Sperm enter bovine uterine glands ex-vivo and initiate a pro-inflammatory response

Ihshan AKTHAR1, Susan SUAREZ2, Vernadyn MORILLO1, Mohamed EZZ1, Motoki SASAKI1, Ken-Ichi TAKAHASHI3, Masayuki SHIMADA4, Mohamed Ali MAREY1,5, Akio MIYAMOTO1

1Obihiro University of Agriculture and Veterinary Medicine, 2Cornell University, 3Genetics Hokkaido Assoc, 4Hiroshima University, 5Damanhur University

The uterus has a well-developed innate immune system that recognizes microbes and sperm. We have recently shown that sperm binding to bovine uterine epithelial cells (BUEC) induces acute inflammatory response. This raises the query of whether the uterus uses a common or similar mucosal immune response to sperm and microbes; however, immune cross-talk between sperm and endometrium is unclear. Our BUEC culture model showed that pro-inflammatory response induced by sperm binding involves TLRs signaling pathway via TLR2; however, immunological functions of the uterus in vivo involve interactions of tissues and mucus coating the endometrial surface could modulate responses. Thus, we developed an ex vivo explant model to investigate the sperm-uterine interaction in vivo. Uterine explants were co-incubated with 10^6/ml washed fresh sperm. JC-1 labeled sperm were used in fluorescence microscopy. Sperm glide over the surface epithelium until they encounter and enter uterine glands. SEM observations show that endogenous neutrophils appeared in uterine glands along with the clusters of sperm; they may initiate sperm clearance. Co-incubation for 2 h resulted in upregulation of TNFA and IL1B mRNA expression, but IL8 expression started to increase earlier at 0.5 h. TLR2 antagonist reduced the sperm numbers in the glands and inhibited the increase of TNFA and IL8 which suggest that the sperm-uterine inflammatory process is at least partly mediated by TLR2 signaling. Our observations suggest that uterine glands serve as a site where sperm interact with glandular epithelium to trigger the innate immune response to rapidly clear sperm from the uterus and thus prepare the endometrium for embryo implantation.