# IVF Bioscience Bovine Slaughterhouse Protocol

Jake Silcock[1]

[1]IVF Bioscience

Jul 02, 2019

Working dx.doi.org/10.17504/protocols.io.4rrgv56

Jake Silcock

## 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** .