Standard operating procedure to isolate bovine oocytes including IVF and metabolic challenge

Research Institute for Farm Animal Biology (FBN)

Institute of Reproductive Biology

RESEARCH INSTITUTE FOR
FARM ANIMAL BIOLOGY

Kits & Chemicals

- PBS (w/o Ca^{2+} and Mg^{2+})
- Pen/Strep
- Wash – Oocyte and embryo wash medium (IVF Bioscience, #51002)
- BO-IVM (IVF Bioscience, #71001)
- BO-IVF (IVF Bioscience, #71004)
- BO-SemenPrep (IVF Bioscience, #71003)
- BO-IVC (IVF Bioscience, #71005)
- Oleic acid
- BSA (fatty acid free)
- Polyvinyl alcohol (PVA)

Isolation and culture of oocytes

1. collect ovaries in DPBS + Pen/ Strep at 37°C
2. aspirate cumulus-oocyte-complexes (COCs) from 2 to 6 mm follicles in Wash – Oocyte and embryo wash medium (IVF Biocsience, #51002) using a 0.8 x 16 mm needle
3. select only COCs surrounded by a closed multicellular cell layer of cumulus cells under microscopic control
4. let oocytes maturate *in vitro* in BO-IVM (IVF Bioscience, #71001) for 22h at 38.8°C and 6% CO_2

***In vitro* fertilization and culture of embryos**

1. thaw and wash sperm in BO-SemenPrep (IVF Bioscience, #71003)
2. fertilize mature oocytes in BO-IVF (IVF Bioscience, #71004) at 38.8°C and 6% CO_2 overnight using $2x10^6$ thawed spermatocytes per ml medium
3. denude fertilized oocytes by vortexing in Wash – Oocyte and embryo wash medium (IVF Biocsience, #51002) for 2 min
4. culture fertilized oocytes in BO-IVC (IVF Bioscience, #71005) at 38.8°C, 6% CO_2 and 6% O_2

Metabolic challenge

1. <u>for challenge with oleaic acid (OA)</u>: culture denuded fertilized oocytes in BO-IVC (IVF Bioscience, #71005) with 100µM oleic acid conjugated with BSA at 38.8°C, 6% CO_2 and 6% O_2
2. <u>as BSA control</u>: culture denuded fertilized oocytes in BO-IVC (IVF Bioscience, #71005) with 0.2% BSA at 38.8°C, 6% CO_2 and 6% O_2

Collection of samples for RRBS

1. after 7 to 9 days post fertilization collect hatched blastocysts
2. wash collected blastocysts twice in PBS with 0.3% polyvinyl alcohol (PVA) at 37°C
3. wash collected blastocysts twice in PBS
4. transfer collected blastocysts with as little as possible PBS into a new tube and shock freeze in liquid nitrogen until further analysis