

SUMMARY

Oocytes obtained from ovaries of slaughtered goats were subjected to in vitro maturation (IVM) and fertilization (IVF), and the subsequent embryos obtained were further cultured (IVC) for cleavage. Goat oocyte is spherical in shape, 110-120 μm in diameter, consisted of spherical mass of cytoplasm and surrounded by a thick transparent structure, zona pellucida (zp). Between the cytoplasm and the zp is a fluid-filled space, the perivitelline space (ps).

At 0 hr of incubation in the in vitro maturation (IVM) medium, the cumulus (cu) and corona cells (co) surrounding the zp were tightly packed. The zp had developed although the zonation was not evident. There are penetration by cumulus cell processes, and these processes were also observed in the perivitelline space. Lipid bodies were present but no mitochondria were observed. At 14 hr, the cumulus cells began to expand and loosen, however, the corona cells remains tightly packed. At 20 hr, both types of cells reached maximum expansion. First polar body can sometimes be seen in the perivitelline space (ps) after removal of cumulus cells. The zp had differentiated into thicker and thinner regions. Clusters of membrane-bound electron-transparent bodies were present in the perivitelline space. The mito-

chondria (mi) were fully developed, distributed evenly and usually in close proximity with dilated endoplasmic reticula. Cortical granules were distributed at the periphery. At 40 hr, the zp appeared thinner but with similar zonation pattern. Lipid bodies were larger than in earlier stages. A number of mitochondria were hooded. In CFO, the cumulus and corona cells were absent. At 0 hr, zonation within the zp was indistinct. Very few vesicles and lipid bodies were observed. At 20 hr, the mitochondria were sparsely distributed, lacking cristae and were not well developed; no hooded mitochondria was observed. At 40 hr, the zp was less compact toward the outside. The membrane-bound electron-transparent bodies were less numerous compared to COC. Endoplasmic reticula (ER) were not dilated and with no associated ribosome. The cortical granules were few and had no definite pattern of distribution. The studies on the morphology of developing oocytes suggest that the COCs are more superior than CFOs in terms of fertilizability. The findings also indicate that 20 hr incubation in the IVM medium was sufficient for the attainment of full development of oocytes.

Studies of chromosomes in developing goat oocytes found that at 0 hr of incubation most of the oocytes remained at prophase, particularly at pachytene stage. At 20 to 30 hr, more than 55% COCs attained metaphase II (MII), whereas for

the CFOs the majority (40%) remained in the germinal vesicle breakdown (GVBD) stage and only 20% attained MII. At 40 to 48 hr, 58.5% COCs and 57.6% CFOs developed into MII. From that study, it is concluded that goat COCs, and not CFOs, incubated between 20 to 30 hr, i.e. the time whereby the oocytes were inseminated, were those which would presumably produce higher fertilization rate in the subsequent IVF procedure.

The oocytes inseminated with heparin-treated sperm but without oviductal epithelial cell co-culture system failed to cleave and the rate of fertilization was 0.0%. This suggests that the growth promoting factor(s) that possibly secreted by the epithelial cells is necessary for the fertilization. The oocytes cultured with oviductal epithelial cells but not inseminated failed to cleave. This suggests that the embryos obtained in the present study was not the result of parthenogenesis. 12.5% (7/56) of the oocytes inseminated with heparin-treated sperm and co-cultured with oviductal epithelial cells were fertilized as shown by the formation of 2-cell embryos. Further culture of these embryos resulted into further cleavage in 5.3% (3/56) of them to 4-cell stage. The present study suggests that the penetration of the oocytes by the heparin-capacitated sperm and the role played by the possible growth promoting

factor(s) secreted by the epithelial cells of the oviduct for the development of embryos has occurred. Since there was no further preimplantation embryonic development beyond 4-cell stage, it is possible that goat embryos obtained through the present study undergone a developmental block between 4- and 8-cell stage.