

## Binding of Lectins to the Zona Pellucida on Sperm-oocyte Interaction in the Pig

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**Objectives:** Lectins are cell-agglutinating and sugar specific proteins or glycoproteins of non-immune origin that precipitate glycoconjugates having saccharides of appropriate complementarity. Because of these properties, plant lectins have been used to help characterize the carbohydrate moieties of glycoproteins in the zona pellucida (ZP) of several mammalian species including pigs. Treatment of oocytes with various lectins blocks sperm binding to the ZP in various mammalian species. This study was undertaken to examine the distribution of sugar residues in the ZP of pig oocytes matured in vitro and the ability of spermatozoa to bind to ZP and in vitro penetration in oocytes treated with fluorescein isothiocyanate (FITC)-labelled lectins

**Materials and Methods:** The lectins of *Bandeiraea simplicifolia* (BS-II, bind to N-acetyl- $\beta$ -D-glucosamine), *Canavalin ensiformis* (Con-A, bind to  $\alpha$ -D-mannos), *Ricinus communis* (RCA-I, bind to  $\beta$ -D-galactose), *Lens culinaris* (LCA, bind to  $\alpha$ -D-mannose) and *Ulex europaeus* (UEA-I, bind to  $\alpha$ -L-fucose) were examined for spermatozoa penetration, binding capacity to ZP and distribution of lectins.

**Results:** The penetration rates were significantly ( $p < 0.05$ ) higher in control oocytes (63%) than those treated with all lectins, but penetration rates (40~49%) were similar in oocytes treated with lectins. The incidence of monospermy was similar in oocytes untreated and UEA-I, but it was higher in oocytes treated with BS-II, Con-A, RCA-I and LCA. The porcine oocytes cultured for 48 h in TC-199 medium were freed from cumulus cells and treated for 30 min with fluorescein isothiocyanate-labelled lectins. When examined under fluorescence illumination, higher ( $p < 0.001$ ) proportions of oocytes showed fluorescence of zona pellucida after treatment with Con-A (93%), RCA-I (93%) and RCA-I (100%) than BS-II (37%) and UEA-I (50%). All of the oocytes treated with RCA-I exhibited strong fluorescence in the outer region of the zona pellucida while those treated with LCA exhibited strong fluorescence throughout the zona pellucida. BS-II bounded mainly to the outer region and UEA-I bound mainly to the inner region of the zona pellucida, with either strong or weak fluorescence. At 120 min after insemination in vitro, fewer spermatozoa were bound to the zona pellucida of oocytes treated with BS-II, Con-A, and RCA-I. Of the lectins, Con-A most inhibited sperm binding.

**Conclusions:** These results suggest that  $\beta$ -D-galactose residues in the porcine zona pellucida may act as primary sperm receptors and inducers of the sperm acrosome reaction and these sugar residues may be involved in the block to polyspermy. This work was supported by Korea Research Foundation Grant (KRF-2000-G00052).