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IVF Bioscience Bovine Slaughterhouse Protocol

Jake Silcock¹

¹IVF Bioscience

Jake Silcock

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Protocol status: Working
We use this protocol and it's working

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Aspiration and IVM

1

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

2

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 µL 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 .