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Abstracts

Section 1, Abstracts 1–6

1.1

IN VITRO CULTURE OF BOAR EPIDIDYMAL EPITHELIAL CELLS

Bassols J, Kádár E, Briz MD, Pinart E, Sancho S, Garcia-Gil N, Badia E, Pruneda A, Coll MG, Bussalleu E, Yeste M, Bonet S

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A protocol to culture boar epididymal epithelial cells from caput, corpus and cauda regions was established to provide a basis for functional investigations of the epididymis in boars.

Boar epididymal epithelial fragments were obtained after dissection and enzymatic digestion with collagenase. About 30 fragments from each epididymal region were cultured in 24-well culture plates with supplemented RPMI-1640 medium at 37 °C, 5% CO_2 in air and 100% humidity. Confluent monolayers of polygonal and tightly packed epitheloid cells from caput, corpus and cauda epididymal fragments were obtained after 12–14 days in culture and maintained in vitro for more than two months. The proportion of epididymal epithelial cells in culture, assessed by indirect immunocytochemical staining assay for cytokeratin, was shown to be >80% during all culture period and in all cultured samples. Non-epithelial cells in these cultures were always in low percentages and cultures with non-epithelial cells overgrowth were not observed in this study. The morphology of caput, corpus and cauda cultured epithelial cells was similar. These cells showed one or two visible nucleoli, contained numerous granules in the cytoplasm and remained in contact through cell bodies and cytoplasmic extensions. As assessed by electron microscopy, ultrastructural characteristics of boar epididymal epithelial cells from the three regions in culture were similar and retained some features that characterize the epididymal epithelium in the intact organ. The apical area of cultured epithelial cells was covered by short stereocilia and epithelial integrity was maintained by junctional complexes situated on the lateral and basal borders of the cells. Their cytoplasm contained a full complement of organelles such as, extensive rough endoplasmic reticulum, well-developed Golgi

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apparatus, numerous mitochondria, bundles of 10 nm filaments and some vesicles, indicating that these cells maintained their functional activities.

In conclusion, this is the first report in boar of a cell culture system that maintains epididymal epithelial cells in vitro for more than two months retaining ultrastructural characteristics of the in vivo epididymis. This model system of boar epididymal epithelial cell culture may be a useful technique to study factors affecting sperm maturation in the epididymis, concretely the specific interactions between boar spermatozoa and epididymal epithelial cells, as well as for identifying and characterizing boar epithelial cell secretions.

1.2

STRUCTURAL AND BIOCHEMICAL ALTERATION OF THE PORCINE ZONA PELLUCIDA DURING IN VITRO MATURATION AND FERTILIZATION

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All vertebrate oocytes are surrounded by an extracellular matrix. In mammals this matrix is known as the zona pellucida (zp). One of its functions is prevention of polyspermy caused by the so-called zona block, a biochemical alteration of the zp glycoproteins induced by release of cortical granule (GC) contents. In the present study, modifications of free thiols in zp glycoproteins were studied during in vitro maturation and fertilization using fluorescent labelling of free thiol groups and laser scanner confocal microscopy. Follicular oocytes collected from prepuberal gilts at a local slaughterhouse were cultured for 46 h in NCSU-37 until the oocytes reached metaphase II. They were then fertilized in vitro. Intact zonae pellucidae collected from immature (GV1), in vitro matured oocytes (26 and 46 h) and fertilized oocytes (after 0, 1, 2, 3, 12 and 18 h) were labelled with iodoacetamide-fluorescein (5-IAF) and assessed employing a laser scanner confocal microscope (LSCM). A multi-comparative, quantitative analysis of the density of labelling in the inner and outer regions of the zona pellucida of all states was performed. Analysis of fluorescent label (5-IAF) revealed that the zona pellucida of oocytes in the GV1 stages showed an approximately uniform distribution of free thiols across the surface, whereas in all other stages a preferential localisation of thiol groups to the inner region of the zona pellucida was observed. During early stages of fertilization (0–3 h), the labelling pattern did not change substantially. However, 12 h and 18 h after fertilization, the intensity of fluorescent label decreased significantly. In control oocytes, where the specificity of fluorescent labelling of free thiol groups was blocked with iodoacetamide prior to the labelling procedure with 5-IAF, no fluorescence was observed. The present results indicate loss of free thiol groups during fertilization, which could be a result of the formation of new