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JOURNAL ARTICLE

Computerized imaging and scanning electron microscope (SEM) analysis of co-cultured fresh and frozen bovine sperm

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Fresh and frozen-thawed bull sperm were incubated with bovine oviductal epithelial cells and segments from the oviducts to examine the usefulness of these culture systems to model sperm changes in vivo. Changes in sperm motion characteristics [computer-assisted sperm analysis (CASA)] and surface morphology [scanning electron microscopy (SEM)] were evaluated. In Experiment 1, fresh and frozen sperm were suspended in the Brackett and Oliphant medium or modified Tyrodes medium (mTALP) and incubated for 0, 3, 6, and 9 hours in direct contact with bovine oviductal-epithelial cell (BOEC) monolayers prepared from oviducts of cows in the periovulatory phase of estrus. The percentage of motile sperm decreased gradually in mTALP, but decreased rapidly in Brackett's defined medium after 3 hours of incubation, with overall averages of 55 and 32%, respectively. The percentage of motile fresh sperm exceeded frozen-thawed sperm under all conditions. In Experiment 2, sperm suspended with mTALP were incubated in dishes without monolayers (control), with monolayers, and within the segments of the oviduct for 0, 3, and 6 hours. In the epithelial cell monolayers, the percentage of motile sperm was similar to the controls throughout incubation, but after 3 hours in the oviductal segments, a decrease, partly associated with more rapid rupture of acrosomal membranes occurred. Sperm velocity was higher (100 microns/second) in fresh sperm than in frozen sperm (85 microns/second). Acrosomal changes, discernible with SEM after 3 hours of incubation, increased with time and were always found more often in frozen than in fresh sperm. The BOEC-monolayer system provided a useful in vitro model to study pre-fertilization changes in sperm.

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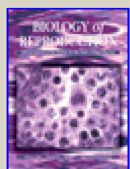

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