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JOURNAL ARTICLE

Addition of specific metabolites to bovine epididymal cell culture medium enhances survival and motility of cryopreserved sperm

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We have developed a cell culture system of bovine epididymal epithelium in which cryopreserved bovine sperm motility was efficiently maintained for many hours. The culture conditions to maintain viable epididymal cells are quite different from conditions normally used to incubate sperm cells. Thus, we have modified a previously described principal cell medium (PCM; Moore et al, 1992) using HEPES as a buffer and supplemented media with myo-inositol, pyruvate, lactate, glycerol, and carnitine to mimic epididymal intraluminal conditions. In the first experiments the effects of PCM and our epididymal cell medium (ECM) on sperm motility were compared in the absence of cells and evaluated by microscopic analysis under a phase contrast microscope or using the Hamilton Thorn Image Analyzer System. Our results showed that motility of cauda epididymal sperm was significantly higher in ECM than in PCM during a 48-hour incubation period when both media were supplemented with 10% fetal bovine serum (FBS). We then replaced FBS with bovine serum albumin (BSA) or no proteins at all to verify if ECM was able to enhance sperm survival. To test this aspect we used frozen-thawed sperm, which survived up to 48 hours when sperm cells were coincubated with epididymal cell monolayers. Hence, PCM, ECM, and different media containing each metabolite of ECM were supplemented with 0.5% BSA to assess motility of thawed sperm after an incubation period of 6 hours. A positive effect on sperm motility was observed in all fresh and unconditioned media containing 1 mM pyruvate. Motion parameters were more efficiently maintained in all conditioned media than in unconditioned media. Our results showed, however, that pyruvate was almost completely oxidized or consumed by epididymal cells during preincubation of culture media. We conclude that motility of frozen-thawed bovine spermatozoa can be improved using a culture medium or a medium conditioned by epididymal cell cultures without carnitine but containing mainly pyruvate, inositol, glycerol, and lactate.

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