FR-AgEncode: a French pilot project to enrich the annotation of livestock genomes

Tissue sampling protocol 3d

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This protocol describes the preparation of oocytes and granulosa cells from ovaries of cows, gilts and goats.

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Objective: to isolate ovocytes and granulosa cells from ovaries of mammals

Reagents

phosphate-buffered saline (PBS)
PBS supplemented with 0.1% bovine serum albumin (BSA)

Instruments

Stereomicroscope
Centrifuge
scalpels, scissors, forceps, tweezers
Platinum loop
Hamilton syringe equipped with a glass capillary
P100 micropipetor
Petri dishes
Pre-labelled 2mL cryotubes
15 mL Falcon tubes
liquid nitrogen in a dewar

Procedure

From the ovaries, follicles were dissected out, measured, grouped according to their diameter and counted. The follicle size varied from 3 to 6 mm in gilt, from 3 to 20 mm in cow, from 2 to 8 mm in goat.

Each follicle was slit with a scalpel and the oocyte-cumulus complex searched in the follicular fluid under a stereomicroscope, then washed in phosphate-buffered saline (PBS) supplemented with bovine serum albumin (BSA). The oocyte-cumulus complex was transferred to a 100 µl PBS-BSA drop and the oocyte was separated from cumulus cells by repeated pipetting. After transfer to three successive PBS-BSA-containing Petri dishes, oocyte denudation was carefully checked under the microscope. Pools of 5 (occasionally 3 to 12) denuded oocytes were transferred to cryotubes and snap frozen in liquid nitrogen.

Granulosa cells were obtained from two 6-8 mm goat follicles or from 10-20 mm follicles for an individual cow. The follicle inner wall was scraped gently and repeatedly with a platinum loop to collect granulosa cells in 300 µl PBS. For small follicles (<10mm), the cell suspensions from 4 to 7 follicles were pooled, whereas one cell suspension was obtained for each large follicle (>10mm).
Each cell suspension was transferred to a cryotube, centrifuged down for 2 minutes at 2000 g, the supernatant was removed and the cells were snapfrozen.

NB: Corpus luteum (for cow and goat only, as none was observed in gilt) was also dissected out. For the ovarian cortex and the corpus luteum, refer to protocol 1.