Vacuuming, Selection and Maturation of Oocytes

The excess tissue was removed from the ovaries with the aid scissors before they were stored in the thermos. Ovaries that had a risk of contamination were discarded. The temperature was kept in check between 25-35°C so that it would not affect the ovaries.

The ovaries were rinsed with saline solution 0.15 M in order to remove as much of the excess blood as possible. This process was repeated and they were covered in the same solution. The recipient was kept closed at all times and transported at room temperature for no more than 2 hours.

For the follicle vacuuming the following preparations were done:

Two dissection trays were cleaned with 70% ethanol and dried with paper towels. They were afterwards covered with paper towels. The vacuuming equipment was set up using a low-power vacuum machine (pressure should be set 2 to 5 mmHg, as it being any higher the oocyte-cumulus cell complex COC's could be separated). An air distributor was connected to two hoses directly connected to the machine. To the air distributor were connected two thinner hoses, which were connected to the stoppers covering the 50 mL Corning tubes. Sterile18G x 1.5 in needles were attached with the bevel side up to the stoppers as well. Before starting the vacuuming process, hands were washed and sanitized and new nitrile gloves were cleaned with 70% ethanol and rinsed with saline solution.

The follicles were vacuumed avoiding direct contact with the needle, that is to say, the needle piercing adjacent areas toward the follicle in order to avoid its rupture and subsequent loss of its content. Once inside, they were vacuumed with circular motions. Follicles that were larger than 10 mm were avoided since they were very likely to contain mature oocytes, which were not needed.

The stoppers were then removed from the corning tube, which were closed with their respective caps and immediately put on a thermic plate.

Using a stereoscope, the oocytes were selected based in morphological characteristics such as having 3-4 compact cumulus cells layers, separated in groups of 50 and stored in a maturing medium for 24 hours.

For the cleansing procedure for the removal of cumulus cells from the mature oocytes petri dishes 35x10 mm were taken and 70 μl drops of PBS-PVP were scattered through its surface. Groups of 10-20 oocytes were taken from the 4 welled nunclon dish with IVM medium were they were stored and were the deposited into 0.5 mL Eppendorf tubes. The tubes were then agitated by vortex for 1 min, after this was done the outside walls were rinsed with PBS-PVP in order to detach the oocytes from the walls. The cell pellet is then removed and placed in one of the PBS-PVP drops under a stereoscope. The cumulus cells were cleansed from the oocyte through pipetting repeatedly, making sure to change from drop when the drop begins to get contaminated with cumulus cells. The clean oocytes were then taken and stored in a 0.5 mL Eppendorf tube.