What if you see ...?

Analgesics must be administered as outlined in the IACUC-approved protocol. When “as needed” analgesics are specified, animals must be monitored for signs of pain, and treated accordingly.

Listed below are easily identifiable indicators of pain in mice. This is neither a comprehensive nor specific list, and as such, other observations should be taken into account when assessing pain status in mice. Please contact ULAM veterinary staff for additional assistance with identification and/or treatment of pain.

**Appearance**

<table>
<thead>
<tr>
<th>Facial Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbital Tightening</td>
<td>There will be a narrowing of the orbital area, with a tightly closed eyelid or an eye squeeze (denoted by a wrinkle around the eye).</td>
</tr>
<tr>
<td>Nose Bulge</td>
<td>A rounded extension of skin is visible on the bridge of the nose.</td>
</tr>
<tr>
<td>Cheek Bulge</td>
<td>There will be a convex appearance of the cheek muscle (between the eye and whisker) from its baseline position.</td>
</tr>
<tr>
<td>Ear Position</td>
<td>Ears pulled apart and back from their baseline position or featuring vertical ridges that form owing to tips of ears being drawn back.</td>
</tr>
<tr>
<td>Whisker Change</td>
<td>Movement of whiskers from their baseline position either backward, against the face or forward, as if standing on end; may also clump together.</td>
</tr>
</tbody>
</table>

**Behavior**

- When mice are experiencing pain, they may demonstrate the following abnormal behaviors:
  - Appetite
    - Reduced food and/or water intake
  - Activity and Posture
    - Reluctance to move
    - Decreased interaction with the environment and/or cage mates
    - Reduced grooming activity
    - Decreased cage organization
    - Hunched posture
    - Increased aggressiveness and/or self-mutilation

**Nest Building**

Static cage side assessment of nesting behavior via nest score:

- **Score 0**: Nestlet not manipulated, possibly dragged around cage.
- **Score 1**: Nestlet slightly manipulated, more than 80% intact, possibly a few shreds picked out.
- **Score 2**: Nestlet noticeably manipulated, less than 80% intact, shreds spread around in one area.
- **Score 3**: Nestlet noticeably manipulated, shreds placed mostly in nest site, hollow building +/- walls.
- **Score 4**: Flat nest, hollow in bedding, walls mainly higher than mice and encasing the nest less than 50%.
- **Score 5**: Complex nest, more than 50% shreds picked out, bowl-shaped nest, walls higher than mice and encasing the nest by more than 50%.

Dynamic cage side tests for pain-related depression of nesting:

**Zone Clearance Test**

1. Remove the existing nest.
2. Cut a nestlet square into 6 pieces and place at 6 zones as pictured below.
3. Measure how many of these zones were cleared after 100 minutes.

Possible scores range from 0 to 5.

**Time to Integrate into Nest Test (TINT)**

1. Add a quarter of a nestlet to the mouse cage.
2. Observe the nest building behavior immediately thereafter.

**Positive TINT** = the square is missing from its original location after 10 minutes.

**Negative TINT** = square remains in the original location after 10 minutes and so, additional observations are needed to determine if the mice require veterinary attention.

For information on how to carry out this test, please watch the following video:

https://www.youtube.com/watch?v=S1012

**References**