ULTRASTRUCTURE OF IMMATURE BOVINE OOCYTES

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Introduction

Fresh ovaries obtained from a slaughterhouse are usually a major source of cumulus-oocyte-complexes (COC) for *in vitro* fertilisation in cattle. In practice, the criteria used to evaluate normal immature COC's is based on the presence of multilayered, compact and transparent cumulus cells and also on the homogeneity and transparency of the ooplasm (de Loos et al. 1989). Cumulus-oocyte-complexes recovered from these ovaries are of heterogeneous quality and have variable developmental potential *in vitro* (Carolan et al. 1996). The current project was undertaken to study the ultrastructure of normal appearing immature COC's as observed in a light microscope which could shed some light on the status of its organelles which would be related to the developmental potential of the oocytes.

Materials and Methods

Bovine ovaries (n=8) were collected on two different days within 30 minutes of slaughter of animals at a local slaughterhouse and brought to the laboratory in a thermos flask containing physiological saline supplemented with antibiotics at 30-35^{0}C. In the laboratory, these were trimmed free of fatty, luteal tissues and dominant follicles (>8 mm) and washed with fresh warm saline and finally dried with a paper towel. Cumulus-oocyte-complexes from medium sized follicles (2-6 mm) were aspirated using TL HEPES supplemented with bovine serum albumin and those (n=32) having more than four layers of compact cumulus cells and a homogeneous ooplasm with overall appearance of the oocyte as light (Madison and Fraser, 1992) were selected and washed in three changes of wash medium. Out of the selected COC's only ten were fixed in 2.5% glutaraldehyde at 4^{0}C for 1-2 hours and further processed for transmission electron microscopy.

Results and Discussion

All the COC's exhibited similar ultrastructural features except some small variations in a few COC's which were not included in the results. Electron micrographs revealed that corona-cumulus cells were vesicular in shape and contained a large distinct nucleus with a well defined nuclear membrane and 1-3 prominent nucleoli indicating active synthesis of RNA for onward transfer to the cytoplasm for the synthesis of proteins. The presence of extensive rough endoplasmic reticulum (RER) and ribosome-lamella complex, probably a modified form of RER, in cumulus cells indicate the importance of these cells in synthesising and transportation of secretory proteins. Higher magnification revealed that corona radiata cells possessed cytoplasmic processes which traversed zona pellucida to establish contacts with oolemma and also the existence of gap junctions between the adjacent cumulus cells indicating the presence of communication between corona-cumulus cells and the oocyte. The association between adjacent cumulus cells is beneficial for growth and maturation the oocyte as the presence of a healthy population of cumulus cells is mandatory to facilitate transport of nutrients and signals into and out of the oocyte (Chian and Niwa, 1994). The incidence of organelles such as mitochondria, Golgi complexes, RER was relatively more in corona cells compared with those present in cumulus cells because of their close association with the oocyte. Outer surface of zona pellucida was porous to accommodate corona cell processes which ultimately made contact with the oolemma. In the ooplasm, numerous mitochondria mostly in aggregates were seen at the periphery indicating their possible involvement in the utilisation of substrates probably transferred via gap junctions, for the production of ATP for metabolic purposes. Mitochondria were generally spherical and majority of them possessed a hood like appendage, which was associated with smooth endoplasmic reticulum. The presence of several well developed Golgi complexes in the peripheral ooplasm and also the presence of vesicles, coated vesicles and endoplasmic reticulum in the vicinity indicate increased participation of Golgi in processing and concentration of secretory products such as cortical granules (CG). The number of CG present in an oocyte is an important indicator of its ability in establishing a block against polyspermy. Cortical granules are electron dense membrane bound organelles present in layers in the cortex of the ooplasm and were of different sizes and electron densities. The difference in the appearance of the CG may be attributed to the difference in their biochemical status (Cran, 1989). Numerous membrane bound vesicles, many of them associated with a laminated structure were observed in the ooplasm. Not much information is available on the functional role of these vesicles may be these are involved in the transportation of materials or proteins in and out of the oocyte. The oocytes also contained few lipid droplets, which were mainly located in the peripheral ooplasm. The lipid droplets in general were associated with mitochondria and vesicles.

Conclusions

In conclusion, electron microscopy of normal immature oocytes reveal the importance of corona-cumulus cells which are in communication with the oocyte and this association is crucial for the growth and maturation of oocyte. It is therefore, suggested that immature bovine oocytes for IVM and IVF should be selected on the basis of number of cumulus cells layers surrounding them.

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