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*Research Article*

# Role of egg surface glycoconjugate in the fertilization of the rock shrimp *Rhynchocinetes typus* (Milne-Edwards, 1837)

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**ABSTRACT.** During first gamete interaction, oligosaccharides on the glycoproteins present in oocyte envelopes of the rock shrimp, *Rhynchocinetes typus*, play an important role in spermatozoon recognition prior to the onset of penetration. These oligosaccharides have terminal monosaccharides that may be recognized by means of specific lectins. A variety of marine species have monosaccharides on their oocyte envelopes, allowing them to interact with the spermatozoa. The number and location of the monosaccharides varies during oocyte maturation. However, this phenomenon has not been studied in shrimp. The present study evaluates the presence and location of monosaccharides on oocyte envelopes during maturation using seven specific lectins conjugated to FITC (GNL, LCA, Con-A BS-1, LTA, GLS-II,DBA); these recognize mannose (α 1-3) mannose, α-mannose, α-glucose, glucose, galactose, fucose, N-acetyl-glucosamine, and N-acetyl-galactosamine, respectively. Participation of these carbohydrates in the fertilization process was determined through the insemination of fresh oocytes with spermatozoa previously incubated with the different monosaccharides that block the active sites of the spermatozoa, inhibiting fertilization. The results showed the presence of N-acetyl-glucosamine and mannose on the oocyte envelopes, indicating that these sugars could be the receptors during the first gamete interaction. The results also suggested that glucose could be a complementary receptor to N-acetyl-glucosamine and mannose since, despite the low concentration of glucose on the envelope, this sugar generated a high degree of fertilization inhibition.

**Keywords**: gametes, oocyte recognition, sperm receptors, lectins, vitelline coat, chorion.

# El rol de los glicoconjugados de las cubiertas ovocitarias en la fecundación del camarón de roca *Rhynchocinetes typus* (Milne-Edwards, 1837)

**RESUMEN.** Durante la primera interacción gamética, los oligosacaridos unidos a las glicoproteínas de las cubiertas ovocitarias del camarón de roca *Rhynchocinetes typus* tienen un importante rol en el reconocimiento del espermatozoide antes de iniciar la penetración. Estos oligosacáridos presentan en su zona terminal, monocacáridos que pueden ser reconocidos por lectinas específicas. Diversas especies marinas tienen monosacáridos en la cubierta externa de sus ovocitos que le permiten interactuar con los espermatozoides y su número y localización presenta cambios durante la maduración, sin embargo este fenómeno no ha sido estudiado en camarones. El presente estudio evalúa la presencia y localización de monosacáridos sobre la cubierta de los ovocitos durante su maduración usando siete lectinas específicas conjugadas con FITC (GNL, LCA , Con-A BS-1, LTA, GLS-II y DBA) las cuales reconocen manosa (α 1-3) manosa, α-manosa, α-glucosa, glucosa, galactosa, fucosa, N-acetil-glucosamina y N-acetilgalactosamina respectivamente. La participación de estos carbohidratos en el proceso de fecundación fue determinada mediante la inseminación de ovocitos frescos con espermatozoides incubados previamente con los diferentes monosacáridos que bloquean los sitios activos de los espermatozoides inhibiéndo la fecundación. Los resultados mostraron la presencia de N-acetil-glucosamina y manosa en la cubierta externa de los ovocitos maduros los cuales podían ser los receptores espermáticos durante la primera interacción gamética. También se sugiere que la glucosa podría ser un receptor complementario a los anteriormente mencionados debido que a pesar que su concentración en las cubiertas es baja, genera una alta inhibición de la fecundación.

**Palabras clave:** gametos, reconocimiento ovocitario, receptores espermáticos, lectinas, cubierta vitelina, corion.

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## INTRODUCTION

The initial interaction between oocytes and spermatozoa involves molecules present on oocyte envelopes and complementary receptors located on the spermatozoa. The most common molecules on the surface of oocyte envelopes are the glycoproteins (Glabe *et al.*, 1982; Ruiz-Bravo & Lennarz, 1989; Focarelli *et al.*, 1990; Xie & Honegger, 1993; Wikramanayake & Clark, 1994), which have terminal oligosaccharides on the surface responsible for sperm recognition through recognition of species-specific polypeptide chains on the spermatozoa (Monroy & Rosati, 1983; Wassarman, 1987; Ruiz-Bravo & Lennarz, 1989; Litscher & Honegger, 1991). In sea urchins, species specific adhesion of spermatozoa to the vitelline envelope is effected by a protein termed *bindin* exposed during spermatozoon acrosoma reaction, which recognizes a 350 kDa glycopeptide on the oocyte envelope (Monroy & Rosati, 1983; Wassarman, 1987; Correa & Carroll, 1997; Hirohashi & Lennarz, 2001). In tunicates, spermatozoa adhesion to the oocyte envelope is mediated by an enzyme-substrate interaction between a sperm surface bound glycosidase and its complementary sugar on the oocyte envelope (Litscher & Honegger; 1991). In *Ascidia nigra* and *Phallusia mamillata* N-acetyl-glucosamine residues were found in the oocyte envelope and N-acetyl-glucosaminidase in spermatozoon membranes (Xie & Honegger, 1993), while for *Ciona intestinalis* and the bivalve *Unio elongatulus,* fucosyl residues on the oocyte envelope are specific sites for sperm adhesion (Rosati & De Santis, 1980; Pinto *et al.*, 1981; Focarelli *et al.*, 1990).

In the Crustacean, studies carried out on the peneaid shrimp *Sycionia ingentis* (Pillai & Clark, 1990; Glas *et al.*, 1996) showed that post-spawning changes occurred in oocyte envelopes due to the exocytose of vesicles similar to cortical granules, which reorganized the oocyte envelopes to form a covering (hatching envelope) which contained sugar moieties including mannose, glucose, N-acetyl-glucosamine and sialic acid (Pillai & Clark, 1990). In *Rhynchocinetes typus*, Palomino (2000) reported that the fertilization percentage *in vitro* significantly decreased when spermatozoa were pre-incubated with solubilized oocyte envelopes, suggesting that molecules from the envelopes could be participating during the fertilization process, reacting with receptors on the spermatozoa which neutralized their subsequent recognition of the receptors on intact oocyte envelopes.

Evidence available from marine organisms has demonstrated that initial gamete interaction involves activities of sugars ligated to proteins. The present study evaluates the presence of oligosaccharide residues during the maturation of the oocyte from de rock shrimp *R. typus*, on intact and isolated envelopes, using various lectins conjugated to fluorescein and their participation during the fertilization process.

## MATERIALS AND METHODS

### Isolation of oocyte envelopes

Oocytes at 5-7 days pre-molt, 1-2 days pre-molt, and 2 days post-molt were obtained by dissection from ovaries of *R. typus (*Dupré & Barros, 1983; Ríos, 1993), washed with filtered sea water, and fixed in 4% formaldehyde for 1-2 h for subsequent screening with lectins. Prior to fixation, some oocyte envelopes were isolated by a 15-30 min treatment with calcium-free artificial seawater containing EGTA (ASW Ca-free: NaCl 0.4 M, KCl 9 mM; $MgCl^2$ 23 mM, $MgSO^4$ 25 mM, $NaHCO_3$ 2 mM, EGTA 10 mM). The envelopes were collected using a micropipette and washed twice with ASW Ca-free. Envelopes were washed with PBS (pH 7.4) and later whit PBS + BSA 3 mg $mL^{-1}$ and placed in a depression slides for lectin treatments.

### Identification of carbohydrates on oocyte envelopes

Batches of 40-50 intact oocyte or isolated enveloped were pre-treated at 21ºC for 30 min in 1 mL phosphate buffer saline (PBS) containing bovine seroalbumin 3 mg $mL^{-1}$ (BSA) and incubated at 18ºC for 20 min in 100 mL of solutions of seven different lectins each dissolved in PBS + BSA 3 mg $mL^{-1}$ conjugated with fluorescein isothiocyanate (FITC). The lectins used were: (1) Concanavalin-A (Con-A) to recognize glucose (Glc), (2) *Glanthus nivalis* (GNL) for mannose (α 1-3) mannose (Man-Man), (3) *Lens culinaris* agglutinin (LCA) for α-mannose (Man) and α-glucose (Glc), (4) *Lotus tetranoglobus* agglutinin (LTA) for fucose (Fuc), (5) *Badeiraea simplicifolia*-I (BS-I) for galactose (Gal), (6) *Griffonia simplicifolia* (GSL II) for N-acetyl-glucosamine (N-AcGlc), and (7) *Dolichos biflorus* agglutinin (DBA) for N-acetyl-galactosamine (N-AcGal) at concentrations of 10 mg $mL^{-1}$ each. They were incubated in dark at 18°C for 20 min, washed in PBS three times, and mounted and examined using a Nikon Fluorophot fluorescence microscope. Samples were photographed using a Nikon FX-35 camera. Control oocytes were prepared using the same procedure except the incubation with FITC-labeled reagents was replaced by PBS. To standardize the fluorescence, samples contained 250 mM of the following monosaccharides solution; Man, Glc, Fuc, Gal, N-AcGlc, N-AcGal, were pre-incubated

for 10 min with the respective lectin prior to incubation with the oocytes envelopes.

Isolated oocyte envelopes were rinsed with three changes of PBS and mounted on microscope slides with 1% polylysine. They were then rinsed with PBS and separate samples were incubated in each of the lectins listed above using the same protocol as that used for the oocytes. Controls were prepared using the same methods and substituting incubation in PBS for that with the lectins. Measurements of fluorescence intensity on both intact and isolated oocyte envelopes were carried out using a Nikon HFX-II automatic photometer attached to the microscope, with a 40X objective. Measurements were made on five separated points selected randomly on each oocyte envelope. Fluorescence intensity index (FI) was calculated by: FI = [(1/exposure time) 100]. The exposure time is the time showed by the photometer of the automatic camera to obtain a picture. Zero luminosity was assumed when no reading was obtained after 500 sec of exposure time.

### Assay for inhibition of fertilization

In the fertilization assays, oocytes from 21 females (3,173 oocytes) were used. Three (with three replica) fertilization assays were performed for each lectin in order to determine which monosaccharides interacted with the spermatozoa during fertilization. *In vitro* fertilization assays were carried out with spermatozoa which had been previously treated with specific monosaccharides to block the specific lectin-like molecules present on the spike of the spermatozoa and thus prevent the specific binding of the sperm to each of the monosaccharides detected in the oocyte envelopes.

Spermatozoa were obtained by means of the electric ejaculation method of Gómez & Dupré (2002). To give a 0.25 M solution, one mL of sperm suspension at 246-498x$10^2$ sperm $mL^{-1}$ was individually diluted in 1 mL of 0.5 M solutions of α-mannose, D-glucose, L-fucose, D-galactose, N-acetyl-glucosamine or N-acetyl-galactosamine which are known competitors of the lectins LCA, Con-A, LTA, BS-1, GSL II and DBA respectively. After the spermatozoa had been incubated in the individual sugar at 21ºC for 30 min, 0.5 mL with 50 oocytes were added to each preparation to give a ratio of 860-1,740 sperm/oocyte. The gametes were then mixed by gentle pipette aspiration for five minutes. After co-incubation of the gametes they were fixed in 4% formaldehyde in 1 mm-filtered seawater. Controls were prepared with gametes by the same methods, but in the absence of competitive sugars.

In order to evaluate the sperm penetration, the oocytes were placed in a solution of 1:1 chloroform-methanol for 5-7 days to render them transparent. After this period this solution was replaced with 50% acetic acid, and the samples were slowly heated (40°C) until complete transparency was obtained. Upon cooling to room temperature the acetic acid was replaced by lactoacetic orcein at 40°C to stain the DNA in both gametes. Later, samples were mounted whole on microscope slides for observation at 100-200X. Penetrated oocytes was recorded when they contained the DNA of one spermatozoon within the oocyte cytoplasm. The control of fertilized oocytes were fixed in 2% glutaraldehyde in seawater, critical point dried with $CO_2$, coated with gold and analyzed by a Jeol JSM-300 scanning electron microscope (SEM).

### Statistical analysis

Luminosity data for both oocytes and isolated envelopes were submitted to two way ANOVA to determine statistically valid differences between preparations ($P < 0.05$). The percentages of inhibition of fertilization were transformed to arcsine and compared by one way ANOVA and Tukey and Kruskal-Wallis test.

## RESULTS

The first sperm-egg interaction between the top of the sperm spike and the oocyte coat was confirmed (Fig. 1).

### Assays with lectins intact envelopes pre and post-molt

All the control oocytes showed autofluorescence of the oocyte yolk with a fluorescence intensity index (FI) of 13.1 ± 4.6. Isolated (Fig. 2) or intact or envelopes treated with each lectins showed some degree of fluorescence. The highest fluorescence was obtained in intact envelopes, of both pre and post-molt stages, incubated with GNL lectin (FI = 67.6 ± 28.5), however the fluorescence was lower in the isolated envelopes. Values for LCA (FI = 43.6 ± 24.6), GSL-II lectins (FI = 33.1 ± 10.9) and BS-1 (FI = 27.7 ± 4.0) were 1.6-2.4 time less than those obtained for GNL. The lowest fluorescence were obtained in intact envelopes treated with DBA (FI = 20.4 ± 9.1), Con-A (FI = 14.8 ± 3.8) and LTA (FI = 14.8 ± 2.9) that produced fluorescence values about 3.5-5.0 time less than GNL. The Kruskal-Wallis and Tukey test showed a significant difference between control and GNL (for man-man), LCA (for Glc and Man), GSL-II (for N-AcGlc) (q 3.85 = 14.82; 20.12 and 9.49 respectively)

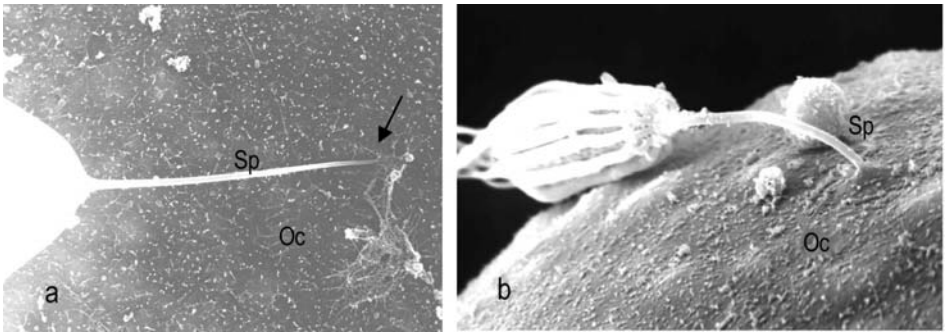


**Figure 1.** Scanning electron microscopy view of the first interaction sperm-oocyte. a) First interaction through the top of the sperm spike (arrow), b) fusion of the spike and the outer coat of the oocyte. Sp: spike of the sperm, Oc: outer coat of the oocyte.

**Figura 1.** Primera interacción espermatozoide-ovocito vista con microscopía electrónica de barrido. a) Primera interacción entre el extremo distal del proceso acicular (flecha), b) cubierta externa del ovocito. Sp: proceso acicular, Oc: cubierta externa del ovocito.

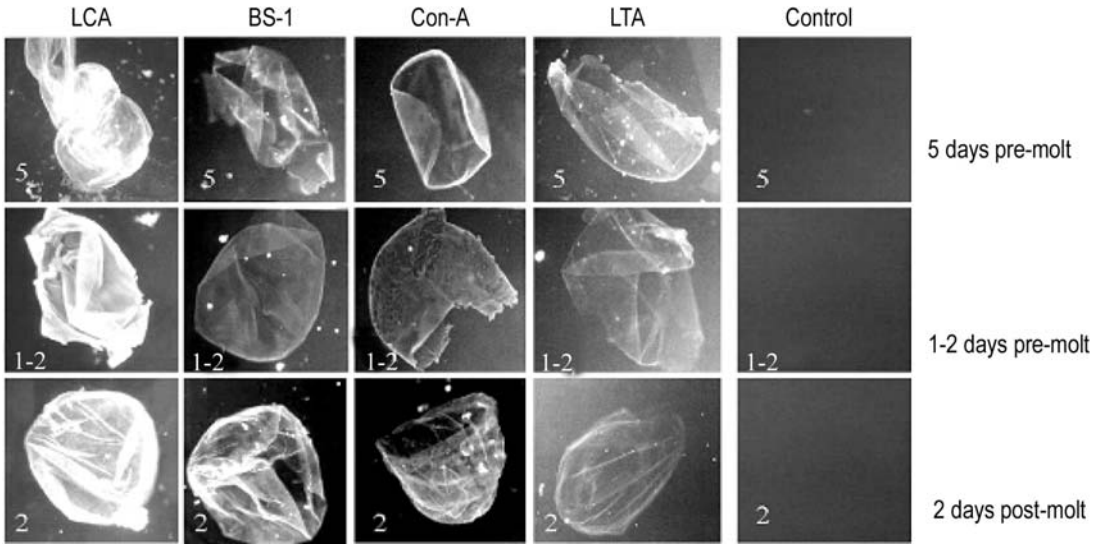


**Figure 2.** Fluorescence micrograph show labeling of isolated envelopes from *R. typus* oocyte on different maturation times pre and post-molt, labeled with different specific lectins as follows: BS-1 for galactose, Con-A for D-glucose, DBA for N-acetyl-galactose, GNL for mannose (α 1-3) mannose, GSL-II for N-acetyl-galactose, LCA for α-mannose and α-glucose and LTA for fucose. 5: five days pre-molt; 1-2: one or two days pre-molt; 2: two days post-molt. (Dupré & Barros, 2011).

**Figura 2.** Micrografías de fluorescencia que muestran la marcación de las cubiertas aisladas de ovocitos de *R. typus* en diferentes estados de maduración pre y post-muda, marcadas con las siguientes lectinas específicas: BS-1 para galactose, Con-A para D-glucose, DBA para N-acetyl-galactose, GNL para mannose (α 1-3) mannose, GSL-II para N-acetyl-galactose, LCA para α-mannose y α-glucose y LTA para fucosa. 5: cinco días pre-muda; 1-2: uno o dos días pre-muda; 2: dos días post-muda. (Dupré & Barros, 2011).

fluorescence. The DBA and BS-I showed only a slight reaction with isolated and intact envelopes and the fluorescence were not significant different from controls ($q3.85 = 3.08$; $P < 0.05$). Comparing the label of the pre-molt and post-molt intact envelopes they showed no significant differences ($F3.85 = 1.27$; $P < 0.05$).

**Isolated envelopes**

The isolated (Fig. 2) and intact envelopes showed similar fluorescence after the treatment with the different lectins, but the intact oocyte showed a weakly higher percentages than isolated envelopes except for LCA (Figs. 2 and 3) which the fluorescence

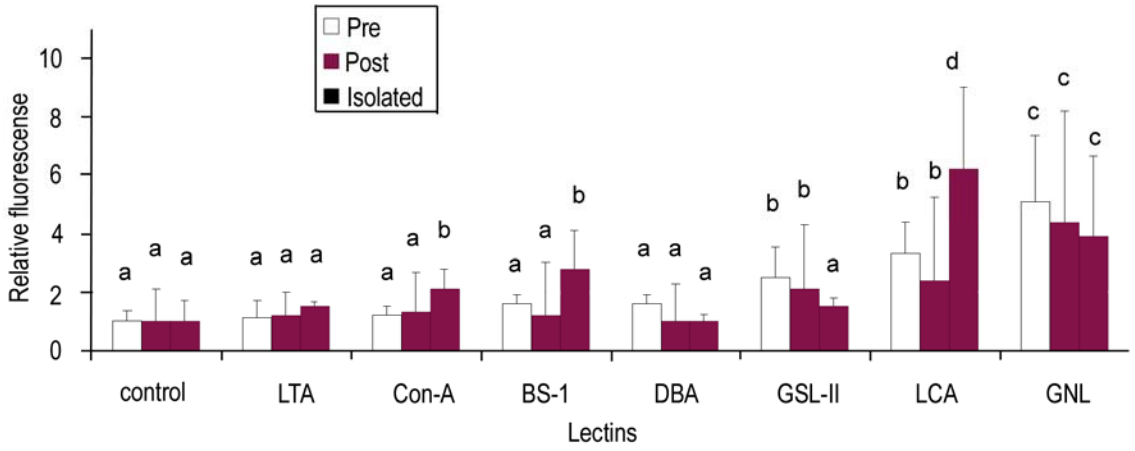


**Figure 3.** Relative index of fluorescence (respect to the controls) of intact and isolated envelopes obtained from the ovary-oocytes post-molt treated with different lectins labeled with FICT-C (BS-1 for galactose, Con-A for glucose, DBA for N-acetyl-galactose, GNL for mannose (α 1-3) mannose, GSL-II for N-acetyl LCA for mannose and glucose; and LTA for fucose). Pre: intact envelopes from pre-spawning oocytes, Post: intact envelopes from post-spawning oocytes, Isolated: isolated envelopes from post-spawning oocytes. Letters a, b, c and d, indicate statistically significant differences.

**Figura 3.** Indice relativo (respecto a los controles) de fluorescencia de cubiertas ovocitarias intactas y aisladas obtenidas del ovarios de hembras mudadas, teñidas con diferentes lectinas específicas marcadas con FICT-C (BS-1 para galactosa, Con-A para D-glucosa, DBA para N-acetilglucosa, GNL para manosa (α 1-3) manosa, GSL-II para N-acetilgalactosa, LCA para α-manosa y α-glucosa y LTA para fucosa). Pre: cubiertas intactas obtenidas de ococitos pre-desove; Post: cubiertas intactas de ovocitos post-desove; Isolated: cubiertas aisladas de ovocitos post-desove. Las letras a, b, c y d indican diferencias estadísticamente significativas.

was a non significative increase in relation to the intact oocyte. The controls of isolated envelopes no fluorescence was observed (Fig. 2).

The highest fluorescence was obtained in the envelopes treated with LCA (FI = 43.7 ± 33.5). The treatment with GNL, BS-1 and Con-A produced fluorescence values of 1.7, 2.1 and 2.9 time less than LCA respectively. Between 4 to 6 time less fluorescence were obtained with GSL-II, LTA and DBA treatments.

The *posteriori* Tukey test showed no significant difference between BS-1, DBA, Con-A, LTA and control ($q3.85 = 3.08$; $P < 0.05$) and between the rest of lectin treatments, all of them showed significant differences ($q3.85 = 5.3$ to $17.1$; $P < 0.05$). Envelopes treated with LCA and GNL, LCA and GSL-II showed marked fluorescence in the three maturational stages evaluated but these differences were not significant ($P < 0.05$).

### Fertilization assay

Figure 4 shows the fertilization and inhibition percentages with respect to the fertilization percentages of the controls oocytes. The highest inhibition percentages occurred when the spermatozoa were treated with the competitive monosaccharide N-acetyl-glucosamine at 0.05 M (80.2 ± 19.5%), glucose 0.05 M (56.9 ± 20.8%) and mannose 0.05 M (52.5 ± 9.9%). No significantly values of inhibition of the fertilization at 0.05 M nor at 0.5 M for N-acetyl-galactosamine 0.5 M (34.4 ± 3.6%), galactose 0.5 M (32.8 ± 6.8%) and fucose 0.5 M (33.4 ± 5.9%) with respect to the control values were obtained ($q = 6.4$; $P < 0.05$). Control values showed about 67% fertilization (33.0 ± 4.1% unfertilized). In spite mannose and glucose showed inhibiting the fertilization, the only significative difference in the inhibition of the fertilization between all the treatments and the controls was the incubation with N-acetyl-glucosamine at 0.25 M ($q6.2 = 0.05$ to $8.4$; $P < 0.05$). The different percentages of inhibition of glucose and mannose showed no significant differences.

## DISCUSSION

The results obtained from the percentage of luminosity permitted comparison of quantities of each of the monosaccharides recognized by the specific lectins at the different times of maturity. The highest luminosity of the oocyte treated with LCA and GNL lectins showed a high concentration (respect to the other monosaccharides and controls) of the Glc and Man respectively on the oocytes at all levels of oocyte

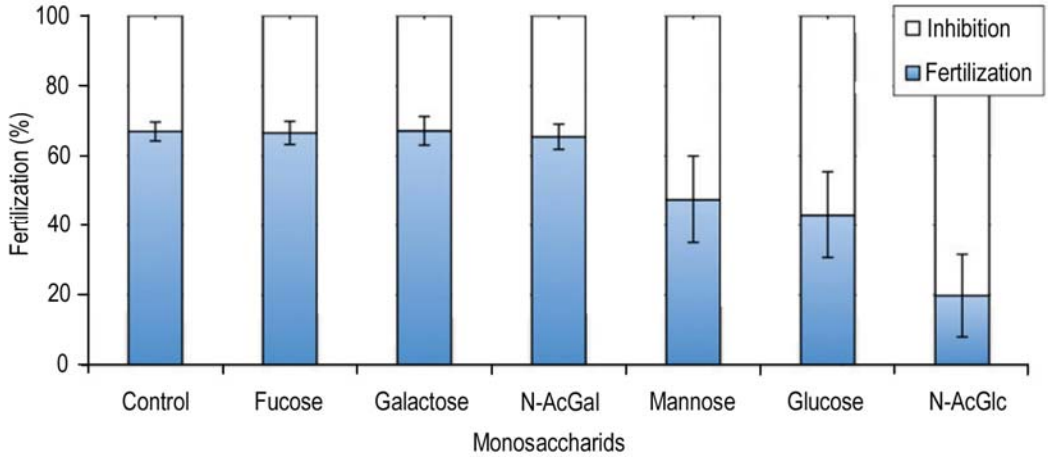


**Figure 4.** Percentage of fertilization (and inhibition) of oocyte inseminated with pre-treated spermatozoa with different monosaccharides. Abbreviations: *Griffonia* (*Badeiraea*) *simplicifolia* lectin-I, Con-A: *Concanavalin-A lectin*; DBA, *Dolichos* biflorus agglutinin; GNL, *Galantus nivalis* lectin; GSL-II, *Gliffonia simplicifolia* lectin-*II*; LCA, *Lens culinaris* aglutinin*;* LTA, *Lotus tetranoglobus* agglutinin.

**Figura 4.** Porcentaje de fecundación (e inhibición) obtenidos de ovocitos inseminados con espermatozoides pre-incubados con diferentes monosacaridos. Abreviaciones: *Griffonia* (*Badeiraea*) *simplicifolia* lectin-I, Con-A: *Concanavalin-A lectin*; DBA, *Dolichos* biflorus agglutinin; GNL, *Galantus nivalis* lectin; GSL-II, *Gliffonia simplicifolia* lectin-*II*; LCA, *Lens culinaris* aglutinin*;* LTA, *Lotus tetranoglobus* agglutinin.

maturation. This result agrees with previous studies carried out on oocyte envelopes in the shrimp *Sicyonia ingentis* (Pillai & Clark, 1990; Glas *et al.*, 1996), the sea urchin *Strongylocentrotus purpuratus* (Correa & Carroll, 1997) and in cattle (Amari *et al.*, 2001), in which the oocyte envelopes had high mannose values.

The high luminosity of the envelopes treated with LCA lectin could be attributed to presence of mannose and glucose because this lectin recognizes both monosaccharides residues. However, this high luminosity is due mainly to the high concentration of mannose on the coat as determined by GNL lectin, which is specific for mannose (α 1-3) mannose.

The major presence of mannose (α 1-3) mannose, detected by GNL lectin, and N-acetyl-glucosamine on the envelopes of different maturation stage oocytes of *R, typus* might be attributable to the eventual participation of these sugars in the primary or secondary gamete interaction as demonstrated in *Phallusia mammillata*, where N-acetyl-glucosamine as well as N-acetyl-galactosamine are implicated in the primary gamete interaction (Honegger, 1982; Litscher & Honegger, 1991) and they are the highest quantity on those oocyte envelopes (Litscher & Honegger, 1991). This was corroborated by Honegger (1982) by inhibition of fertilization using the WGA lectin, which adheres specifically to N-acetyl-glucosamine where it blocks sperm adhesion sites.

By analogy with present data, we now suggest that N-acetyl-glucosamine and mannose may be involved in the sperm-oocyte interaction in *R. typus*. This suggestion is supported by the strong inhibition of the fertilization when spermatozoa were pre-treated with N-acetyl-glucosamine.

Galactose was another monosaccharide detected on the oocyte envelope (reactive with BS-1). This monosaccharide, however, does not apparently have a direct role in the fertilization, as no inhibition of fertilization was observed in our tests. It may however, aid in oocyte recognition, as observed in mice where the terminal galactose of a 3-5 kDa oligosaccharide belonging to the ZP3 glycoprotein of the zona pellucida, was necessary for recognizing the spermatozoa as well as with N-acetyl-glucosamine (Miller & Ax, 1990).

It is known that not all monosaccharides present in oocyte envelopes are directly involved with gamete interactions (Focarelli *et al.*, 1988; Litscher & Honegger, 1991; Kitazume-Kawaguchi *et al.*, 1997). The fucose, galactose and α-GalNAc detected in lowest concentration in oocyte envelopes of *R. typus* may be only a portion of structural oligosaccharide chains of glycoconjugates due to its low quantity in the envelope and absence of inhibition in the fertilization inhibition tests, and probably do not interact in the gamete recognition as suggested by Litscher & Honegger (1991) in oocytes of *Phallusia mamillata* and *Ascidia malaca* by O'Dell *et al.* (1974). Different is the situation of the α-N-acetyl-glucosamine detected in *Strongylocentrotus purpu-*

*ratus*, which did not participate directly in primary gamete interaction (Correa & Carrol, 1997), but in *R. typus* this sugar has a great importance in the fertilization process because in spite of its small concentration (similar to that of L-fucose, D-galactose and α-GalNAc) in the intact oocyte and isolated envelopes it was the strongest inhibitor of fertilization together with glucose and mannose among the seven sugars tested in this study. Both monosaccharides, glucose and mannose participate in the gamete interaction in the anuran *Bufo arenarum* (Del Pino & Cabada, 1987), as well as that of the sea urchin *Arbacia punctulata* (Schmell *et al.*, 1977) as demonstrated by the inhibition of fertilization of oocytes treated with Con-A. Present data on inhibition of fertilization suggest that glucose and mannose act in sperm recognition and adhesion in *R. typus*. Glucose and N-acetyl-glucose may occur in the distal portion of oligosaccharide chains present on the oocyte envelope, which also contain a large amount of mannose (Miller & Ax, 1990).

A preceding study in *R. typus*, the fertilization was inhibited by the solubilized extract of oocyte coat (Palomino, 2000), but the molecules that participate in this inhibition were not determined. The presents study showed that the inhibitor molecules could be the α-N-acetyl-glucosamine.

The result obtained permit to suggest that the mechanism of the inhibition could be explained by the existence of a lectin-like molecule on the tip of the spike of the spermatozoa (the distal end of which interacts with envelope of the oocyte; Dupré & Barros, 1983, Barros *et al*., 1986; Dupré, 1991; Ríos, 1993; Palomino, 2000; Bustamante *et al.*, 2001) capable of recognizing the oocyte sugars, which were specifically blocked during the incubation of the sperm with the corresponding monosaccharide.

A protein on the shrimp sperm analogous to *bindin* of sea urchin sperm was postulated by different before studies (Barros *et al*., 1986; Dupré, 1991; Ríos, 1993; Bustamante *et al*., 2001), since a strong union was observed between the extreme distal portion of the sperm spike and the external envelope or chorion of mature oocytes in the shrimp. This union could not be broken by strong physical agitation of the oocyte with attached sperm. This phenomenon was again observed in the present study, and based on the results of inhibition of fertilization by mannose and glucose, it is suggested that the molecule responsible for recognition of carbohydrates on the oocyte envelope is a protein similar to *bindin* of sea urchin spermatozoa (Glabe *et al.*, 1982).

According to Glabe *et al*. (1982) who proposed that *bindin* was a surface lectin, an explanation for the inhibition of the fertilization by the α-N-acetil-glucosamine and α-mannose and α-D-glucose could be that free monosaccharides in the incubation medium adhere to the *bindin*-type protein located in the spike of the spermatozoa and block the active site to adhere to oocyte ligand on the envelope.

The inhibition of the fertilization does not determine if the interaction is associated with the primary or secondary sperm-oocyte interaction, nor if the most external or the inner most zone of the coat bound to the spermatozoa as showed in the shrimp *Sycionia ingentis* (Wikramanayake *et al.*, 1994).

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