Protocol for density gradient centrifugation of Bovine sperm

Materials required
- BoviPure™
- BoviDilute™
- BoviWash™
- Conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor

Components (for eg. 10 mL)

<table>
<thead>
<tr>
<th>Component</th>
<th>Dilution titers</th>
<th>Top layer</th>
<th>Bottom layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BoviPure™</td>
<td>4 mL</td>
<td>8 mL</td>
<td></td>
</tr>
<tr>
<td>BoviDilute™</td>
<td>6 mL</td>
<td>2 mL</td>
<td></td>
</tr>
</tbody>
</table>

Protocol
Depending on the volume ejaculate to process, different sizes of tubes can be used. See table below.

<table>
<thead>
<tr>
<th>Ejaculate size</th>
<th>Tube size (mL)</th>
<th>Bottom layer volume (mL)</th>
<th>Top layer volume (mL)</th>
<th>Extended ejaculate volume (mL) on the gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>10-15</td>
<td>2</td>
<td>2</td>
<td>1-3</td>
</tr>
<tr>
<td>Medium</td>
<td>50</td>
<td>5</td>
<td>5</td>
<td>3-4</td>
</tr>
<tr>
<td>Large</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>7-8</td>
</tr>
</tbody>
</table>

- Prepare the appropriate amount of solutions according to the instructions above.
- Bring all materials to room temperature.
- Extend the ejaculate with BoviWash™ (max conc 250 million/mL).

1. Using a sterile pipette, transfer BoviPure™ Bottom Layer to a conical centrifuge tube (see volumes and sizes above).
2. Using a new sterile pipette, layer BoviPure™ Top Layer carefully over the BoviPure™ Bottom Layer, taking care not to disrupt the gradient layers.
3. Layer extended semen on top of the gradient taking care not to disrupt the layers.
4. Centrifuge at 300 g for 20-25 minutes (longer time for larger volumes) at room temperature in a centrifuge with a swing-out rotor.
5. Carefully remove ejaculate, BoviPure™ Top Layer and most of the BoviPure™ Bottom Layer.
6. Using a new sterile pipette, transfer and resuspend sperm pellet in 1 mL BoviWash™ in a new sterile tube (10-15 mL).
7. Dilute to desired concentration with BoviWash™.

Note: we recommend preparing two BoviPure™ gradients for each sample, to reduce the risk of overloading a single gradient and to provide two tubes to balance the centrifuge rotor.