1. PURPOSE

This Standard Operating Procedure (SOP) intends to describe methods of assessing pain in rabbits and mitigating pain by administering analgesic medications.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. GENERAL CONSIDERATIONS

3.1. A procedure expected to be painful in humans is considered painful in animals.

3.2. When there is a question of whether a procedure is painful, the animal should benefit from analgesia.

3.3. Analgesia should be provided at an appropriate dose and frequency to control pain.

3.4. Any deviation from this procedure must be justified by the investigator and approved by the BGU ethical committee.

4. PAIN RECOGNITION AND ASSESSMENT

4.1. Adapt the observation frequency to the procedure's invasiveness (minimum once a day).

4.2. Start by observing the animal from a distance, so the observer's presence does not alter the animal's behavior. Then proceed to observe the animal more closely.

4.3. Look for any changes in the behavior. Report animals that appear to be in pain to the vets.

4.4. Common clinical signs of pain or distress include (but are not limited to): avoidance, vocalization, aggressiveness, teeth grinding, isolation from the group, low spontaneous activity, hunched posture, lack of grooming, reduced appetite, and weight loss, and no feces in pen.

Note: The most reliable signs of pain and distress are changes in behavior and no manipulation of enrichment materials.

4.5. The Rabbit Grimace Scale (Keating et al. 2012): A poster demonstrating the scale is posted in Appendix 1.

5. ANALGESIA PLAN

5.1. Specify the analgesia plan in your animal protocol.

5.2. Provide analgesia just before the painful stimulus whenever possible, as it is more effective in preventing
5.3. Use a combination of analgesics, often more effective than a single agent.

5.4. Extend analgesia from pre-op to 72 hours post-op for surgical procedures unless specified otherwise in the Animal Use protocol and approved by the BGU Ethical committee.

6. LOCAL ANESTHESIA

6.1. Infiltrate or apply local analgesics to areas where a painful stimulus may be induced. Repeat the application of local agents at specified intervals to maintain analgesia. In some cases, a sedative is recommended when using local analgesia.

<table>
<thead>
<tr>
<th>Analgesic</th>
<th>Dose</th>
<th>Route</th>
<th>Duration</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>&lt; 2 mg/kg</td>
<td>SC, Infiltration of surgical wounds</td>
<td>30–60 min.</td>
<td>Use lidocaine HCl 2% (20mg/ml) injectable solution. Because this drug is acidic, it is recommended to dilute it 3:1 with sodium bicarbonate injectable solution (at 5 or 8.4%). Dilution must be prepared immediately before use and should not be stored. A diluted solution is as effective, but induction of analgesia is slightly prolonged. *Dilution with sodium bicarbonate is unnecessary if lidocaine is administered to an anesthetized animal.</td>
</tr>
<tr>
<td>EMLA cream</td>
<td>Thick spread</td>
<td>Topical</td>
<td>30–60 min.</td>
<td>Apply only to intact skin. Shave or pluck the fur. Ideally, 10 minutes before the painful procedure</td>
</tr>
<tr>
<td>Localine eye drops</td>
<td>1-2 drops</td>
<td>Ocular</td>
<td>30-60 min.</td>
<td></td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>&lt; 2 mg/kg</td>
<td>SC, Infiltration of surgical wounds</td>
<td>3–4 hrs.</td>
<td>Use bupivacaine HCl 0.50% (5mg/ml) injectable solution. Same comment as for lidocaine.</td>
</tr>
</tbody>
</table>

7. SYSTEMIC ANALGESIA

7.1. Administration of non-steroidal anti-inflammatory drugs (NSAIDs):

7.1.1. NSAIDs include carprofen, ketoprofen, meloxicam, and dipyrone.

7.1.2. Ensure good water intake and monitor hydration status during the treatment period.

7.1.3. Suspend water restriction before administration of NSAIDs.

7.1.4. Do not administer NSAIDs to neonatal rodents.
<table>
<thead>
<tr>
<th>Analgesic</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.1 mg/kg</td>
<td>SC</td>
<td>4–8 hrs.</td>
<td>Mild to moderate pain. Controlled drug.</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.65 mg/kg</td>
<td>SC</td>
<td>72 hrs</td>
<td>Mild to moderate pain. Controlled drug.</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2 mg/kg</td>
<td>SC, PO</td>
<td>12–24 hrs.</td>
<td>Use carprofen 50mg/ml injectable solution. To prepare a 4mg/ml dilution: add 0.8 ml of carprofen 50mg/ml to 9.2 ml of sterile water for injection. Administer 5µL/g of body weight. Store at room temperature. Discard dilution after one month.</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1.5 mg/kg</td>
<td>SC, PO</td>
<td>24 hrs.</td>
<td>Mild to moderate pain.</td>
</tr>
<tr>
<td>Ketoprophene</td>
<td>15 mg/kg</td>
<td>SC</td>
<td>12-24 hrs.</td>
<td>Mild to moderate pain.</td>
</tr>
<tr>
<td>Dipyroone</td>
<td>50 mg/kg</td>
<td>PO</td>
<td>In water bottle</td>
<td>Mild to moderate pain.</td>
</tr>
</tbody>
</table>

8. Appendix

Appendix 1: The Rabbit Grimace Scale

SOP 105 RABBIT ANALGESIA
Written on (dd,mm,yyyy) 01.11.2022
Revised on (dd,mm,yyyy) 13.03.2023
Approved by the BGU Animal Policy and Welfare Oversight Committee