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## IVF Bioscience Bovine Slaughterhouse Protocol

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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:** bovine

## Troubleshooting

## Aspiration and IVM




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### Dish and Media Preparation

- 1.1 Preheat the Wash medium to  36 °C
- 1.2 Prepare and equilibrate IVM wells at  38.8 °C in 6% carbon dioxide in a humidified atmospheric air (21% oxygen) for 02:00:00 before use
- 1.3 Add  500 µL of BO-IVM medium per well to 4-well plates without an Oil overlay and place in the incubator
- 1.4 Additionally, prepare one 35 millimetre dish with  3 mL of BO-IVM per 4-well plate and place in the incubator without an Oil overlay




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### Aspiration

- 2.1 Set the heating plate to  34 °C and connect the vacuum equipment
- 2.2 Warm the saline solution to  32 °C
- 2.3 Take note of the arrival time and temperature of the ovaries
- 2.4 Wash the ovaries in the warmed saline and hold in a thermo-container of warmed saline
- 2.5 Add  140 µL of heparin to the 50 ml tube prior to aspiration

- 2.6 Aspirate all follicles between 2-15 millimetres using an 18-guage needle connected to the pump system. The opening of the needle should face downwards
- 2.7 Aspirate no more than  20 mL of follicular fluid per tube and note the number of ovaries aspirated on the lid of the tube
- 3 Collection and Maturation
  - 3.1 Use 9 cm Petri dishes marked with a square grid pattern
  - 3.2 Prepare one dish per tube of follicular fluid. Mark the dish with the corresponding tube number.
  - 3.3 Add  7 mL of Wash to each dish
  - 3.4 Prepare three 35 millimetre dishes with  2.5 mL of Wash in each. Mark these dishes as 1, 2 and 3
  - 3.5 Transfer the oocyte pellet from the bottom of the 50 ml tube into the larger petri dish using a plastic transfer pipette
  - 3.6 Search through the dish systematically and transfer all oocytes to the smaller dish marked 1
  - 3.7 Repeat the transferring of the oocyte pellet and search, twice for each tube
  - 3.8 Once all oocytes have been collected, wash through dish 2 and then 3. Count the number of collected oocytes
  - 3.9 Rinse once through the 35 millimetre dish containing BO-IVM
  - 3.10 Once rinsed, transfer the oocytes to the 4-well plate containing BO-IVM. Do not transfer more than 45 oocytes per  500  $\mu$ L well.



3.11 Incubate at  38.8 °C and 6% carbon dioxide in humidified atmospheric air (21% oxygen) for between  21:00:00 and  24:00:00 .