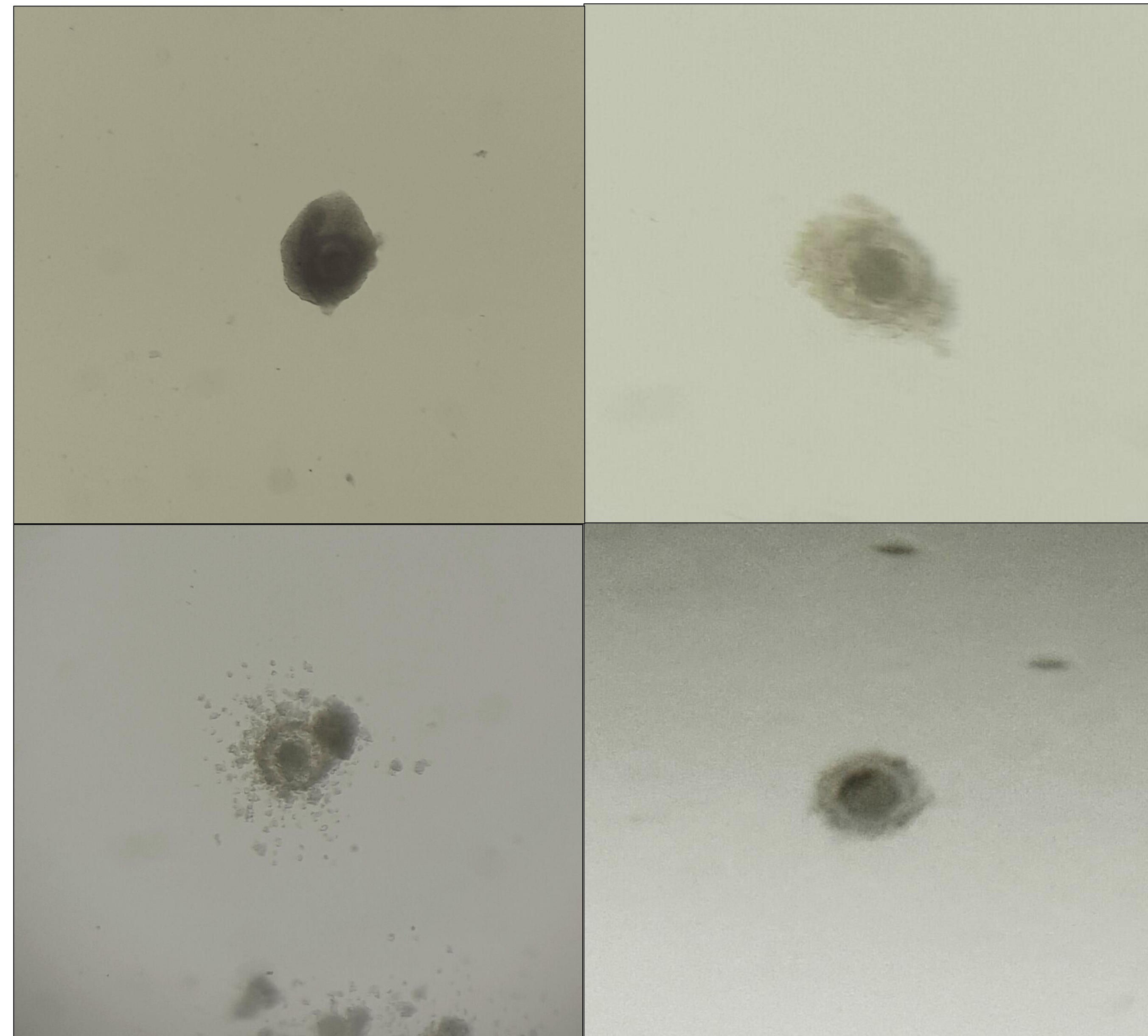


Observation of Fertilized Bovine Oocytes

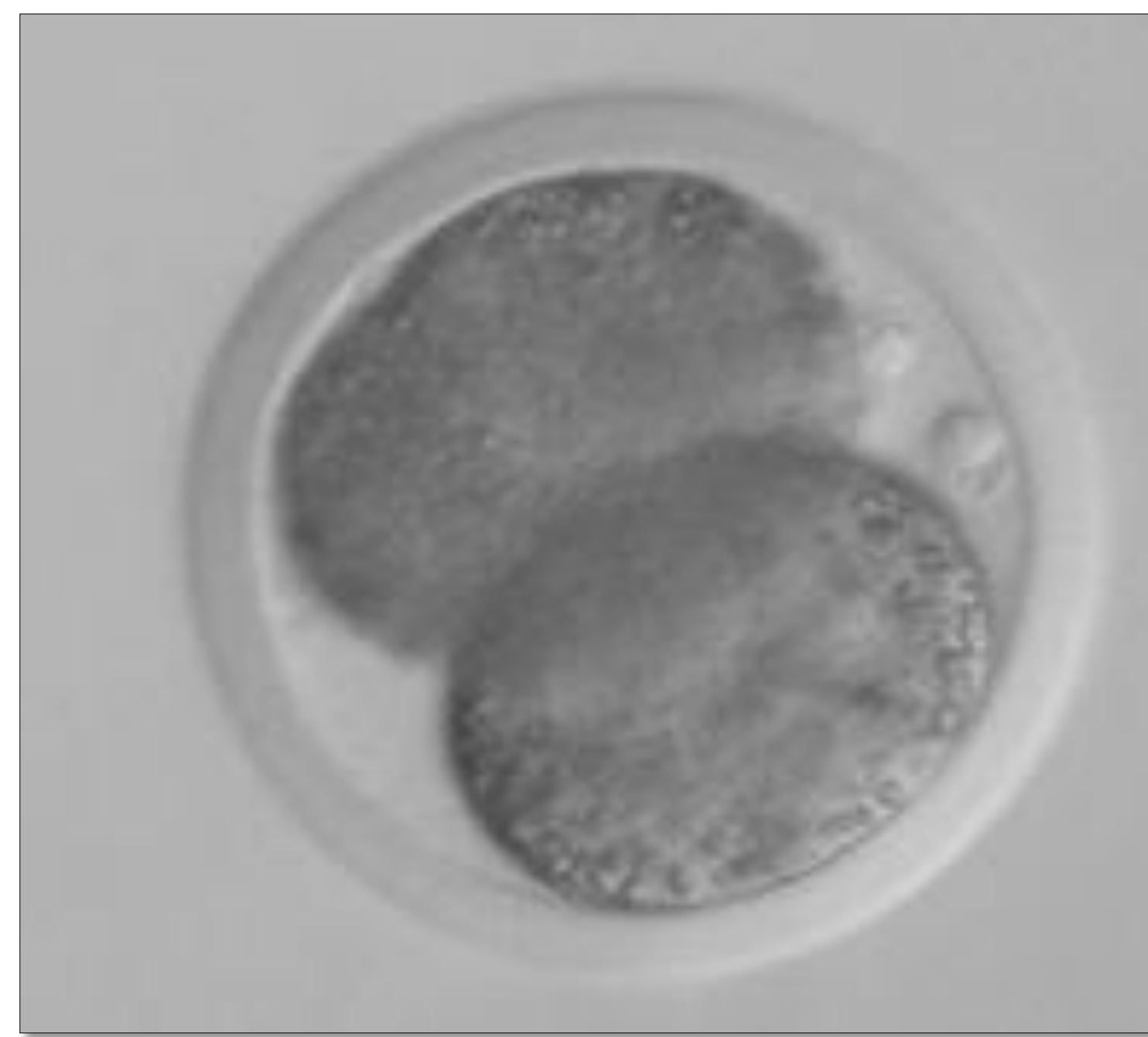
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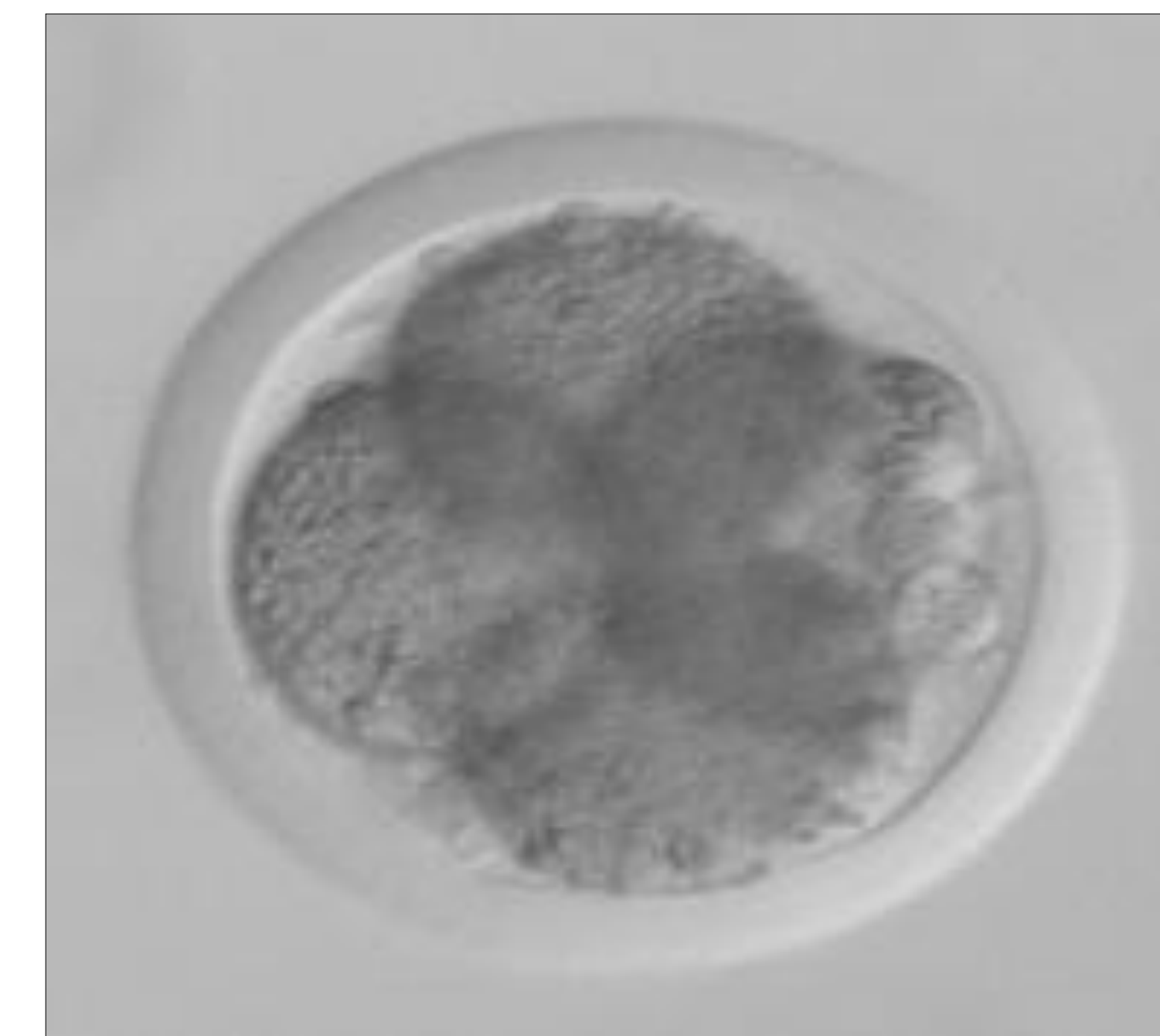


Grade A: 3 or more layers of cumulus cells. Grade B: 1-2 layers of cumulus cells.
Grade C: 1 layer of loose cumulus cells. Grade D: No cumulus cells.

Oocytes were extracted from bovine ovaries by perforation of the follicle wall. Oocytes extracted were chosen for culture based on the cumulus-oocyte complex (COC), graded by the density of cumulus cells on a scale of A-D, in vitro fertilization (IVF) was performed on COC's rated A or B. Healthy COC were placed into a maturation media for incubation at 29 degree C with a 5 percent CO₂ air mixture for twenty four hours. After the maturation period the chosen oocytes were fertilized by IVF using frozen/thawed semen from one bull.



First cell division creating two distinct blastomeres within the trophoblast membrane. Approximately 24 hours after fertilization.



Second division creating four blastomeres. Approximately 36 hours after fertilization.

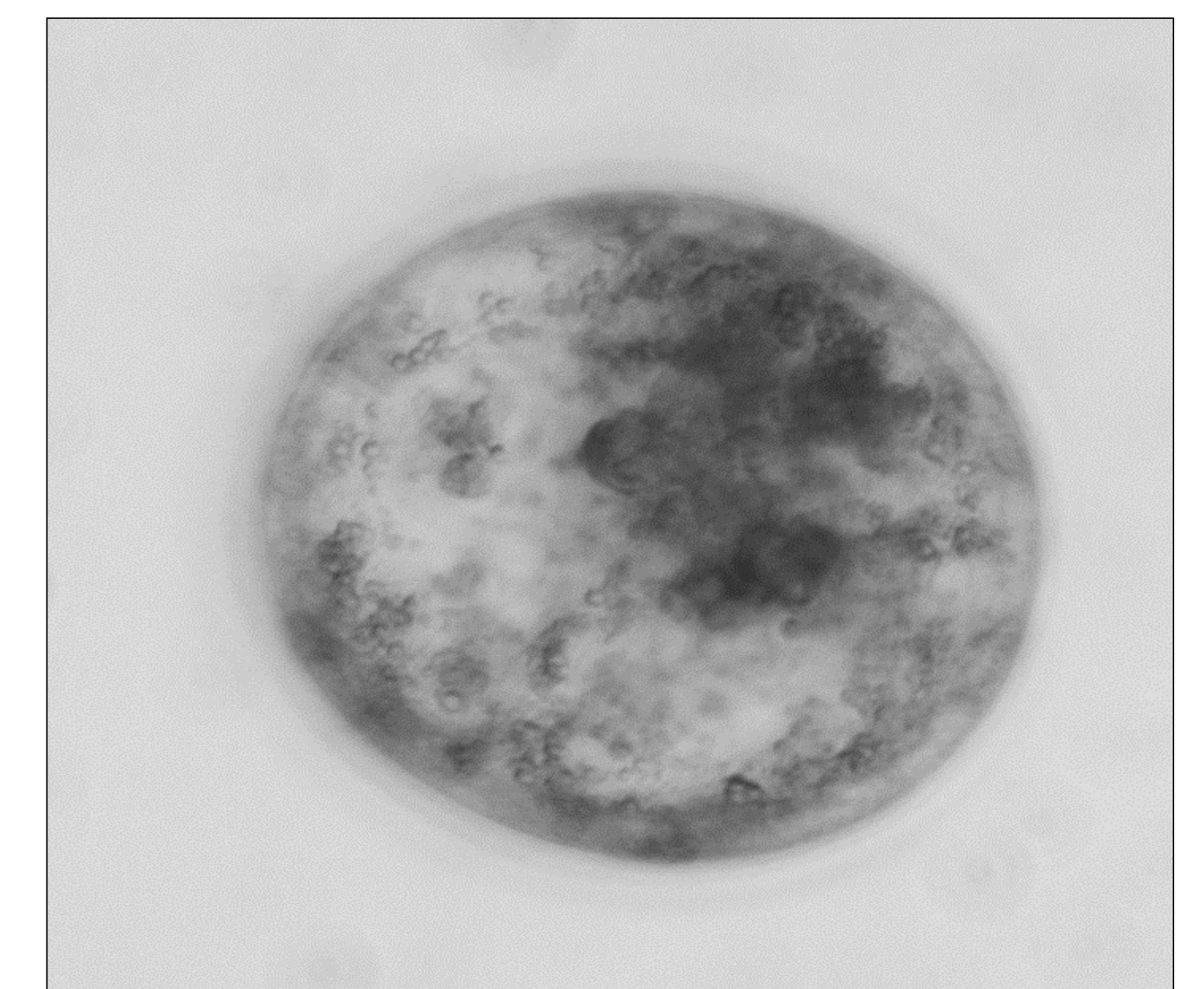


48 to 60 hours after fertilization eight cells can be observed within membrane.

- 24 hours after IVF the zygotes were observed for sperm penetration and the fertilization rate was obtained. First cleavage can be easily observed, which creates two distinct blastomeres within the trophoblast.
- 36 hours after fertilization a third and fourth blastomere develops within the trophoblast dividing along symmetrical planes of cleavage. Cleavage between the blastomeres is known as holoblastic cleavage, or total cleavage between cells.
- A third set of cell divisions creates eight individual cells within the trophoblast. Each cell is roughly the same in size and continues to divide the remaining cytoplasm into smaller portions for the next blastomeres to be developed.
- A fourth stage of cell division creates the morula 72 hours after initial fertilization, which consists of 16 distinct individual cells within the trophoblast.
- Another cell division occurs 96 hours after fertilization and forms a blastocyst, a small cavitation is also visible in early blastocyst development.



72 hours after fertilization a morula is formed. 16 individual cells within the membrane.



84-96 hours after fertilization a blastocyst is visible.