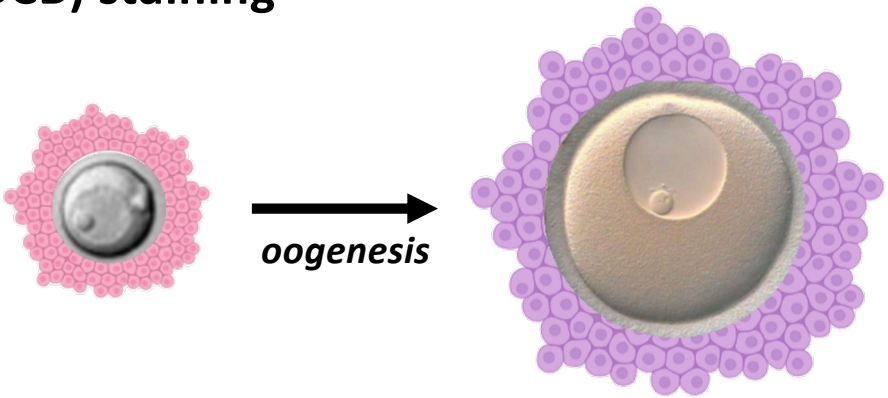
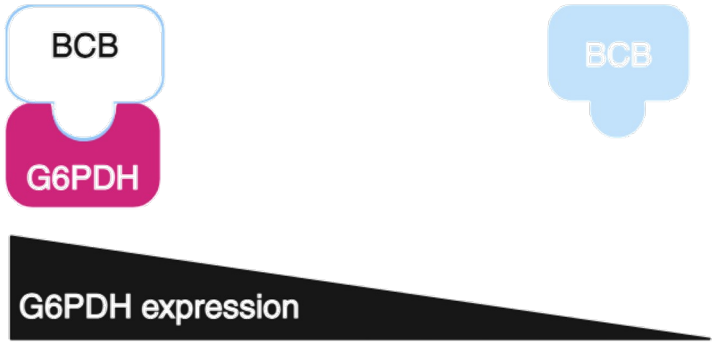
Oocyte Quality Assessment by Brilliant Cresyl Blue (BCB) staining

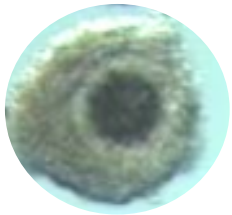
AIM OF THE STAINING:
Used to select more competent oocytes prior to IVM in various species, including pigs, mice, goats, cattle, and buffaloes

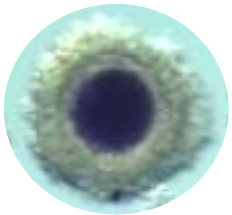
HOW BCB WORKS:
The staining allows for the determination of the glucose-6-phosphate dehydrogenase (G6PDH) activity, which converts BCB dye from blue to colorless.
The oocytes, which are still in the growth phase, have a high G6PDH activity and show a colorless cytoplasm (BCB-). However, oocytes that have completed their growth have low levels of G6PDH and show a blue coloration of the cytoplasm (BCB+).

Reserch studies have demonstrated that BCB+ oocytes have a significantly higher blastocyst developmental rate than BCB- oocytes, suggesting that the quality of BCB+ oocytes is higher than that of BCB- oocytes.






BCB-


BCB+

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BCB Molecular weight (MW): 385.96 g/mol

- Prepare a BCB stock solution 1mg/mL, this correspond to a concentration of 2.6mM.
  Let's verify:

$$g = MW \times V \times M \longrightarrow M = \frac{g}{MW \times V}$$

- To use the formula we need to express "mg as g" and "mL as L":

1mg/mL = 0.001g/0.001L

- Let's put the values in the formula:

$$M = \frac{0.001\ g}{385.96 \times 0.001L} = 0.0026\ M \longrightarrow 2.6\ mM$$

- The BCB powder should be dissolved in 10mL of PBS at the concentration 1mg/mL to have the stock solution.

How many gr?

…….

- The medium for incubation of CoC with BCB is PBS + 0.5% BSA (mPBS). Prepare 10mL of this solution. How many gr of BSA?

…….

- BCB Working solution for pig CoC → 65µM

Calculate the dilution factor for BCB:

…….

How many ul of BCB stock solution per 10mL of mPBS (to have the BCB working solution)?

…….

WE ARE READY FOR STAINING CoC:

1. Collect CoC in a multi-well plate
2. Wash twice CoC with mPBS (0.5mL each)
3. Move CoC into the BCB working solution and incubate 1h 38°C
4. Wash twice CoC with complete growth medium (alphaMEM + 10% FBS + 1%P/S + 1% Amphothericin)
5. Observe CoC with a stereomicroscope

Oocyte nuclei staining with Propidium Iodide (PI)

AIM OF THE STAINING:
Chromatin configuration characterization to distinguish fully grown oocytes from growing ones

PROTOCOL:

1.Denude the oocytes.

2.Transfer them to PBS.

3.Fix in 1% PFA in PBS for 15 minutes.

4.Wash twice with PBS.

5.Permeabilize with 0.5% Triton X-100 in PBS for 20 minutes.

6.Wash twice with PBS.

7.Stain with 1 mg/mL Propidium Iodide for 30 minutes in the dark. The stock solution is 1

g/mL.

8.Wash twice with PBS.

9.Mount the stained oocytes on a slide and cover with a coverslip.

10.Observe using a fluorescence microscope or a confocal station.