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INTRODUCTION

Sperm-egg interaction is a complex process mediated by receptors and ligands present in both gametes, spermatozoon and oocyte. The important role played by the carbohydrates in the gamete interaction is well documented. However, the identification of the molecules involved in this process

remains unsolved. A carbohydrate modification in the sialic acid contents of the bovine zona pellucida (ZP) post fertilization has been previously reported. However, no evidence exists on the implication of this carbohydrate in the sperm-ZP interaction. In this study, we investigate the role played

by the sialic acid residues in the sperm-ZP binding by means of different sperm binding competition and IVF assays with different sialic acid specific lectins, neuraminidase enzyme and antibodies.

MATERIAL AND METHODS

Bovine oocytes with 4 or more cumulus cells layers and a homogeneous cytoplasm were isolated from ovarian follicles (2-6 mm diameter). In experiment 1, oocytes were fixed in glutaraldehyde and processed for ultrastructural cytochemistry using lectins (LFA, MAA, SNA) and

antibodies (sialyl-lewisa and x epitopes) with a specific affinity for different sialic acid residues. In experiment 2, ZP were mechanically isolated and incubated with the lectins or the neuraminidase and further incubated with bovine sperm to investigate the number of sperm

bound to the ZP. In experiment 3, oocytes were in vitro matured and incubated with neuraminidase to test if the IVF was affected. In experiment 4, immature and mature activated oocytes were used to detect seven different glycosidase activities.

RESULTS

A strong reactivity was observed in the ZP with the lectins; however, no labelling was detected with the antibodies against sialyl-lewis epitopes. The sperm-ZP binding was 73% and 81% inhibited with LFA and MAA, respectively. This inhibition was dose dependent. No

effect was observed with the SNA lectin. A 60% and 95% inhibition of the sperm binding to the ZP was observed when the ZP was treated with the neuraminidase at 1U/ml and 10U/ml, respectively. A 81% inhibition of fertilization was detected in IVF experiments using

oocytes previously incubated with neuraminidase. Only neuraminidase activity was detected in the oocyte. This activity was mainly associated with the oocyte exudates obtained after activation with the calcium ionophore.

CONCLUSIONS

The bovine sperm-ZP interaction is a carbohydrate dependent process probably mediated by sialic acid

with an alpha 2,3 linkage to lactosamine contained in the ZP glycoprotein. Neuraminidase is

present in the bovine oocyte and is mainly associated with the cortical granules.