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Effect of *Varroa jacobsoni* parasitization in the glycoprotein expression on *Apis mellifera* spermatozoa

E Del Cacho, JI Marti, A Josa, J Quílez, C Sánchez-Acedo

Department of Animal Pathology, Faculty of Veterinary Science,
Zaragoza University, Zaragoza, Spain

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Summary — In this article we describe, at both light and electron-microscopic levels, the distinctive distribution of binding sites to biotinylated BPA, Con A and PNA lectins in the male gametes of *Apis mellifera*. BPA was reactive with the plasma membrane of the entire head, Con A with the acrosomal and postacrosomal region, and PNA with the acrosomal cap. Comparison of the lectin-binding patterns to the spermatozoa of non-parasitized drones and to those cells of drones parasitized with *Varroa jacobsoni* showed similar staining patterns in both cases. However, the number of labelled cells was lower in parasitized than in non-parasitized drones, suggesting that *V jacobsoni* may alter the expression of glycoproteins on the spermatozoa.

***Varroa jacobsoni* / *Apis mellifera* / lectin / spermatozoa**

INTRODUCTION

Scientists have long postulated that cell surface carbohydrates are involved in specific cell–cell recognition. In many cells, surface carbohydrate binding proteins (lectins) mediate cell recognition and adhesion in vivo and in vitro. However, with the notable exception of sperm–egg binding, well-defined biological functions of such lectin–carbohydrate pairs have proved elusive (Brandley et al, 1990).

The participation of specific glycoproteins in the cell–cell recognition that occurs

between gametes during fertilization has been well analysed (Rosati, 1985). Reports on spermatozoon surface glycoproteins have referred to mammals (Eddy, 1988) and to invertebrate species in which surface glycoproteins also seem to be an important factor in the recognition between gametes during fertilization, as has been well documented in ciona (Rosati, 1985), *Limulus* (Barnum and Brown, 1983) and the sea urchin (Ruiz-Bravo and Lennarz, 1989). In insects, analyses on *Orthoptera* and *Drosophila* have recently shown the presence of different terminal sugars in the male

gamete (Esponda and Guerra, 1991; Perotti and Riva, 1988a), suggesting species-specific differences.

Descriptions of the structure of the mature *Apis mellifera* spermatozoa have been based on optical microscopy (Kurennoi, 1954) and on observations with an electron microscope (Lensky et al, 1979). Despite these studies, there seems to be a lack of information about the distribution of glycoproteins in the *A mellifera* spermatozoa.

The present study was undertaken in order to obtain data concerning the pattern of sugar residues in *A mellifera* spermatozoon plasma membrane. We have used a series of biotinylated-labelled lectins to histochemically study honey bee spermatozoa from seminal vesicles and ejaculated spermatozoa. The labelled lectins used in our study were the following: *Canavalia ensiformis* (Con A), *Bauhinia purpurea* (BPA) and *Arachis hypogaea* (PNA).

In addition, Ritter (1988) has reported the existence of an atrophic process in the seminal vesicles of *A mellifera* bees parasitized with *Varroa jacobsoni*, that may induce pathological features in the maturation of the spermatozoa. For this reason, we have analysed the changes occurring in some plasma membrane glycoproteins of the spermatozoa of parasitized and non-parasitized honey bees.

MATERIALS AND METHODS

A total of 100 sexually-mature adult drone honey bees were taken from colonies in Madrid, Spain. Fifty drones were naturally parasitized with *V jacobsoni* and 50 drones were non-parasitized. The seminal vesicles of 50 drones (25 parasitized and 25 non-parasitized) were removed by tearing the last abdominal segments away from the body, detaching them from the mucus glands with fine forceps. The seminal vesicles were then punctured with a fine needle to allow sperm to be released. For the subsequent treatments the

sperm sample from each drone was smeared on both poly-L-lysine-coated slides and formvar-coated grids.

Semen was collected from 50 sexually-mature drones (25 parasitized and 25 non-parasitized) using the method developed by Kaftanoglu and Peng (1980). Drones were everted and ejaculated by hand as described by Mackensen and Tucker (1970). The everted bulb of the drone's endophallus was brought into contact with diluent in the funnel and the semen obtained from each drone was smeared on both coated slides and coated grids. Prior to staining, the spermatozoal spot was washed twice with PBS. In order to demonstrate the presence of glycoproteins, each sample was incubated with one or more drops of the biotinylated lectin solution for 30 min at room temperature in a moist chamber. Biotinylated DBA, PNA and Con A (Sigma) were diluted to 100 µg/mL in PBS. After the incubation, each sample was thoroughly washed three times in PBS and incubated in avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA) for 45 min at room temperature. The peroxidase reaction was developed with diaminobenzidine (DAB) and hydrogen peroxide solution (20 mg DAB in 100 mL of Tris-HCl buffer pH 7.6 containing 0.005% hydrogen peroxide) for 5 min. Control samples were prepared by incubating the spermatozoa with the corresponding inhibitory sugar (0.3 M).

Examination and photography of the preparations were performed using a Jeol T7 electron microscope and a Nikon Optiphot optical microscope. To determine the number of labelled cells, 500 nuclei were counted in each of the slides or grids prepared from each drone. Data were analysed by the Kruskal-Wallis test (Kruskal and Wallis, 1952) and all means were separated for significance according to the Kolgomorov-Smirnov test (Smirnov, 1939).

RESULTS

Honey bee spermatozoa were long and filamentous. The head and tail regions were approximately of the same diameter, making it difficult to readily distinguish between the two by optical microscopy. Thus all samples were examined by optical and electron microscopy in parallel, so that structures

expressing the binding sites could be clearly defined, and binding sites present even in minor quantities could be detected.

Binding sites for BPA, Con A and PNA were detected only in very restricted regions (figs 1 and 2). This is shown by the selective staining of the head by the biotinylated lectins. Furthermore, the distribution of the lectin binding sites in the spermatozoa cytoplasmic membranes was significantly different. Biotinylated BPA did not stain any part of the spermatozoa cytoplasmic mem-

brane apart from the whole surface of the head. The acrosomal and postacrosomal regions bound prominently with Con A. Intense staining of the acrosomal cap was visible when the spermatozoa were stained with biotinylated PNA.

Table I summarizes the results of the binding of the biotinylated lectins to the spermatozoa of parasitized and non-parasitized honey bees. As shown in table I, quite different results were obtained between the spermatozoa of parasitized and non-pa-

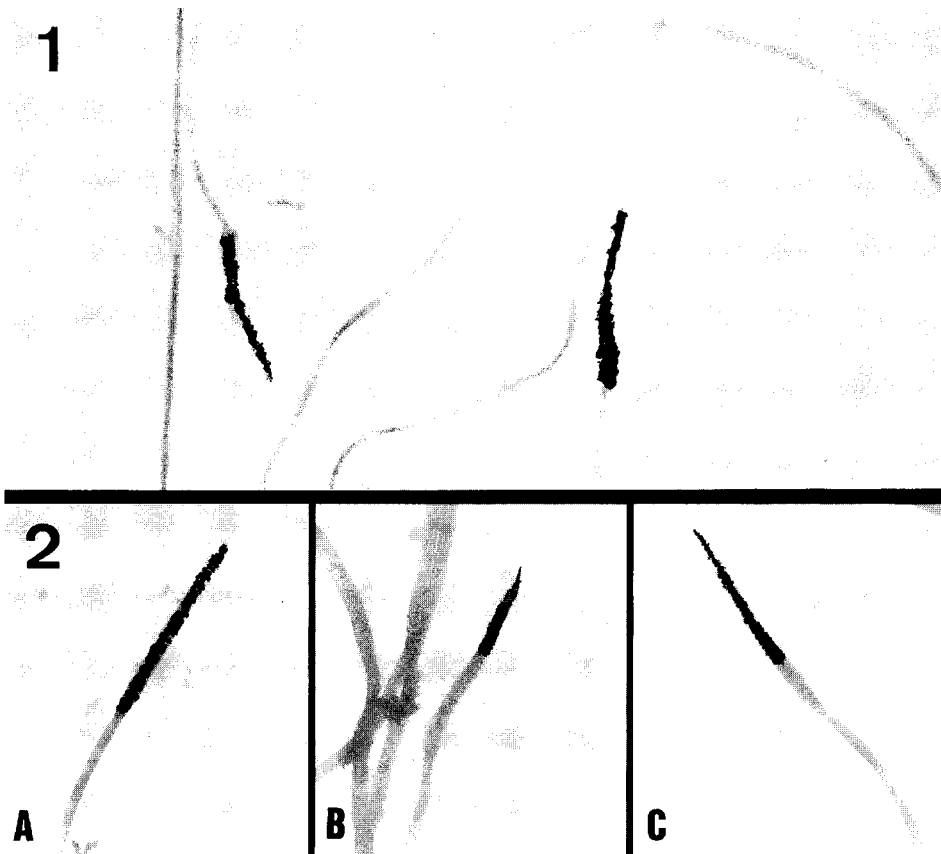


Fig 1. Light micrograph of *A mellifera* spermatozoa labelled with biotinylated BPA. 2 000 x.

Fig 2. Electron micrographs of *A mellifera* ejaculated spermatozoa labelled with biotinylated lectins. A) BPA binds to the plasma membrane overlying the entire head. B) PNA labels the plasma membrane overlying the acrosome. C) Con A labels the acrosomal and the postacrosomal regions. 2 000 x.

Table 1. Mean of the number and percentage of labelled spermatozoa counted in 500 cells from each sample.

| <i>Lectins</i> | | <i>Non-parasitized</i> | <i>Parasitized</i> |
|----------------|-------|-----------------------------------|-----------------------------------|
| S vesicles | BPA | ^a 472.8 + 37.4 (94.5%) | ^b 174.8 + 29.6 (34.9%) |
| | Con A | ^a 457.8 + 35.6 (91.5%) | ^b 195.2 + 49.2 (39%) |
| | PNA | ^a 397.9 + 37.2 (79.5%) | ^b 69.4 + 24.3 (13.8%) |
| Ejaculated | BPA | ^a 484.3 + 45.7 (96.8%) | ^b 169.4 + 34.6 (33.8%) |
| | Con A | ^a 428.3 + 27.9 (85.6%) | ^b 212.4 + 40.5 (42.4%) |
| | PNA | ^a 410.4 + 30.2 (82%) | ^b 75.2 + 19.1 (15%) |

Each mean represents 50 observations and is included with its standard error. Within each column, means not possessing the same superscript are significantly different at the 5% level.

sitized drones. There were no differences in the distributions of lectin binding sites. However, positive cells were more frequently seen in non-infected drones than in infected insects. On the other hand, findings with spermatozoa of seminal vesicles were similar to those obtained with ejaculated spermatozoa. All spermatozoa examined showed the same reactivity pattern and the number of labelled cells detected in seminal vesicles was similar to that counted in ejaculated semen.

DISCUSSION

Based on the staining pattern of the head region of the ejaculated spermatozoa of mammals, lectins were divided into four groups by Lee and Damjanov (1985); 1) lectins reactive with the plasma membrane of the entire head including WGA and BPA; 2) lectins reactive with the acrosomal cap and postacrosomal region including Con A; 3) lectins reactive with the acrosomal cap region including PNA; and 4) lectins reactive with the midregion of the sperm head.

A similar pattern of distribution for the four kinds of binding sites was observed in

the present investigation and in that of Esponda and Guerra (1991) on insect spermatozoa. Therefore, although the spermatozoa of different species show different morphological features, there are many similarities between the spermatozoa of insects and mammals in the distribution of their sugar residues. In this article we describe the distinctive distribution of binding sites to BPA, Con A and PNA in the spermatozoa of *A. mellifera*. Despite the distinctive distribution of the binding sites of each of the three lectins, the acrosomal cap strongly expressed binding sites to all of them. Therefore, a large quantity of sugar residues appears exclusively in the acrosomal area of the cellular plasma membrane. This result agrees with the findings of Esponda and Guerra (1991) and Perotti and Riva (1988b), who demonstrated the presence of a large quantity of sugar residues in the acrosomal area of several invertebrate and vertebrate species. In insects, data concerning fertilization phenomena are scarce. It is known that in *Drosophila* spermatozoa the membrane glycoproteins might play a role in sperm-egg recognition, since a large quantity of sugar residues appears exclusively in the acrosomal area of the plasma membrane, and the binding sites for these sugars

are present on the micropylar region of the egg (Esponda and Guerra, 1991). In addition, data by Wassarman (1988) and Ruiz-Bravo and Lennarz (1989) suggest that spermzoon surface glycoproteins may function as receptors that initiate the acrosomal reaction: an exocytotic phenomenon that is a prerequisite for sperm penetration. Therefore the large quantity of sugar residues detected in the acrosomal area of *A. mellifera* might be connected with a similar function.

Comparison of the lectin-binding patterns to the spermatozoa of non-parasitized and parasitized drones showed similar staining in both cases. However, the number of labelled cells was lower in parasitized than in non-parasitized drones, suggesting that *V. jacobsoni* alters the expression of glycoproteins in the spermatozoa.

In our results, ejaculated spermatozoa and spermatozoa stored in the seminal vesicles showed similar features following biotinylated BPA, PNA and Con A labelling. This finding suggests that in sexually mature drones there are no changes while the spermatozoa are stored in the seminal vesicles. However, Marti et al (unpublished data) demonstrated that spermatozoa maturation occurs in the seminal vesicles of immature drones a few days after they emerge. Therefore, the pathological process produced by *V. jacobsoni* in the seminal vesicles (Ritter, 1988) includes alterations in the maturation of the spermatozoa as shown by a lack of expression of glycoproteins in the spermatozoa of parasitized drones. These alterations probably correlate with stages of maturation of the spermatozoa, suggesting that the spermatozoa of parasitized drones may not be able to fertilize female gametes, since glycoproteins are operative during sperm maturation, motility acquisition capacitation and ultimately sperm-egg fusion, as has previously been discussed.

Résumé — Influence du parasitisme par *Varroa jacobsoni* sur le développement des glycoprotéines sur les spermatozoïdes d'*Apis mellifera*. La description de la structure des spermatozoïdes mûrs d'*Apis mellifera* repose sur des observations faites en microscopie optique et électronique. Il semble y avoir un manque d'informations concernant la répartition des glycoprotéines à la surface des spermatozoïdes d'*Apis mellifera*. Nous avons étudié en microscopie optique et électronique la répartition des sites de liaison aux lectines biotinylées BPA (*Bauhinia purpurea*), Con A (*Canavalia ensiformis*) et PNA (*Arachis hypogaea*). BPA a marqué sélectivement la membrane plasmique sur toute la tête du spermatozoïde, Con A, la région acrosomienne et postacrosomienne et PNA, la calotte acrosomienne (fig 2). La carte de répartition des sites de liaison sur les spermatozoïdes des mâles parasités par *V. jacobsoni* est semblable à celle des mâles non parasités. Néanmoins le nombre de cellules marquées est plus faible chez des mâles parasités que chez les non parasités (tableau I) ; ceci laisse à penser que *V. jacobsoni* peut modifier le développement des glycoprotéines sur les spermatozoïdes.

***Varroa jacobsoni* / *Apis mellifera* / lectine / spermatozoïde**

Zusammenfassung — Einfluß einer Parasitierung durch *Varroa jacobsoni* auf die Bildung von Glycoproteinen auf den Spermatozoen von *Apis mellifera*. Beschreibungen der Struktur reifer Spermatozoen von *A. mellifera* beruhten bisher vorwiegend auf licht- und elektronenmikroskopischen Beobachtungen. Zur Verteilung von Glycoproteinen auf den Spermatozoen von *A. mellifera* liegen bisher keine Angaben vor. In dem vorliegenden Beitrag wird die unterschiedliche Verteilung der Bindungsstellen für biotinierte BPA-, Con A- und PNA-Lektine auf den männlichen

Gameten von *A mellifera* beschrieben. Diese Verteilung wurde sowohl licht- als auch elektronenmikroskopisch ermittelt. BPA reagierte mit der Plasmamembran des gesamten Kopfes, con A mit der akrosomalen und postakrosomalen Region und PNA mit der akrosomalen Hülle (Abb 2). Spermatozoen von unparasitierten Drohnen und von Drohnen aus mit *Varroa jacobsoni* parasitierten Drohnen zeigten zwar ähnliche Lektinbindungsmuster, allerdings war die Anzahl markierter Zellen bei parasitierten Drohnen geringer als bei nicht-parasitierten (Tabelle I). Dies legt nahe, daß die Ausbildung von Glycoproteinen auf den Spermatozoen durch den Befall mit *Varroa jacobsoni* verändert wird.

***Varroa jacobsoni* / *Apis mellifera* / Lektine / Spermatozoen**

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