Asian-Aust. J. Anim. Sci.
Vol. 20, No. 3 : 351 - 358
March 2007

www.ajas.info

Effect of Monosaccharide L-fucose and Polysaccharide Fucoidan on Sperm α-L-fucosidase Activity and Relation to Sperm-oocyte Interaction in Pig

X. X. Song[1], C. K. Park*, Y. J. Piao[1] and K. Niwa[2]

[1] Faculty of Animal Science and Technology, Laiyang Agriculture College, Qingdao 266109, P. R. China

ABSTRACT : Carbohydrate-protein interactions are known to be important in gamete interactions. Several evidence indicated that a fucose-containing sulfated polysaccharide fucoidan was potential inhibitor of fertilization *in vitro* and thus fucose seemed to be part of the recognition signal of gamete interaction in mammals. In recent investigation we found that α-L-fucosidase activity was present in boar spermatozoa and it was related to sperm binding to and penetration into zona pellucida (ZP) *in vitro*. The objective of this study was to determine the effects of monosaccharide L-fucose and polysaccharide fucoidan on sperm α-L-fucosidase activity and relation to sperm-oocyte interaction in pig. Results indicated that the activity of sperm α-L-fucosidase was largely inhibited (62%) when sperm suspension was treated with monosaccharide L-fucose. It also significantly inhibited the number of sperm binding to ZP (32%) and penetration into zona-intact oocytes (72%), but did not inhibit penetration into zona-free oocytes when fertilization medium contained L-fucose. The chlorotetracycline (CTC) assessment showed that L-fucose did not affect induction of sperm capacitation and acrosome reaction. In contrast, the activity of sperm α-L-fucosidase was not inhibited when sperm suspension was treated with polysaccharide fucoidan but sperm-ZP binding was greatly inhibited (85%) and completely blocked sperm penetration into zona-intact or zona-free oocytes. The CTC assessment showed that fucoidan increased the F pattern and decreased the AR pattern sperm. These results suggested that the different inhibitory mechanisms were present between monosaccharide L-fucose and polysaccharide fucoidan on sperm-oocyte interaction, the inhibition effect of α-L-fucose on sperm binding and penetrating into ZP caused sperm α-L-fucosidase inhibited by α-L-fucose. (**Key Words :** Pig Oocytes, Chondroitin Sulfate, Sperm Penetration, *In vitro*)

INTRODUCTION

Fertilization in mammals involves complementary recognition and fusion between two specialized and morphologically disparate cells, the sperm and the egg (Yanagimachi 1981). The attachment and the species-specific binding of the spermatozoa to the oocyte zona pellucida is prerequisite for the penetration of the zona pellucida and finally for the successful fertilization of the oocyte (for review, Hinrichsen-Kohane et al., 1984). It is a cell-cell complementary recognition event that involves specific receptors on the surface of each gamete. However, the nature of these complementary recognition signals and the molecular details of the mechanisms involved are still poorly understood although there is growing evidence showed that carbohydrate moieties on surface membrane glycoconjugates play a key role. As demonstrated in several biological systems, the carbohydrate-protein interaction plays an essential role in cell differentiation, cell adhesion and cell recognition (Frazier and Glaser, 1997). In mammalian, several sugars have been shown to inhibit the sperm-zona binding (Ahuja 1982, 1985). In the different mammalian species such as mouse (Moreno et al., 2001), guinea pig (Huang et al., 1982), hamster (Ahuja, 1985; Moreno et al., 2001), rat (Shalgi et al., 1986) and human (Huang et al., 1982; Mahony et al., 1991; Oehninger et al., 1992), the sperm-zona binding is strongly inhibited by polysaccharide fucoidan, an algal sulfated L-fucose polymer. Thus, fucose seemed to be part of the recognition signal of gamete interaction in mammals (Huang et al., 1982; Huang and Yanagimachi, 1984; Ka and Bazer, 2005).

In boar, it was also reported that fucoidan inhibit sperm-zona binding (Peterson et al., 1981; 1984). The mechanism of fucoidan inhibition effect of sperm-zona binding has been shown that fucoidan binds proacrosin, the zymogen

* Corresponding Author. Choon-Keun Park. College of Animal Life Sciences, Kangwon National University, Chuncheon 200-701, Korea. Tel: +82-33-250-8627, Fax: +82-33-244-2532, E-mail: parkck@kangwon.ac.kr

[2] Division of Animal Science and Technology, Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan.

Received February 6, 2006, Accepted October 2, 2006

form of the acrosomal protease acrosin (Jones and Brown, 1987) and inhibits acrosin amidase activity (Urch and Hedrick, 1988; Lo Leggio et al., 1994). However monosaccharide L-fucose was not inhibitor for acrosin amidase activity (Urch and Hedrick, 1988) and completely inhibited ^{125}I-labeled fucoidan for binding to proacrosin (Jones, 1990; Urch and Patel, 1991). Up to now, little is known about the effect of L-fucose on sperm-zona binding, and about both L-fucose and fucoidan on sperm penetration into zona-intact and zona-free pig oocytes.

In recent investigation, we found α-L-fucose residues distributed on inner region of pig ZP (Song et al., 1999) and α-L-fucosidase presented in boar spermatozoa and released by acrosome reaction (Song et al., 2000). When oocytes are labeled with specific lectin or added with α-L-fucosidase inhibitor to the fertilization medium, the numbers of sperm-binding to ZP and penetrating into the oocyte are significantly inhibited. It suggested that α-L-fucosyl residues present in ZP and α-L-fucosidase present in sperm might involve in sperm-oocyte interaction in pig. For further identify between α-L-fucosyl residues and α-L-fucosidase in sperm-oocyte interaction, the present study was designed to confirm this hypothesis by examining 1) the effect of monosaccharide L-fucose and polysaccharide fucoidan on activity of α-L-fucosidase, and 2) the binding and penetration *in vitro* of spermatozoa to zona-intact and zona-free oocytes in the presence of L-fucose and fucoidan.

MATERIALS AND METHODS

Media

Unless stated, all chemicals used in this study were purchased from Sigma Chemical Company (St. Louis, MO). The basic medium (TCM-199B) used for manipulation of oocytes and spermatozoa was tissue culture medium (TCM) 199 with Earle's salts (Gibco Lab., Grand Island, NY, USA) supplemented with 3.05 mM D-glucose, 0.91 mM sodium pyruvate, 75 μg/ml potassium penicillin G, 50 μg/ml streptomycin sulfate and 10% (v/v) fetal calf serum (FCS, Gibco).

Treatment of spermatozoa for α-L-fucosidase assay

Frozen ejaculated pig spermatozoa (2 pellets, each 100 μl in volume) were thawed in 2 ml of TCM-199B (pH 7.8) at 39°C for 1 min. The medium was previously equilibrated in an atmosphere of 5% CO_2 in air at 39°C for about 3 h. After thawing, 6 ml of the same medium was added and the spermatozoa were washed three times by centrifugation at 550×g for 5 min each. The final sperm pellet was resuspended in the same medium supplemented with 5 mM caffeine sodium benzoate and 30 mM L-fucose or 1.0 mg/ml fucoidan to give a concentration of 2×10^7 spermatozoa/ml. Multiple aliquots (1 ml) of the sperm suspension were introduced into 1.5 ml polypropylene microcentrifuge tubes. After incubated in an atmosphere of 5% CO_2 in air at 39°C for 2 h, the sperm suspension was assayed for α-L-fucosidase activity.

Measurement of α-L-fucosidase activity

The activity of α-L-fucosidase was measured by the liberation of *p*-nitrophenol from *p*-nitrophenyl-derived substrates (α-L-fucoside). After the addition of 100 μl substrate solution (pH 5.2) containing 2 mM of substrate to 100 μl of sperm supernatant, the mixture was kept for 2 h in air at 39°C; the reaction was then stopped by addition of 1 ml of 1 M Na_2CO_3. The substrate solution was composed of citrate-phosphate buffer (60 mM trisodium citrate, 40 mM NaH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM KCl and 1 mg/ml BSA) supplemented with protease inhibitor cocktail (PIC) (Miller et al., 1992, 1993). Absorbance of the product (*p*-nitrophenol) was measured at a wave length of 405 nm using a spectrophotometer (Du-65; Smithkline Beckman Co., Tokyo, Japan).

Preparation of oocytes and spermatozoa for sperm-binding/penetration assay

Ovaries were collected from prepubertal gilts at a local slaughterhouse and cumulus-oocyte complexes (COCs) were aspirated from follicles of 2-5 mm in diameters. After washing three times with maturation medium (TCM-199B, pH 7.4, supplemented with 10 IU/ml eCG and 10 IU/ml hCG), 10-15 COCs were transferred to a 100 μl drop of the same medium, which had been previously covered with warm paraffin oil in a polystyrene culture dish (35×10 mm; Falcon No. 1008, Becton Dickinson Labware, Lincoln Park, NJ, USA) and equilibrated in 5% CO_2 in air for at least 3 h, and cultured for 36 h at 39°C under the same atmospheric conditions. After culture, oocytes were freed from cumulus cells with 0.1% (w:v) hyaluronidase or freed from ZP by 0.1% (w:v) protease followed by repeated passage through a fine pipette. After washing three times, 10-15 zona-intact or zona-free oocytes were placed into a 50 μl drop of the fertilization medium, TCM-199B (pH 7.8) supplemented with 30 mM fucose or 1.0 mg/ml fucoidan and 10 mM caffeine in culture dish and kept in a CO_2 incubator (5% CO_2 in air at 39°C) for about 30 min before spermatozoa was added. Frozen ejaculated spermatozoa were thawed, washed and resuspended in fertilization medium without L-fucose or fucoidan and caffeine with the same procedures as in the glycosidase assay.

Assessment of sperm penetration and sperm-zona binding

Sperm penetration of oocytes were examined 12 h after insemination. Oocytes were mounted on slides, fixed for about 72 h in 25% (v/v) acetic acid in ethanol at room

temperature, stained with 1% (w/v) orcein in 45% (v/v) acetic acid, and examined under a phase-contrast microscope at a magnification of ×200 or ×400. Oocytes were considered as penetrated when they had one or more decondensing sperm nuclei and corresponding sperm tail(s). Sperm-zona binding was examined 2 h after insemination. Oocytes were picked out and passaged 30 times through a fine pipette with an inner diameter of about 250 μm to remove weakly attached spermatozoa from the surface of the zona pellucida (ZP). The oocytes then were fixed for 30 min in 3% (v/v) paraformaldehyde, stained with 10 μg/ml bis-benzimide (Hoechest 33342) in PBS for 3-5 min, placed on a glass slide, and counted the number of spermatozoa firmly bound to the ZP under a fluorescent microscope with DM 400 filter (Nikon, Tokyo, Japan) at a magnification of ×200.

Chlorotetracycline assessment of spermatozoa

Methods for chlorotetracycline (CTC) assessment were essentially the same as described by Wang et al. (1995). Briefly, 4 μl Hoechst solution, containing 100 μl/ml Hoechst bis-benzimide 33258 (Sigma) in TC-199 medium for spermatozoa washing was added to 396 μl sperm suspension. After mixing and gently vortexing, the suspension was incubated for 3 min at room temperature in the dark, layered onto 4 ml of 3% (w/v) polyvinylpyrolidone (PVP-40; Sigma) in PBS and centrifuged at 500×g for 6 min. The pelleted spermatozoa was resuspended in 50 μl of TC-199 medium. From this suspension, 45 μl was added to 45 μl CTC solution, and mixed together with 8 μl of 12.5% (w/v) paraformaldehyde in 0.5 mM Tris-HCl (pH 7.4). The CTC solution was prepared on the day of use and contained 750 μM CTC (Sigma) in a buffer of 130 mM NaCl, 5 mM cysteine and 20 mM Tris-HCl, pH adjusted to 7.8. This solution was kept wrapped in foil at 4°C until just before use. Slides were prepared by placing 10 μl of the stained, fixed suspension on a slide. One drop of 0.22 M 1,4-diazabicyclo(2.2.2) octane dissolved in glycerol:PBS (9:1) was carefully mixed to retard fading of the fluorescence. A cover slip was placed on top. The slide was compressed firmly between tissues to remove any excess fluid and to maximize the number of spermatozoa lying flat on the slide. The cover slip was then sealed with colourless nail varnish and refrigerated and wrapped in foil.

An assessment was carried out either on the same or on the following day using an Olympus BHS microscope equipped with phase-contrast and epifluorescent optics. Cells were assessed for CTC staining using violet light. The excitation beam passed through a 405 nm bandpass filter and fluorescence emission was observed through a DM 455 dichroic mirror. In each sample, 600 live cells were assessed for CTC staining patterns. There were three main patterns of CTC fluorescence which could be identified: F, with uniform fluorescence over the entire head, characteristic of uncapacitated, acrosome-intact cells; B, with a fluorescence-free band in the post-acrosomal region, characteristic of capacitated, acrosome-intact cells; and AR, with a dull or absent fluorescence over the sperm head, characteristic of capacitated, acrosome-reacted cells. Bright fluorescence on the midpiece could be seen at all three stages.

Experimental design

In experiment 1, to examine the effects of monosaccharide L-fucose or polysaccharide fucoidan on the activity of sperm α-L-fucosidase, 0.5 ml fertilization medium containing 30 mM L-fucose or 1.0 mg/ml fucoidan was added into a 0.5 ml sperm suspension (2×10^7 cell/ml) in 1.5 ml polypropylene microcentrifuge tubes and the sperm suspension was incubated for 2 h at 39°C in 5% CO_2 in air without being covered with paraffin oil and cap. For enzyme assays, a 100 μl incubated sperm suspension was added an equal volume of 2 mM substrate. After 2 h of coincubation at 39°C, the activity of α-L-fucosidase was measured.

In experiment 2, to examine the effects of L-fucose and fucoidan on sperm binding and sperm penetration, *in vitro* matured oocytes with or without the ZP were introduced into a 50 μl fertilization medium containing 60 mM L-fucose or 2.0 mg/ml fucoidan. For insemination, 50 μl of sperm suspension was added to give a final sperm concentration of 1×10^7 cell/ml and 30 mM L-fucose or 1.0 mg/ml fucoidan. After 2 and 12 h of co-incubation, sperm binding to the ZP and sperm penetration into zona-intact or zona-free oocytes were examined, respectively.

In experiment 3, to examine the effects of L-fucose and fucoidan on sperm motility, capacitation and acrosome reaction, spermatozoa (1×10^7 cell/ml) were incubated up to 4 h in fertilization medium containing each saccharide at the same concentration as in Experiment 1. Sperm motility was estimated subjectively with a small drop of sperm suspension under a cover slip using a bright-field microscpe (×100) equipped with a heated microwarm plate (39°C; MP 10-DM, Kitazato Supply Co., Shizuoka, Japan). The evaluation of sperm capacitation and acrosome reaction was made by CTC staining.

Statistical analysis

All proportional data was compared by Student's t-test.

RESULTS

Effect of L-fucose and fucoidan on sperm α-L-fucosidase activity

As shown in Figure 1, the activity of sperm α-L-

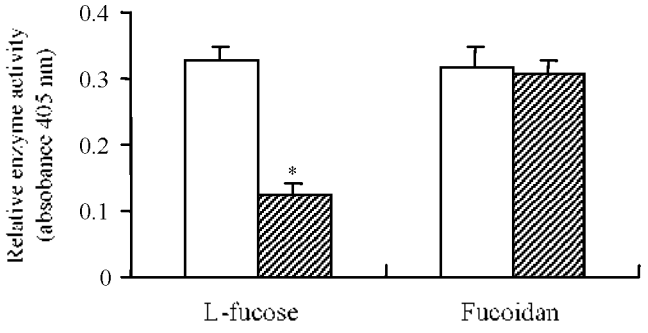

Figure 1. Activity of fucosidase in the absence (open bars) and presence (solid bars) of L-fucose or fucoidan with frozen-thawed pig ejaculated sperm suspension. Sperm suspension (1×10^7 cell/ml) were incubated for 2 h in 5% CO_2 in air at 39°C in the absence or presence L-fucose (30 mM) or fucoidan (1.0 mg/ml). The activity of α-L-fucosidases was measured by the absorbance of the product (p-nitrophenyl) at a wave length of 405 nm. Data are shown as means±SDM of six trials. * $p<0.001$ compared with the values in the absence of saccharide. The percentage superscripts on the bars are inhibitory rate.

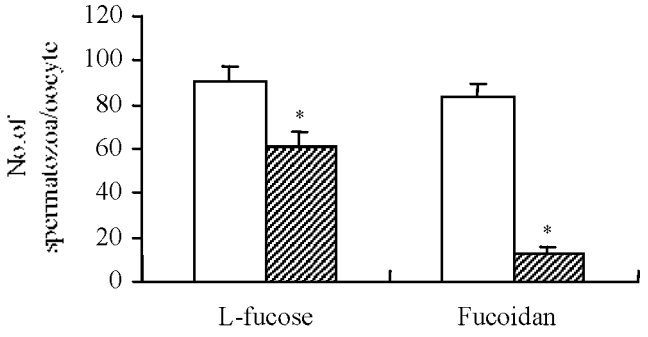

Figure 2. Binding of spermatozoa *in vitro* to the zona pellucida of *in vitro* matured pig oocytes after incubation in the absence (open bars) and presence (solid bars) of L-fucose or fucoidan. Frozen-thawed pig ejaculated spermatozoa (1×10^7 cell/ml) were incubated with oocytes for 2 h in 5% CO_2 in air at 39°C in a 100 μl drop of fertilization medium containing L-fucose (30 mM) or fucoidan (1.0 mg/ml). Data are shown as means±SDM of six trials. * $p<0.001$ compared with the values in the absence of saccharide. The percentage superscripts on the bars are inhibitory rate.

fucosidase was strongly inhibted (60.6%) when fertilization medium contained L-fucose compared with control. However, fucoidan did not significantly inhibit α-L-fucosidase activity after incubation for 2 h compared with control.

Effect of L-fucose and fucoidan on sperm-ZP binding and sperm penetration into ZP intact or ZP free oocytes

As shown in Figure 2, the number of spermatozoa bound to the ZP was inhibited 31.5% when fertilization medium contained L-fucose compared with unadded control (61 vs. 91 spermatozoa/oocyte) and inhibited 85.2% when

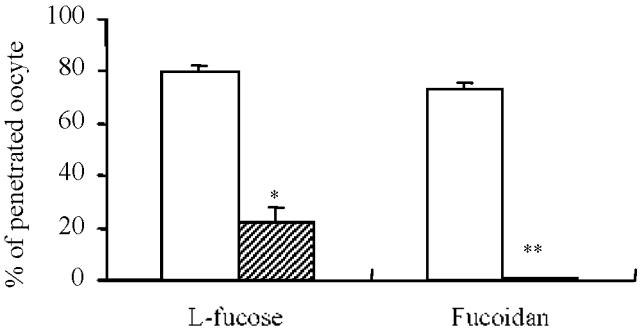

Figure 3. Penetration of spermatozoa into *in vitro* matured zona-intact pig oocytes after incubation in the absence (open bars) and presence (solid bars) of L-fucose or fucoidan. Frozen-thawed pig ejaculated spermatozoa (1×10^7 cell/ml) were incubated with oocytes for 12 h in 5% CO_2 in air at 39°C in a 100 μl drop of fertilization medium containing L-fucose (30 mM) or fucoidan (1.0 mg/ml). Data are shown as means±SDM of seven trials. * $p<0.01$, ** $p<0.001$ compared with the values in the absence of saccharide. The percentage superscripts on the bars are inhibitory rate.

fertilization medium contained fucoidan compared with unadded control (12 vs. 83 spermatozoa/oocyte).

As shown in Figure 3, the percentage of sperm penetrated into zona-intact oocytes was inhibited 71.7% when fertilization medium contained L-fucose compared with unadded control (22% vs. 79.6%) and inhibited 100% when fertilization medium contained fucoidan compared with unadded control (0% vs. 72.7%).

When ZP free oocytes were fertilized, a lot of sperm bound to oocytes. When fertilization medium contained L-fucose, it seemed not significantly affect sperm binding to oocyte membrane, but about a half of sperm bound to oocyte were inhibited when fertilization medium contained fucoidan (data not shown).

As shown in Figure 4, the number of sperms penetrated into ZP-free oocytes were not significant inhibited when fertilization medium contained fucose (88.8%) compared with control (92.3%), but completely blocked sperm penetration when fertilization medium contained fucoidan compared with control (90.5%).

Effect of L-fucose and fucoidan on sperm motility, capacitation and acrosome reaction

As shown in Figure 4, when fertilization medium was added 30 mM L-fucose, sperm penetration ability was not significant difference. The fucose also did not affect induction of capacitation and acrosome reaction of spermatozoa compared with unadded control. However, fucoidan (1.0 mg/ml) increased ($p<0.05$) significantly in F pattern of sperm, and decreased penetration rates and AR pattern sperm cell compared with unadded control from 1 to 4 h, but it did not affect capacitation B pattern sperm cell (Figures 4 and 5).

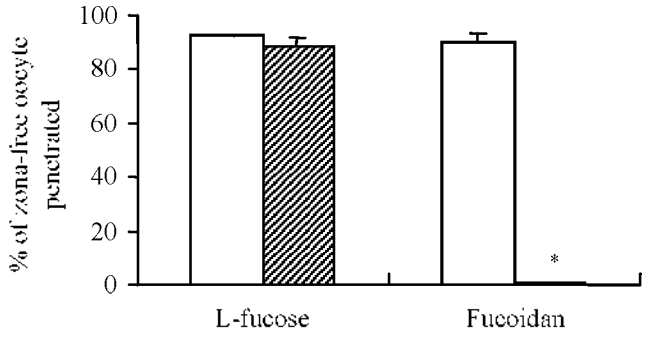

Figure 4. Penetration of spermatozoa into *in vitro* matured zona-free pig oocytes after incubation in the absence (open bars) presence (solid bars) of L-fucose or fucoidan. Frozen-thawed pig ejaculated spermatozoa (1×10^7 cell/ml) were incubated with oocytes for 12 h in 5% CO_2 in air at 39°C in a 100 μl drop of fertilization medium containing L-fucose (30 mM) or fucoidan (1.0 mg/ml). Data are shown as means±SDM of six trials. * $p<0.001$ compared with the values in the absence of saccharide. The percentage superscripts on the bars are inhibitory rate.

DISCUSSION

The results on the present study indicated that the activity of sperm α-L-fucosidase was largely inhibited when sperm suspension treated with monosaccharide L-fucose. It also significantly inhibited the number of sperm binding to ZP and penetration into zona-intact oocytes, but did not inhibit penetration into zona-free oocytes when fertilization medium contained L-fucose. The CTC assessment showed that L-fucose did not affect induction of capacitation and acrosome reaction. The activity of sperm α-L-fucosidase was not inhibited when sperm suspension treated with polysaccharide fucoidan, but sperm-ZP binding was greatly inhibited and completely blocked sperm penetration into zona-intact or zona-free oocytes. The CTC assessment showed that fucoidan increased the F pattern and decreased the AR pattern of spermatozoa. These results suggested that a different inhibitory mechanisms were presented between L-fucose and fucoidan on sperm-oocyte interaction.

Many studies have implied the macromolecules, such as lectin-like proteins and enzymes in the plasma membrane and acrosomes of spermatozoa were concerned with recognition of oligosaccharides of ZP glycoproteins. Among them, certain sperm glycosidases were implicated in sperm-oocyte interaction. It is believed to form stable enzyme-substrate complexes by binding with high specificity and affinity to the oligosaccharide residues of glycoproteins of the ZP without acting as a catalyzer (for review, Macek and Shur, 1988). However, certain sperm-associated glycosidases have been localized in the acrosome (for revew, Bellve and O'Bren, 1983) and this acrosomal localization

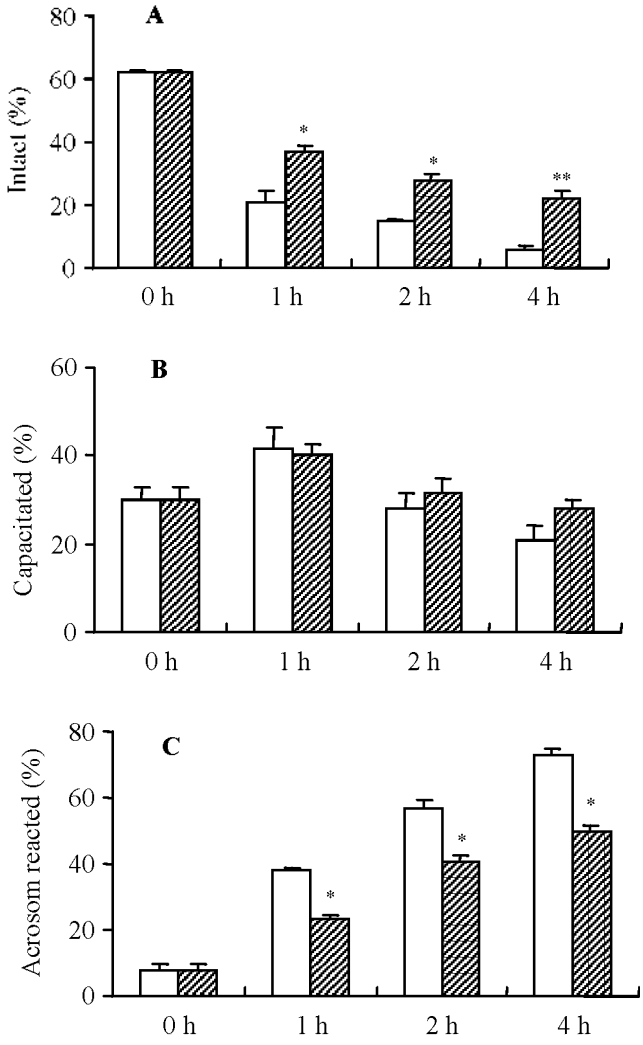

Figure 5. CTC staining pattern of spermatozoa incubated with (solid bars) and without (open bars) fucoidan. Frozen-thawed ejaculated pig spermatozoa (1×10^7 cell/ml) were incubated for up to 4 h in 5% CO_2 in air at 39°C in the absence or presence fucoidan (1.0 mg/ml). Data of intact (A), capacitated (B) and acrosome reacted (C) spermatozoa are shown as means±SDM of six trials. * $p<0.01$, ** $p<0.01$ compared with the values in the absence of saccharide at each time of incubation.

has led to the hypothesis that glycosidases released as a consequence of the acrosome reaction might act to degrade glycoconjugates of the investments of the oocyte and thus facilitate sperm penetration. It was reported sperm-associated α-L-fucosidase has been proposed to act as a noncatalytic binding protein that might mediated sperm-oocyte recognition in ascidian *Ciona intestinalis* (Rosati and DeSantis, 1980; Hoshi et al., 1985; Hoshi, 1986; Matsumoto et al., 2002). In mammals, α-L-fucosidase activity has been detected in bull (Zahler and Doak, 1975; Jauhiainen and Vanha-Perttula, 1986), human (Alhadeff et al., 1999; Khunsook et al., 2003) sperm. However, the biological function of α-L-fucosidase remains unknown. In

recent investigation, we found α-L-fucose residues present in pig ZP (Song et al., 1999), and the activity of α-L-fucosidase was present in boar spermatozoa and was released by acrosome reaction (Song et al., 2000). When specific lectin labeled oocyte or fertilization medium containing α-L-fucosidase inhibitor was used, sperm binding to ZP and penetration to oocyte were significantly inhibited. It is suggested that α-L-fucose residues presented in ZP and α-L-fucosidase presented in spermatozoa involved sperm-oocyte interaction in pig. In the present study, L-fucose inhibited 61% of α-L-fucosidase activity and inhibited 30% of sperm-ZP binding or 72% of sperm penetration into ZP. These results are same as the result from our previous investigation with deoxyfuconojirimycin hydrochloride (DFM-H) as an α-L-fucosidase inhibitor (Song et al., 2000). Integrating these observations, we propose that α-L-fucosidase presented in pig spermatozoa specific to α-L-fucose residues presented in the ZP may play roles in sperm-ZP interaction, the inhibition of sperm binding to and penetration through the ZP by L-fucose is due to the inhibied activity of α-L-fucosidase.

Sperm-zona interaction involves binding of the sperm to species-specific receptor molecules presented within the zona (Yanagimachi, 1981; Wassarman, 1987). A number of studies have indicated that carbohydrate moieties presented within the ZP regulate this interaction. Competitive inhibition studies using various monosaccharides or polysaccharides have been used to investigate sperm-zona interactions. It has been reported that monosaccharide L-fucose at higher concentrations(50 mM) than those used in our study, was used to inhibit sperm-zona binding in the guinea pig (Huang et al., 1982) , rat (Shalgi et al., 1986) and bovine (Tanghe et al., 2004). In the present study indicated that the number of sperm bound to ZP was inhibited 32% when fertilization medium contained 30 mM L-fucose. It was same as the results from guinea pig (Huang et al., 1982), rat (Shalgi et al., 1986) and bovine (Tanghe et al., 2004) had minor extent effect for sperm-ZP binding. In contrast, the number of sperm bind to ZP inhibited 85% when fertilization medium containing fucoidan. Its also same as the results from the guinesa pig (Huang et al., 1982), rat (Shalgi et al., 1986) , pig (Peterson et al., 1981; 1984) and bovine (Tanghe et al., 2004) .

In the present study, fucoidan showed a stronger inhibition on sperm-oocyte binding and sperm penetration through the ZP than L-fucose. One would expect that the fucoidan may inhibit sperm α-L-fucosidase activity more strongly, but the expected inhibitory effect was not observed. It was reported that a group of fucose-binding proteins with molecular weights of 53 KD was described on boar spermatozoa (Toepfer-Petersen et al., 1985), very similar to those of a zona-binding protein (Brown and Jones, 1987) and later identified as proacrosin, the zymogen form the acrosomal protease acrosin (Jones and Brown, 1987). Urch and Hedrick (1988) reported fucoidan can inhibit acrosin amidase activity and thus propose it is related to the inhibition of sperm binding to the zona pellucida. However monosaccharide fucose did not inhibit acrosin amidase activity (Urch and Hedrick, 1988; Lo leggio et al., 1994). It was suggested that different inhibition mechanisms were present between L-fucose and fucoidan.

Fertilization in mammals can be viewed specific cellular interactions between the sperm and egg. During the fertilization process, the sperm cell must successfully interact with two primary surface constituents of the egg, the zona pellucida and the egg plasma membrane (Yanagimachi, 1981). It is reported that, certain saccharides and glycoproteins would competitively inhibit fertilization of zona-intact hamster oocytes but not zona-free oocytes (Ahuja, 1982). Studies of zona-free hamster (Hirao and Yanagimachi, 1978) or mouse (Boldt et al., 1988) oocyte or hamster spermatozoa (Ahuja, 1982; 1985) treated with exoglycosidic enzymes prior to insemination also indicated failure of inhibition of sperm-oocyte fusion. However, fertilization medium containing polysaccharide fucoidan significantly inhibited sperm-oocyte fusion with hamster (Drabland and Mortimer, 1988) and mouse oocytes (Boldt et al., 1989). In the present study, the fertilization of zona-intact oocytes were strongly inhibited, but did not inhibited zona-free oocytes by L-fucose. In contrast, fucoidan was not only completely blocked fertilization of zona-intact but also completely blocked fertilization of zona-free oocytes. Since fucoidan could inhibit acrosin amidase activity (Urch and Hedrick, 1988), it may considered that acrosin amidase activity were related to sperm-oocyte fusion.

It is hypothesized that, the primary molecules in the plasma membrane were implicated in low affinity attachment of spermatozoa, while ZP kept sufficient strength to resist lateral displacement forces generated by the motile tail. This initial attachment immediately triggers the acrosome reaction and thereby acrosomal contents including proacrosin and acrosin begin to disperse. At this stage, a second phase of sperm binding with high affinity takes place. Thus, proacrosin and acrosin are considered as secondary molecules implicated in sperm-oocyte binding (Jones, 1990). In the present study, since L-fucose inhibited sperm bind to ZP only 32% but strongly inhibit fertilized oocyte 72%, it could reflect lack of L-fucosyl residual for sperm binding sites on the surface of ZP. This inference was supported by our previous investigation that α-L-fucose specific lectin UEA-I mainly labeled the inner region of the ZP (Song et al., 1999). Integrating these observations and with the hypothesis of Jones (1990) described above, we

propose that α-L-fucosidase may play a role in hydrolysis between spermatozoa and zona pellucida for maintaining the sperm penetration on the ZP, α-L-fucosidase and proacrosin may be play a role for cycles of hydrolysis and binding that facilitate sperm penetration of the ZP (Urch, 1991).

In conclusion, data presented here suggest that different inhibition mechanism of the sperm-oocyte interaction are present between monosacchride L-fucose and polysaccharide fucoidan.

REFERENCES

Ahuja, K. K.1982. Fertilization studies in the hamster: The role of cell surface carbohydrates. Exp. Cell Res. 140:353-362.

Ahuja, K. K. 1985. Carbohydrate determinants involved in mammalian fertilization. Am. J. Anat. 174:207-223.

Alhadeff, J. A., S. Khunsook, K. Choowongkomon, T. Baney, V. Heredia, A. Tweedie and B. Bean. 1999. Characterization of human semen alpha-L-fucosidases. Mol. Hum. Repro. 5:809-15.

Bellve, A. R. and D. A. O'Bren. 1983. The mammalian spermatozoon: structure and temporal assembly. In: (Ed. J. F. Hartmann) Mechanism and Control of Animal Fertilization. New York: Academic Press; pp. 55-137.

Boldt, J., A. M. Howe, J. B. Parkerson, L. E. Gunter and E. Kuehn. 1989. Carbohydrate involvement in sperm-egg fusion in mice. Biol. Reprod. 40:887-896.

Boldt, J., A. M. Howe and J. Preble. 1988. Enzymatic alteration of the ability of mouse egg plasma membrane to interact with sperm. Biol. Reprod. 39:19-27.

Brown, C. R. and R. Jones. 1987. Binding of zona pellucida proteins to a boar sperm polypeptide of Mr 53000 and identification of zona moieties involved. Development 99:333-339.

Drabland, J. E. and D. Mortimer. 1988. Role for fucose-sulfate-rich carbohydrates in the penetration of zona pellucida free hamster eggs by hamster spermatozoa. Gamete Res. 21:353-358.

Frazier, W. and L. Glaser. 1979. Surface and cell recognition. Annu. Rev. Biochem. 48:491-523.

Hinrichsen-Kohane, A. C., M. Hinrichsen and W. B. Schill. 1984. Event leading to fertilization - a review. Andrologia 16:321-341.

Hirao, Y. and R. Yanagimachi. 1978. Effects of various enzymes of the ability of hamster egg plasma membranes to fuse with spermatozoa. Gamete Res. 1:3-12.

Hoshi, M. 1986. Sperm glycosidase as a plausible mediator of sperm binding to the vitelline envelope in ascidians. Adv. Exp. Med. Biol. 207:251-260.

Hoshi, M., R. D. Santis, M. R. K. Pinto, F. Cotelli and R. Rosati. 1985. Sperm glycosidases as mediators of sperm-egg binding in the ascidians. Zool. Sci. 2:65-69.

Huang, T. T. F., E. Ohzu and R. Yanagimachi. 1982. Evidence suggesting that L-fucose is part of a recognition signal for sperem-zona pellucida attachment in mammals. Gamete Res. 5:355-361.

Huang, T. T. F. and R. Yanagimachi. 1984. Fucoidan inhibits attachment of guinea pig spermatozoa to the zona pellucida through binding to the inner acrosomal membrane and equatorial domains. Exp. Cell Res. 153:363-373.

Jauhiainen, A. and T. Vanha-Perttula. 1986. α-L-fucosidase in the reproductive organs and seminal plasma of the bull. Biochem. Biophys. Acta. 880:91-95.

Jones, R. and C. R. Brown. 1987. Identification of a zona-binding protein from boar spermatozoa as proacrosin. Exp. Cell Res. 171:505-508.

Jones, R. 1990. Identification and functions of mammalian sperm-egg recognition molecules during fertilization. J. Reprod. Fert. 42:89-105.

Lo Leggio, L., R. M. Williams and R. Jones. 1994. Some effects of zona pellucida glycoproteins and sulfated polymers on the autoactivation of boar sperm proacrosin and activity of β-acrosin. J. Reprod. Fert. 100:177-185.

Ka, H. and F. W. Bazer. 2005. Effects of keratinocyte growth factor on the uterine endometrial cells in pigs. Asian-Aust. J. Anim. Sci. 18:1708-1714.

Khunsook, S., B. S. Bean, S. R. McGowan and J. A. Alhadeff. 2003. Purification and characterization of plasma membrane-associated human sperm alpha-L-fucosidase. Biol. Reprod. 68:709-716.

Macek, M. B. and B. D. Shur. 1988. Protein-carbohydrate complementarity in mammalian gamete recognition. Gamete Res. 20:93-109.

Mahony, M. C., S. Oehninger, G. F. Clark, A. A. Acosta and G. D. Hodgen. 1991. Fucoidin inhibits the zona pellucida-induced acrosome reaction in human spermatozoa. Contraception 44:657-65.

Matsumoto, M., J. Hirata, N. and M. Hoshi. 2002. Sperm-egg binding mediated by sperm alpha-L-fucosidase in the ascidian, Halocynthia roretzi. Zoolog. Sci. 19:43-8.

Miller, D. J., X. Gong and B. D. Shur. 1993. Sperm require b-N-acetylglucosaminidase to penetrate through the egg zona pellucida. Development 118:1279-1289.

Miller, D. J., M. B. Macek and B. D. Shur. 1992. Complementarity between sperm surface β-1,4- galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. Nature 357:589-593.

Moreno, R., P. Orihuela and C. Barros. 2001. Differential effects of polysulphates between mouse and hamster during *in vitro* fertilization. Andrologia 33:19-25.

Oehninger, S., G. F. Clark, D. Fulgham, P. F. Blackmore, M. C. Mahony, A. A. Acosta and G. D. Hodgen. 1992. Effect of fucoidin on human sperm-zona pellucida interactions. J. Androl. 13:519-25.

Peterson, R. N., L. Russell, D. Bundmann, M. Conway and M. Freund. 1981. The interaction of living boar sperm and sperm plasma membrane vesicles with the porcine zona pellucida. Dev. Biol. 84:144-156.

Peterson, R. N., L. Russell and W. P. Hunt. 1984. Evidence for specific binding of uncapacitated boar spermatozoa to porcine zonae pellucidase *in vitro*. J. Exp. Zool. 231:137-147.

Rosati, F. and R. DeSantis. 1980. Role of the surface carbohydrates in sperm-egg interaction in *Ciona intestinalis*. Nature 283:762-764.

Shalgi, R., A. Mitityahu and L. Nebel. 1986. The role of carbohydrates in sperm-egg interaction in rats. Biol. Reprod. 34:446-452.

Song, X. X., X. Z. Yang, K. Iga and K. Niwa. 1999. Binding of lectins to the zona pellucida of *in vitro* matured pig oocytes and sperm-oocyte interaction *in vitro*. J. Mamm. Ova Res. 16:23-30.

Song, X. X., P. X. Liu, K. W. Park, K. Iga and K. Niwa. 2000. Identification, localization and involvement of glycosidases in sperm-zona interaction using frozen-thawed ejaculated pig spermatozoa. J. Reprod. Develop. 45:115-125.

Tanghe, S., A. Van Soom, L. Duchateau and A. De Kruif. 2004. Inhibition of bovine sperm-oocyte fusion by the p-aminophenyl derivative of D-mannose. Mol. Reprod. Dev. 67:224-32.

Toepfer-petersen, E., A. E. Friess, H. Nguyen and W. B. Schill. 1985. Evidence for a fucose-binding protein in boar spermatozoa. Histochem. 82:139-145.

Urch, U. A. 1991. Biochemistry and function of acrosin. In the Biology and Chemistry of mammalian Fertilization (Ed. P. M. Wassarman). Chicago: CRC Press, pp. 233-248.

Urch, U. A. and J. L. Hedrick. 1988. The inhibition of boar acrosin amidase activity by sulphated polysaccharides. Biol. Chem. Hoppe-Seyler 369:727-732.

Urch, U. A. and H. Patel. 1991. The interaction of boar sperm proacrosin with its natural substrate, the zona pellucida, and with polysulfated polysaccharides. Development 111:1165-1172.

Wang, W. H., L. R. Abeydeera, L. R. Fraser and K. Niwa. 1995. Functional analysis using chlortetracycline fluorescence and *in vitro* fertilization of frozen-thawed ejaculated boar spermatozoa incubated in a protein-free chemically defined medium. J. Reprod. Fertil. 104:305-313.

Wassarman, P. M. 1987. The biology and chemistry of fertilization. Science NY, 235:553-560.

Yanagimachi, R. 1981. Mechamisms of fertilization in mammals. In fertilization and embryonic development *in vitro* (Ed. L. Mastroianni and J. D. Biggers), New York: Plenum Press, pp. 32-182.

Zahler, W. L. and G. A. Doak. 1975. Isolation of the outer acrosomal membrane on bull sperm. Biochem. Biophys. Acta. 406:479-488.