale from 0 to 10 (0=no pain, 10=full pain) and sedation based on another
scoring system (0=fully alert, 1=alert but unable to walk, 2=drowsy and unable to stand and 3=heavily sedated/asleep).

After surgery, the dog’s posture, behaviour, vocalisation, and food/water consumption, the willingness to move and the pattern of locomotion and the response to the palpation of the incision area were noted.

<table>
<thead>
<tr>
<th>Main findings: (relevant to PICO question):</th>
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<tr>
<td>• Maximal pain score (mean ± SD) for B-pre: 3.67 ± 1.11, B-post: 5.20 ± 1.30, NS-pre: 6.67 ± 1.59, NS-post: 6.53 ± 1.13.</td>
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<td>• Time points of pain scores: 2 hrs after surgery in 48/60 dogs, 3 hrs after surgery in 55/60 dogs and within 4 hrs in 60/60.</td>
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<td>• Statistically significant difference was the mean maximal pain score between the B-pre group and the other three groups.</td>
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<td>• B-pre group was also statistically different from the other three groups concerning less additional postoperative analgesia (p&lt;0.0005).</td>
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<th>Limitations:</th>
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<td>• No power calculation.</td>
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<td>• The choice of the pain score as it was based on behavioural criteria.</td>
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<td>• The duration of the surgery was not recorded but the duration of anaesthesia was monitored.</td>
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<tr>
<th>Fitzpatrick et al. (2010)</th>
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<td><strong>Population:</strong></td>
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<td><strong>Sample size:</strong></td>
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<td><strong>Intervention details:</strong></td>
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<tr>
<td>Outcome studied:</td>
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| Main findings: (relevant to PICO question): | - Incision length was significantly greater in Group III.  
- Glasgow pain scores were significantly decreased from 4 to 24 hrs in Groups I, III and IV. No statistically significant decrease for the same hours in Group II (p=0.125).  
- No dog with Glasgow pain score >10.  
- Von Frey filament score did not differ significantly for Group I, III and IV from 4 to 24 hours. This score was significantly decreased in Group II (p=0.031).  
- There was a higher rate of complications by the time of the suture removal in dogs that received pre-incisional infiltration of bupivacaine compared with dogs that received no injection at the incision site. However, bupivacaine was not indicated for the higher rate of complications in this group. |
| Limitations: | - ovariohysterectomy was performed by non-experienced 4th year veterinary students and this may have affected the results as they had a greater inexperience.  
- No equal number of dogs in each group because of scheduling conflicts and the availability of investigators.  
- High dose of morphine as premedication and thiopental for induction.  
- Postoperative analgesia with hydromorphone, buprenorphine and carprofen in all dogs independently of the pain scores may have affected the results as none of the dogs required rescue analgesia. |

Campagnol et al. (2012)

<table>
<thead>
<tr>
<th>Population:</th>
<th>Client owned rescue dogs undergoing ovariohysterectomy</th>
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<tbody>
<tr>
<td>Sample size:</td>
<td>30 female dogs randomly assigned to three groups</td>
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</table>
| Intervention details: | - The treatments consisted of administration of 0.9% NaCl and/or 0.5% bupivacaine with epinephrine either by the IP and/or INC route.  
- Control group (n=10) – saline solution IP and INC.  
- IP group (n=10) – bupivacaine 5 mg/kg IP and saline solution INC.  
- INC group (n=10) – saline solution IP and bupivacaine 1 mg/kg INC.  
- Acepromazine 0.05 mg/kg and butorphanol 0.2 mg/kg as premedication.  
- Thiopental 10 mg/kg intravenously for induction.  
- Blinded single surgeon performed all the procedures and the administration of the solutions. |
**Study design:**
Prospective, blinded, randomised clinical study

**Outcome studied:**
- During postoperative period, any signs of local anaesthetic toxicity were recorded.
- Postoperative pain and sedation were evaluated 1, 2, 3, 4, 6, 12 and 24 hrs after extubation. It was evaluated through NRS from 0–29 and VAS from 0–10.
- VAS scores for both pain and sedation were performed without interaction with the dog.
- Non-interactive parameters were evaluated before interactive parameters during NRS scoring.
- Client-owned rescue dogs continued to be scored throughout the 24 hr observation period and data obtained from these dogs were included in the statistical analysis.

**Main findings:**
(relevant to PICO question):
- 28 mixed-breed and 2 Rottweilers were included in the study
- Sedation scores did not differ amongst groups throughout the observational period.
- Sedation was not evident in any of the dogs at 24 hrs.
- At 1 hr, VAS pain scores were lower in IP compared to the control group (p<0.05). Medians were: 6.4 in control group, 0.3 in IP group and 0.0 in INC group.
- From 2–24 hrs, VAS and NRS scores did not differ amongst groups.
- Rescue analgesia was given to 7/10, 3/10 and 5/10 dogs in control, IP and INC groups during the first 24 hrs respectively.
- Dogs in control group (7/10) tended to receive rescue analgesia one hr after anaesthesia than in dogs in the IP group (3/10) and INC group (5/10).
- Rescue analgesia was administered more than once in 3/7, 2/3 and 5/5 dogs in the control, IP and INC groups.
- Administration of IP bupivacaine provided adequate analgesia in most dogs.
- Pain scores were lower in dogs given the combination of IP and INC bupivacaine than in the control group.
- Anaesthesia with acepromazine, butorphanol, thiopental and halothane seemed not to provide adequate analgesia in this study as the pain scores in the control group were higher than IP and INC groups and supplemental analgesics were needed during the postoperative period.

**Limitations:**
- Small number of subjects in each group without power calculation.
- Client owned rescue dogs usually excluded from the study as this may affect the overall results.
- Large volume of bupivacaine is necessary for the IP administration as the dose of bupivacaine was five times higher than that used in the INC group.
### Morgaz et al. (2014)

<table>
<thead>
<tr>
<th>Population</th>
<th>Female dogs undergoing ovariohysterectomy</th>
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<tbody>
<tr>
<td>Sample size</td>
<td>38 dogs randomly allocated in two groups</td>
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**Intervention details:**
- Lidocaine group (L) n=19 – received a continuous lidocaine infusion (2 mg/kg/hr) through a wound catheter in the peritoneal space.
- Control group n=19 – received methadone at 0.2 mg/kg IM every 4 hours after premedication.
- Medetomidine at 3 μg/kg IM and methadone at 0.3 mg/kg IM were given as premedication.
- Induction with propofol and maintenance of anaesthesia with isoflurane.
- Rescue analgesia with methadone at 0.3 mg/kg IV was given when CMPS-SF score >6 or DIVAS >50mm.

**Study design:**
- Prospective, non-blinded, randomised clinical study

**Outcome studied:**
- HR, RR, EtCO₂, SpO₂ and non-invasive arterial blood pressure were recorded.
- Pain assessment at baseline, 2, 4, 6, 18 and 24 hrs after the end of anaesthesia.
- Three pain assessment systems: DIVAS, Glasgow scale (CMPS-SF) and MWTs.
- Sedation assessment at baseline, 2, 4, 6, 18 and 24 hrs.
- Lidocaine and cortisol levels were measured at baseline, 2, 6, 18 and 24 hrs after the completion of the surgery.

**Main findings:**
- No difference in analgesic parameters between the two groups.
- In control group, methadone was given at 1 and 5 hrs after the end of the surgery.
- Rescue analgesia was given to 4/19 animals in control group and 0/19 in the L group.
- Mean lidocaine continuous rate infusion (CRI) volume was 3.17 ± 0.89 mL/hr.

**Limitations:**
- Absence of blinding.
- Two dogs removed the wound catheters before the end of the study.
- Dysphoria was the most frequent adverse effect.
- No statistically significant differences between the groups.

### McKune et al. (2014)

<table>
<thead>
<tr>
<th>Population</th>
<th>Healthy female dogs undergoing ovariohysterectomy</th>
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<tr>
<td>Sample size</td>
<td>59 dogs randomly allocated in to three groups</td>
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</table>

**Intervention details:**
- All three groups were sedated with acepromazine at 0.03 mg/kg SC.
- Induction with propofol and maintenance with isoflurane.
- L/B (lidocaine/bupivacaine) group (n=20): line block prior to the incision with lidocaine at 4 mg/kg and 1 mg/kg of...
bupivacaine. Saline at 0.05 mg/kg SC was given at the same time with acepromazine.

- **L/BM (lidocaine/bupivacaine-morphine) group (n=19):** line block prior to the incision with lidocaine at 4 mg/kg and 1 mg/kg of bupivacaine. Morphine at 0.5 mg/kg SC was given at the same time with acepromazine.

- **SS (normal saline/normal saline) group (n=20):** 0.275 ml/kg of normal saline prior to surgery in the INC area and 0.05 mg/kg of saline SC at the same time with acepromazine.

- Pain was assessed prior to the sedation (time negative zone), zero time (time of extubation), 2, 4, 6, 8 and 24 hrs post-surgery.

- One blinded veterinarian.

- Rescue analgesia was morphine at 0.5 mg/kg IM and given to:
  - any animal that achieved a maximum score in any one category of the GCPS;
  - any animal with a pain score of 8 or greater on the GCPS;
  - any animal who did not improve over time as compared to pre-sedation GCPS score;
  - any animal developing aggression;
  - or any animal with a combination of these previous factors.

<table>
<thead>
<tr>
<th><strong>Study design:</strong></th>
<th>Prospective, randomised, blinded clinical trial</th>
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<tbody>
<tr>
<td><strong>Outcome studied:</strong></td>
<td>HR, RR and systolic blood pressure were recorded Four pain score systems: VAS, Glasgow scale (GCPS), UMPS and von Frey</td>
</tr>
</tbody>
</table>
| **Main findings:**  
(relevant to PICO question): | • 20/59 dogs required rescue analgesia (7/20 in L/B group, 3/19 in L/BM group and 10/20 in SS group).  
• VAS, GCPS and UMPS analyses showed no significant differences in pain score systems between groups.  
• No statistically significant difference between the positive (L/BM) and negative group (SS) at any given time point. |
| **Limitations:** | • No significant difference between pain scores for any treatment group as the sample size was small. The initial sample size calculations hindered the study in two ways. In order to correctly calculate the initial sample size, the p-value should be corrected to 0.017 and this was not done.  
• Low pain scores may be due to inherent insensitivity of the measurement techniques preventing a significant difference between negative and positive controls.  
• Von Frey may not be appropriate for assessing sensitivity of clinical wounds according to previous studies. |
Appraisal, application and reflection

The purpose of this Knowledge Summary was to look at the evidence for the use of bupivacaine through the line block in dogs undergoing midline coeliotomy. Seven papers were identified as relevant to this question.

Five out of seven were blinded clinical trials (Carpenter et al., 2004; Savvas et al., 2008; Fitzpatrick et al., 2010; Campagnol et al., 2012; and McKune et al., 2014), four out of seven were prospective clinical studies (Carpenter et al., 2004; Campagnol et al., 2012; Morgaz et al., 2014; and McKune et al., 2014) and one was an experimental, pharmacology, non-blinded, non-controlled study.

Only one study (Savvas et al., 2008) evaluated the effectiveness of pre-emptive incisional block with bupivacaine on postoperative pain and opioid requirements after coeliotomy in dogs. According to this, preoperative wound infiltration with bupivacaine is an effective technique to reduce postoperative pain but further studies are needed.

Back to 2004, there were two clinical studies. The first one, Wilson et al. (2004), determined the safe dose of intraperitoneal and incisional lidocaine applied in dogs undergoing ovariohysterectomy but no evaluation of pain scores was reported. The second was by Carpenter et al. (2004) where they found that intraperitoneal and incisional bupivacaine provided effective analgesia following ovariohysterectomy in dogs. In contrast, Fitzpatrick et al. (2010) found that there is no any additional analgesic benefit of infiltration with bupivacaine at the incision site, given either pre-emptively or following surgery when used as part of multimodal analgesic protocol for dogs undergoing routine ovariohysterectomy.

Another study (Campagnol et al., 2012) compared the effect of intraperitoneal or incisional bupivacaine on pain and analgesic requirement after ovariohysterectomy in dogs without any statistical difference.

There was only one clinical trial (Morgaz et al., 2014) where lidocaine was used through the intra-peritoneal continuous wound infusion for pain control following ovariohysterectomy in dogs, but the results were not statistically significant as the postoperative analgesia was similar to the analgesia provided by methadone.

Lidocaine and bupivacaine were used as a pre-incisional local anaesthetic block in dogs undergoing ovariohysterectomy (McKune et al., 2014). However, even in this prospective, blinded, randomised clinical trial, there were no firm conclusions about whether or not this line block is effective.

In conclusion, it is not clear if the use of a local anaesthetic including bupivacaine or lidocaine as incisional blocks minimises the postoperative pain especially in the first 24 hours, as the results are not statistically significant between the groups. As the sample sizes were so small and multiple analgesics were used in the clinical trials, further studies are necessary.
Methodology Section

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<th>Search Strategy</th>
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<tr>
<td><strong>Databases searched and dates covered:</strong></td>
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<td><strong>Search terms:</strong></td>
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<td><strong>Dates searches performed:</strong></td>
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<th>Exclusion / Inclusion Criteria</th>
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<td><strong>Total relevant papers when duplicates removed</strong></td>
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


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