Differential Effects of Specific Phosphodiesterase Isoenzyme Inhibitors On Bovine Oocyte Meiotic Maturation, Gap Junctional Communication, and Developmental Competence

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Abstract

Induction of oocyte maturation *in vivo* is associated with increased follicular cAMP levels, however intra-oocyte cAMP decreases prior to germinal vesicle breakdown (GVBD). The aim of this study was to examine whether the differential regulation of cAMP levels within the oocyte and somatic (cumulus) cell compartments of the follicle regulates bovine oocyte meiotic maturation as a result of the specific cell-type localisation of phosphodiesterase (PDE) isoenzymes. Selective PDE inhibitors were used to modulate cAMP levels in each of the two follicular compartments and to examine their effects on oocyte meiotic maturation.

The type 3 PDE inhibitor, but not the type 4, prevented spontaneous meiotic maturation and elevated intra-oocyte cAMP in cultured oocytes in a dose dependant manner. While the type 4 PDE inhibitor had no effect on the oocyte, it dose dependently elevated mural granulosa and cumulus cell cAMP production. These results indicate that specific PDE isoenzymes are differentially localised within the two compartments of the bovine follicle – the type 3 PDE in the oocyte and the type 4 PDE in the granulosa cells. In addition, results showed oocyte cAMP levels to be primarily regulated in bovine oocytes by its degradation by PDE, whereas granulosa cell cAMP levels are controlled mainly by active adenylate cyclase - with both sources able to participate in oocyte meiotic regulation. This study also demonstrated that FSH, but not forskolin, was able to override the meiotic arrest at the immature GV stage caused by milrinone treatment – suggesting the existence of a form of induced oocyte maturation in the bovine species.

In the growing follicle, communication between the oocyte and its surrounding follicular cells is essential for normal oocyte and follicular development. Gap junction channels metabolically couple the oocyte and the follicular cells to each other, allowing inter-cellular communication and transfer of low molecular weight (<1000 Mr) substrates such as ions, nucleotides, amino acids, metabolites and regulatory molecules between the cells that are important for oocyte growth. Maturation of the fully-grown oocyte in vivo is associated with loss of cumulus cell-oocyte gap junctional communication, preventing entry of meiotic-modulating factors such as cAMP into the oocyte. An assay designed to measure gap junctional communication between the oocyte and its surrounding cumulus cell vestment using the fluorescent dye calcein-AM was developed and validated. In control cumulus-oocyte complexes (COCs), dye transfer from cumulus cells to the oocyte fell progressively from 0 to 9 h of maturation, after which oocyte-CC GJC was completely lost. Loss of gap junctional communication was significantly attenuated ($P<0.05$) by treatment with the oocyte type 3 PDE inhibitor, and also to a lesser extent by the cumulus cell type 4 PDE inhibitor. Importantly, all treatments that prolonged GJC also delayed meiotic resumption, with meiosis generally resuming when fluorescence had fallen to ~40% of initial levels. These results demonstrate that treatments which maintain/elevate cumulus cell and/or oocyte cAMP levels result in prolonged oocyte-cumulus cell communication and delayed meiotic resumption. It was hypothesised that this may have a positive effect on the capacity of an oocyte to undergo cytoplasmic maturation and therefore may improve oocyte developmental potential. Inclusion of isoenzyme-specific PDE inhibitors to bovine oocyte IVM media (containing FSH) was shown to cause a significant delay in the progression of oocyte meiosis to the metaphase II stage, and resulted in a 15% increase in the proportion of cleaved embryos that proceeded to the blastocyst stage of development compared to controls.

Keywords: oocyte, meiotic maturation, meiotic inhibition, cAMP, phosphodiesterase, granulosa cell, gap junction, developmental competence, embryo development.