

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

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Objective: 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 *Bandieriaea simplicifolia* (BS-II, bind to β -D-N-acetylglucosamine), *Canavalin ensiformis* (Con A, bind to α -D-Mannose), *Lens culinaris* (LCA, bind to α -D-Mannose), *Ricinus communis* (RCA-I, bind to β -D-Galactose) and *Ulex europaeus* (UEA-I, bind to α -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 group 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 fluorescein illumination, higher ($p < 0.001$) proportions of oocytes showed fluorescein of zona pellucida after treatment with Con A (93%), LCA (93%) and RCA-I (100%) than BS-II (37%) and UEA-I (50%). All of the oocytes treated with RCA-I exhibited strong fluorescein in the outer region of the zona pellucida while those treated with LCA exhibited strong fluorescein throughout the zona pellucida. BS-II bounded mainly to the outer region and UEA-I bounded mainly to the inner region of the zona pellucida, with either strong or weak fluorescein. At 120 min after insemination *in vitro*, fewer spermatozoa were bound to the zona pellucida of the oocytes treated with BS-II, Con-A and RCA-I. Of the lectins, Con A most inhibited sperm binding.

Conclusions: These results suggest that β -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.

Key Words: *In vitro*, Lectin, Pig, Sperm-oocyte interaction, Zona pellucida (ZP)

hydrate chain , 가 lectin

lypeptide chain oligosaccharides 가 po- gly- coprotein ,⁵ oligosacchar- ides - tin fluorescein isothiocyanate (FITC)-labelled lec- tin , 가 lectin 가

glycoprotein .³ glycoprotein zona binding

1 2 1. 1) 35~37 75 µg/ml penicillin, 50 µg/ml streptomycin sulfate가 가 0.9% (w/v) 2 18-gage 10-ml 2~6 mm .

charides 가 . monosac- 10 IU/ml eCG hCG가 가 6,7,18 가 가 .⁴ (TC-199 , pH 7.4) 3 paraffin oil .⁸ Lectin , 10 5% CO₂ 39 100 µl 가 lectin 42~44 .

glycoconjugates .¹² 3.05 mM D-glucose, 0.91 mM sodium pyruvate, 75 µg/ml penicillin, 50 µg/ml streptomycin sulfate 10% (v/v) fetal calf serum (FCS) 가 TC-199 .^{11,13,14,23} lectin- 3) binding , glycoprotein carbo-

Table 1. Lectins used for identifying carbohydrate components in the zona pellucida of pig oocytes matured *in vitro*

Lectin origin	Common name	Acronym	Major sugar specification
<i>Bandeiraea simplicifolia</i>		BS-II	β -D-N-acetylglucosamine
<i>Canavalin ensiformis</i>		Con A	α -D-Mannose
<i>Lens culinaris</i>	Common Lentil	LCA	α -D-Mannose
<i>Ricinus communis</i>	Castor Bean	RCA-I	β -D-Galactose
<i>Ulex europaeus</i>	Gorse	UEA-I	α -L-Fucose

Soejima	Almlid	Johnson	Lee	가	.
				2)	Fluorescein Isothiocyanate (FITC)- Labelled Lectins
			BTS		0.1% (w/v) hyaluronidase가
1:1	6 × 10 ⁹	spermatozoa		가	Dulbecco's phosphate buffered saline (D-PBS) pipetting
	1,500 rpm	10			D-PBS
			5 ml		
			50-ml tube	3	3% (w/v) paraformaldehyde가 가
1~2	4				30
	5 ml	1		가	D-PBS 3 1 μM
2		4	40		FITC-labelled lectin 가 D-PBS 30
		straw			lectin
		LN ₂	5 cm		
	20		LN ₂	Table 1	
				3)	Lectin
				FITC-labelled lectin	가 D-
2.				PBS 3	slide glass
				Cover-glass	가 가
1)				가	
					1 μM
FITC-labelled lectin	가	D-PBS			FITC-labelled lectin 가
30			3		100 W mercury lamp DM 510 filter
			2.92 mM		FITC-labelled lectin positive ne-
hemicalcium lactate	10 mM	caffeine sodium benzo-			gative , positive
ate가 가	TC-199	5% CO ₂	39		
			3		
10	50 μl	drop		4) Lectin	
가	30	CO ₂		가	
				2	
					250
	6 ml		가	μm	30 pipetting
10	1,500 rpm	2			3% (v/v) paraformaldehyde 30
		25 × 10 ⁶	spermatozoa		가 10 μg/ml
		2 μl	50 μl		bis-benzimide가 가 D-PBS 3~5

Table 2. Spermatozoa penetration *in vitro* of porcine oocytes treated by lectins

Lectins (1 μ M)	No. of oocytes examined	No. of oocytes penetration	
		Total (%)	Polyspermy (%)
Control	97	61 (63) ^a	8 (13) ^{ab}
<i>Bandeiraea simplicifolia</i>	100	47 (47) ^b	1 (2) ^a
<i>Canavalin ensiformis</i>	98	58 (49) ^b	12 (21) ^b
<i>Lens culinaris</i>	102	51 (40) ^b	13 (25) ^b
<i>Ricinus communis</i>	104	43 (41) ^b	4 (9) ^{ab}
<i>Ulex europaeus</i>	99	43 (43) ^b	6 (14) ^{ab}

^{a,b} Values with different superscripts within each column differ significantly. $p < 0.05$

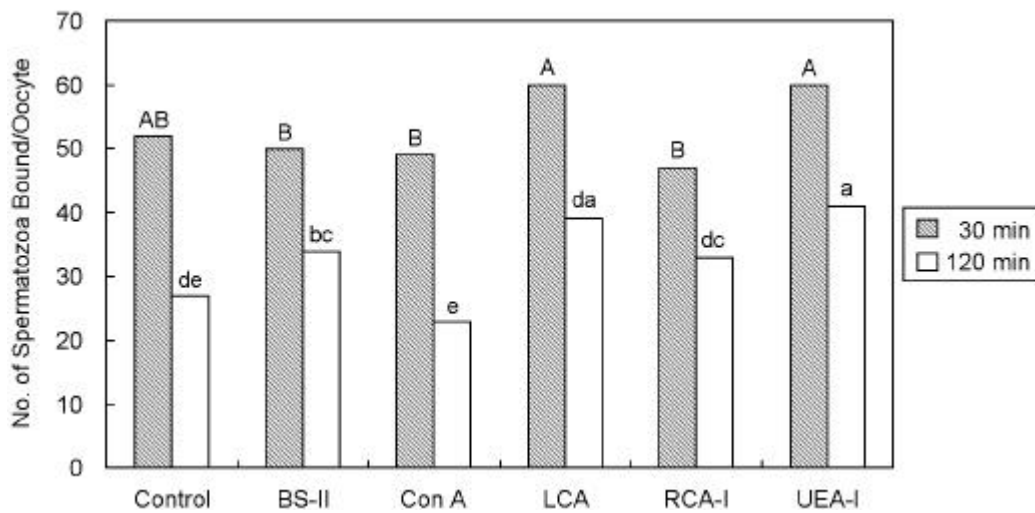


Figure 1. Effect of incubation periods and lectins on sperm binding to the zona pellucida in oocytes matured *in vitro* in the pig. Bars with different letter differ at 30 or 120 min of incubation period. $p < 0.05$

slide glass 63%

DM 400 filter가 (61/97) lectin 가

($\times 200$) (40~49%)

200 μ m (p<0.05). lectin

12 가

. *Canavalin ensi*

. *formis* (21%, 12/58) *Lens culinaris* (25%, 13/51)

가 *Bandeiraea simplicifolia* (2%, 1/47) 가

(p<0.05)

가

lectin lectin

. , Table 2 Figure 1 30

Table 3. Distribution and binding ability of lectins in the zona pellucida of porcine oocytes matured *in vitro*

Lectins	No. of oocytes examined	No. (%) of oocytes with ZP stained with	
		Positive (strong/weak)	Negative
<i>Bandeiraea simplicifolia</i>	46	17 (37) ^c 10 (59) ^B /7 (41)	29 (63)
<i>Canavalin ensiformis</i>	43	40 (93) ^a 28 (70) ^B /12 (30)	3 (7)
<i>Lens culinaris</i>	44	41 (93) ^a 26 (63) ^B /15 (37)	3 (7)
<i>Ricinus communis</i>	44	44 (100) ^a 44 (100) ^A /0 (0)	0 (0)
<i>Ulex europaeus</i>	44	22 (50) ^b 10 (45) ^B /12 (55)	22 (50)

^{A,B; a,b,c} Values with different superscripts within each column differ significantly. p<0.05

lectin 120 (100%) strong positive Ulex europaeus 22 가 (weak) 12 (55%)
 culinaris Ulex europaeus 가 30 Lens lec- (strong)
 tin 가 (p<0.05). 120
 Lens curinaris Ulex europaeus control
 Canavalin ensiformis group (p<0.05). fluorescein isothiocyanate (FITC)-labelled
 lectin lectin 가 lectin
 Table 3 Canavalin ensi-
 formis (93%), Lens culinaris (93%) Ricinus com-
 munis (100%) Banderiaea simplicifolia (37%)
 Ulex europaeus (50%) positive 가
 가 (p<0.05). posi-
 tive Ulex europaeus lectin
 lectin , Ricinus com- 가
 munis lectin ,
 (p<0.05).
 , positive ,
 (Figure 2). Strong 가 ,
 , weak ,
 가 Ricinus 12,24

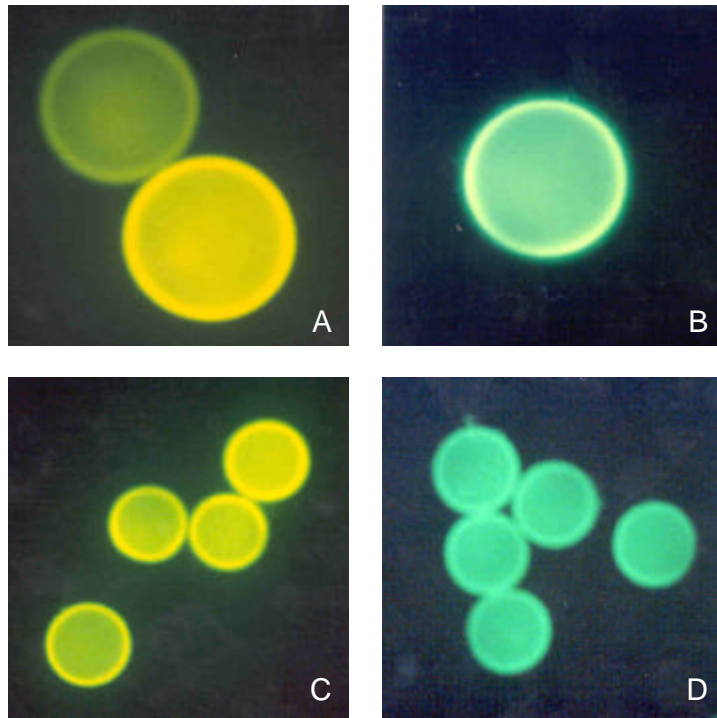


Figure 2. Lectin-binding to the zona pellucida of oocytes matured *in vitro* in the pig. **A:** Oocytes bounded with and without fluorescence in the *Ulex europaeus*. **B:** *Lens culinaris* bound mainly to the outer region of the zona pellucida with strong fluorescence. **C:** Oocytes bound with strong fluorescence by *Lens culinaris* agglutinin to out region of the zona pellucida. **D:** Oocytes bound throughout the zona pellucida with weak fluorescence by *Canavalin ensiformis*.

ZP1, ZP2 ZP3
 ,
 ZP3
 ,²² lectin
 , lectin
 , lectin
 lectin 가
 ,
 lectin
 , β -D-Galactose
 a-D-Mannose 가
 ,
 30 lectin
 가
 ,
 120 lectin

lectin
 . lectin
 , lectin
 ,
 ,¹⁹
 lectin
Canavalin ensiformis (93%), *Lens culinaris* (93%)
Ricinus communis (100%) *Banderiaea simplicifolia* (37%)
Ulex europaeus (50%) positive
 가 . positive
Ulex europaeus lectin
 , *Ricinus communis*
 lectin

가
 guinea pig,²⁵,^{1,15-17,20} 10
 ,¹ 11,13,14 9
 .
 lectin
 lectin
 가 .

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