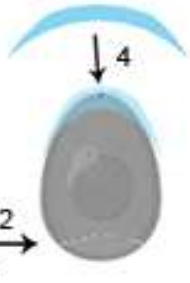
ESM_3 Illustration of the shell-less CAM technique: 1.egg is disinfected; 2. line drawn over the air pocket boundary; 3-4. air hole created at narrow apex and covered with tape; 5-6. egg shell removed within air pocket boundary; 7-9. meniscus formed after removal of tape; 10. Membrane is ruptured; 11.egg contents carefully transferred to dish; 12. dish covered with breathable sheet

1. Disinfect eggs with 70% alcohol or germicidal cloths.

2. On broad apex, spot air sac using a flashlight. Draw a line over air sac boundary.

3. On narrow apex, use an 18G needle to drill a hole. Use slight pressure and rotation motion.



4. Cover small hole with scotch tape.

Air pocket boundary drawn on broad apex.

Hole covered with scotch tape on narrow apex.

5. Turn egg over. Puncture the eggshell using an 18G needle. Remove pieces of eggshell within air pocket boundary using disinfected square tip forceps.

6. Alternatively, first cover broad apex with tape. And use scissors to cut along air pocket boundary.

Eggshell has been removed along air pocket using step5 technique.

7. Turn egg over. Remove scotch tape at narrow apex.

8. Meniscus will form at bottom apex.

9. Position egg on top of and close to the dish.

10. Allow embryo to move up towards narrow apex. Remove membrane with square tip forceps.

11. Allow egg contents to slowly slip onto dish.

Egg contents are transferred to dish.

12. Cover dish with breathable sheet. Return to incubator.