

Are cumulus cells additional players in calcium signalling during cattle oocyte fertilisation?

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Upon sperm entry within the oocyte, pulsatile calcium release occurs, facilitating activation of the oocyte. While calcium signalling has been characterised in mouse oocytes, patterns within the whole cumulus oocyte complex (COC) and in larger mammals such as cow, are yet to be determined. The aim of this study was to investigate calcium changes in media, cumulus and oocytes during in vitro fertilisation in cattle oocytes.

Cattle COCs were inseminated in vitro following IVM. Approximately 3 hr post-insemination (negative control=no sperm), COCs were cultured for 30 mins in 5uM Fluo-4AM (calcium indicator), washed and transferred into glass-bottomed confocal dishes maintained at 38 C. Fluorescence intensity was captured using confocal microscope every 2 mins, between 200-510 mins post-sperm addition (8.5h). Fluorescence intensity was determined within regions of the oocyte, cumulus vestment and surrounding medium. Successful fertilisation was determined by DAPI fluorescence and visualisation of female/male pronuclei.

A peak and then decrease in Fluo-4AM intensity occurred within the oocyte and then cumulus vestment, corresponding with previously reported timing of sperm entry in cattle (399-405 mins), followed by 2-4 fold increase 8-16 mins later in intensity of probe in the media of fertilised COCs. In contrast, oocyte, cumulus and media fluorescence remained relatively constant in the no sperm group. These results suggest the intensity and pattern of calcium release from the oocyte, cumulus and into the media indicates successful fertilization, and demonstrates that calcium signalling at fertilization extends beyond the oocyte.