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Roles of glycodelin in modulating sperm function

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Abstract

Glycodelin is a glycoprotein with three well-defined isoforms. They are named as glycodelin-S, glycodelin-A and glycodelin-F. The three isoforms have similar protein core but different carbohydrate moieties. Glycodelin-S is abundant in the human seminal plasma. It suppresses sperm capacitation and in doing so, it maintains the spermatozoa in an uncapacitated state before they enter into the uterine cavity. Glycodelin-A is abundant in the amniotic fluid. It is also secreted from endometrial glands into uterine fluid and is produced by the fallopian tube. Glycodelin-A is the first endogenous glycoprotein that was found to inhibit the binding of spermatozoa to the zona pellucida. The immunosuppressive properties of glycodelin-A suggest that the molecule may protect the spermatozoa from immune attack in the maternal reproductive tract. Glycodelin-F was first found in the follicular fluid, hence its name. It also inhibits spermatozoa-zona pellucida binding. In addition, glycodelin-F suppresses progesterone-induced acrosome reaction, and may serve to prevent premature acrosome reaction. Preliminary findings suggest possible presence of yet another glycodelin isoform in the extracellular matrix of cumulus oophorus. Unlike glycodelin-A and -F, it stimulates spermatozoa-zona pellucida binding. In summary, different isoforms of glycodelin have different biological roles on sperm function, and they act in succession to contribute to the success of fertilization.

Keywords: Acrosome reaction; Capacitation; Glycodelin; Spermatozoa; Zona pellucida

1. Introduction

Fertilization is the key step in the human propagation. Proper functioning of the male and female gametes is critical to ensure the success of fertilization. It is well established that the reproductive tract modulates various sperm functions. This is likely to prepare the spermatozoa for the fertilization process.

Glycodelin is a glycoprotein belonging to the lipocalin protein family. It has been previously known as chorionic α2-globulin, placental α2-microglobulin, α-uterine protein, placental protein 14, pregnancy-associated α2 globulin, progestagen-dependent endometrial protein and progesterone-associated endometrial protein. For the role of glycodelin in reproduction and oncology, interested readers are referred to previous reviews (Seppala et al., 2001, 2002; Seppala, 2004). Recent additional data suggest that that this molecule has important actions on sperm function. The present article will concentrate on the recent data on different glycodelin isoforms in modulating diverse events that take place in spermatozoa before fertilization.

2. Interaction between the reproductive tract and the spermatozoa

Spermatozoa are produced in the testis and are deposited with seminal plasma into the female vagina during intercourse. The sperm cells then migrate through the uterine cervix into uterine cavity and eventually to the oviduct where fertilization takes place. During passage through the female reproductive tract, the spermatozoa gain their fertilization capacity by an ill-defined process known as capacitation. After ovulation, the oocyte, its associated cumulus cell mass and follicular fluid surrounding and trapped within the cumulus–oocyte complex are transported to the oviduct (Fraser, 1985). The spermatozoa have to traverse through the cumulus cell mass before one of them can eventually fertilize the oocyte.

There are delicate interactions between oviductal cells, cumulus cells, follicular fluid and spermatozoa at the fertilization site. As in the other mammalian species, the human oviduct acts as

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a sperm reservoir (Yao et al., 1999a) to maintain their motility and viability until the arrival of the oocyte. Human follicular fluid inhibits spermatozoa-zona pellucida binding (Yao et al., 1996), but this inhibitory effect is reduced by the cumulus cells (Hong et al., 2003). Interestingly, human oviductal cells have been found to inhibit whereas human follicular fluid stimulates the spermatozoa–oocyte fusion (Yao et al., 1999b). The follicular fluid, probably together with progesterone stimulates the fertilization activity of oviductal spermatozoa (Yao et al., 1999b). It has been suggested that the cumulus oophorus may be involved in selecting spermatozoa with normal morphology (Carrell et al., 1993; Hong et al., 2004) and intact acrosome (Yanagimachi, 1994). However, the molecular mechanisms of these interactions are relatively unclear.

3. Glycodelin isoforms

The glycodelin gene is localized on chromosome 9, band q34 (Van Cong et al., 1991). It has seven exons and six introns (Vaisse et al., 1990). Based on the cDNA sequence of glycodelin, the estimated molecular mass of the glycodelin protein core is 18,787 Da (Julkunen et al., 1988). The size of the secreted glycodelin is 27–30 kDa (Seppala et al., 2002; Chiu et al., 2003a). Thus the carbohydrate moieties of glycodelin occupy a significant portion of the molecule. Indeed, the function of the molecule on sperm function is glycosylation dependent (see below).

Glycodelin has three well-defined isoforms. They are named according to their source where they are most abundantly found: glycodelin-A from amniotic fluid, glycodelin-S from seminal plasma and glycodelin-F from follicular fluid. The three glycodelin isoforms have the same protein core but different glycosylation. The N-glycans of glycodelin-A and -S have been determined by mass spectrometry (Dell et al., 1995; Morris et al., 1996). Glycodelin-S contains many more fucose residues than glycodelin-A. It also differs from glycodelin-A in having no sialylated glycans, an unusual feature among secreted human glycoproteins (Morris et al., 1996). Although the glycan structure in glycodelin-F is not yet known, lectin binding characteristics and fluorophore-assisted carbohydrate electrophoresis show that the carbohydrate moieties of the molecule differ from those of the other two isoforms (Chiu et al., 2003a).

4. Expression of glycodelin isoforms in the reproductive tract

Glycodelin-A is abundant in decidualized endometrium of early pregnancy, and it is present at a high concentration in the amniotic fluid (125 mg/l) in the first half of pregnancy (Bohn et al., 1982; Julkunen et al., 1985). It is also highly expressed in the secretory human endometrium. Several microarray analyses showed that the expression of glycodelin mRNA in the endometrium in the peri-implantation period is strongly upregulated compared to non-receptive endometrium (Kao et al., 2002; Borthwick et al., 2003; Horcajadas et al., 2004). Glycodelin protein expression has been localized to the secretory endometrial glands and the decidualized endometrium (Julkunen et al., 1986a; Waites et al., 1988; Waites and Bell, 1989). Proliferative endometrium contains little or no glycodelin except in the first 2–3 days of the cycle (Julkunen et al., 1986a,b; Seppala et al., 1988a). Endometrial glycodelin becomes detectable from the fourth day after luteinizing hormone surge (LH + 4) and is present in all the endometrial glands on LH + 10 (Seppala et al., 1988b; Brown et al., 2000). The above observations are supported by in vitro studies. Explants of human secretory endometrium and pregnancy decidua synthesize glycodelin (Julkunen, 1986; Julkunen et al., 1986a), and monolayer cultures of decidua from early pregnancy secrete glycodelin into the culture medium (Fay et al., 1990; Laird et al., 1993). Based on physicochemical analyses it has been concluded that the glycodelin isoforms from secretory endometrium, pregnancy decidua, amniotic fluid and pregnancy serum are sufficiently similar so that they may be called glycodelin-A, although slight interindividual differences in the glycan structures may be detected (Koistinen et al., 2003).

The fallopian tube (Julkunen et al., 1986b; Saridogan et al., 1997), ovary (Kamarainen et al., 1996; Tse et al., 2002) and cervix (Connor et al., 2000) also express glycodelin mRNA. Human uterine tubal epithelial cells produce glycodelin in vitro (Laird et al., 1995). In these studies, glycodelin expression was determined either by mRNA analysis or immunological techniques. These studies addressed nucleotide sequence of mRNA, whereas the carbohydrate moieties of the molecule were not investigated. Although there are many antibodies against glycodelin, they do not distinguish between various glycodelin isoforms that differ in their glycan parts only. Thus, the profile of glycodelin isoforms produced in these tissues remains to be resolved. We have successively used anti-glycodelin affinity chromatography and ion-exchange chromatography to analyze the glycodelin isoforms produced by the human oviductal cells in culture; these unpublished observations show that the oviductal cells produce glycodelin-A and -F in about equal proportions. On the other hand, follicular fluid contains mainly glycodelin-F. The regulation of synthesis of these different isoforms is not clear, although glycodelin-A appears to be progesterone-regulated, and glycodelin-F mRNA is expressed in luteinized granulosa cells, indicating association with progesterone secretion.

In the male, glycodelin-S is synthesized in the epithelial cells of the seminal vesicles (Julkunen et al., 1984; Koistinen et al., 1996). It is secreted into human seminal plasma in large quantity (Julkunen et al., 1984; Koistinen et al., 2000), where it may make up to 2.5% of the total protein (Bolton et al., 1986). As the levels of glycodelin-S in seminal plasma are similar in vasectomized and non-vasectomized men, it is unlikely that glycodelin-S is produced in the testis or epididymis (Julkunen et al., 1984; Bell and Patel, 1987). No glycodelin mRNA has been found in these tissues (Koistinen et al., 1997).

5. Encounter of the spermatozoa with glycodelin

Available data suggest that spermatozoa interact with different glycodelin isoforms during their journey to fertilize an oocyte. After ejaculation the spermatozoa first come into contact with glycodelin-S in seminal plasma. Sperm-bound glycodelin-

S is then removed during the passage of the spermatozoa through the cervical canal (Chiu et al., 2005). There is glycodelin in the cervical mucus (Connor et al., 2000; Pockley et al., 1989). However, the isoform in the cervical mucus and its functional role in modulating sperm function are not known. It is possible that part of the glycodelin found in cervical mucus is sperm-derived.

Glycodelin-A is cyclically secreted from endometrial glands into uterine fluid. Its absence in the endometrium in the periovulatory period suggests that spermatozoa are unlikely to encounter glycodelin-A in the uterine cavity. Instead, spermatozoa come into contact with glycodelin in the fallopian tube. Glycodelin is produced in the fallopian tube, both in the proliferative and secretory phases of the cycle, and its concentration being higher during the secretory phase (Julkunen et al., 1986b). Our unpublished data from in vitro studies suggest that human oviductal cells secrete both glycodelin-A and -F isoforms into the culture medium. Thus, the oviductal spermatozoa are likely to interact with both.

Glycodelin-F is the main glycodelin isoform in the follicular fluid (Chiu and Yeung, unpublished data). Some of the follicular fluid is transported to the oviduct with the oocyte–cumulus cell mass after ovulation. Therefore, oviductal spermatozoa will be exposed to more glycodelin-F as they swim towards the oocyte–cumulus cell mass.

Both glycodelin-A and -F inhibit binding of spermatozoa to the zona pellucida (Chiu et al., 2003a; Oehninger et al., 1995), and both need to be removed before fertilization. This appears to take place when the spermatozoa migrate through the matrix of cumulus oophorus, as evidenced by the observations that the cumulus/corona cells reduce the zona binding inhibitory activity of follicular fluid (Hong et al., 2003) and the gain in zona binding capacity of spermatozoa passing through the cumulus oophorus (Hong et al., 2004). While details of this process remain to be elucidated, we have preliminary data suggesting that spermatozoa may encounter yet another isoform of glycodelin in the cumulus/corona cell matrix (see below).

6. Role of glycodelin-S

Capacitation enables the spermatozoa to fertilize an oocyte. However, the fertilizing ability of capacitated human spermatozoa is short-lived, and after 240 min, the capacitated spermatozoa cannot undergo zona pellucida-induced acrosome reaction, an essential event in fertilization (Cohen-Dayag et al., 1995). Therefore, control of the timing of capacitation is important for successful fertilization. Our recent study indicates that glycodelin-S modulates sperm capacitation.

Glycodelin-S binds to spermatozoa via two binding sites, but it is readily removed during migration of the spermatozoa through cervical fluid (Chiu et al., 2005). Although the binding of glycodelin-S to spermatozoa is specific, its affinity is low (Table 1, Chiu et al., 2005). Compared with the binding kinetics of the other glycodelin isoforms, glycodelin-S binds to and detaches from spermatozoa at a faster rate (Table 1). This fast kinetics is important for the function of the molecule as the spermatozoa will be in contact with seminal plasma only briefly after ejaculation before their passage through the cervix. The weak binding of glycodelin-S explains why sperm-associated glycodelin immunoreactivity can be demonstrated only when spermatozoa are treated with high physiological but not with low concentration of glycodelin-S (Chiu et al., 2003a, 2005). It also explains the readiness of removal of bound glycodelin-S from spermatozoa during migration through the cervical mucus.

Glycodelin-S suppresses capacitation of human spermatozoa. This is suggested by the observations that glycodelin-S inhibits albumin-induced cholesterol efflux from spermatozoa (Chiu et al., 2005), and loss of cholesterol from the sperm membrane initiates capacitation (Visconti et al., 2002; Cross, 1998). Albumin is present at high concentration in the uterine fluid, but is almost absent in the seminal plasma (Setchell, 1974). Therefore, the removal of sperm-bound glycodelin-S by mucus surrogates (Chiu et al., 2005) may have relevance for what happens during sperm migration through the in cervical fluid. It is possible that removal of glycodelin-S from spermatozoa in cervical mucus would allow uterine albumin to induce cholesterol efflux and initiate capacitation. The possible involvement of other constituents of cervical mucus in this process remains to be addressed.

Deglycosylation has been shown to reduce glycodelin-S binding on spermatozoa and to abolish the inhibitory effect of glycodelin-S on albumin-induced capacitation (Chiu et al.,

Table 1
Kinetic data on glycodelin-spermatozoa binding

	Glycodelin-S	Glycodelin-A	Glycodelin-F
High affinity			
K_D (nM)	104 ± 20	N/A	3.94 ± 0.08
B_{max} (pmol/10^6 spermatozoa)	4 ± 0.5	N/A	1.36 ± 0.03
Association half-time (min)	6	N/A	4
Dissociation half-time (min)	99	N/A	247
Low affinity			
K_D (nM)	1413 ± 316	20.76 ± 2.15	24.98 ± 2.36
B_{max} (pmol/10^6 spermatozoa)	8.5 ± 1.5	3.13 ± 0.11	3.37 ± 0.06
Association half-time (min)	11	13	16
Dissociation half-time (min)	25	197	182

The kinetic data are extracted from Chiu et al. (2003b, 2005), and recalculated using the data sets derived from these two studies. All the data were expressed as mean and standard error of the mean (S.E.M.). Each result represents the mean ± S.E.M. of four experiments performed in triplicate. N/A: not available; K_D: equilibrium dissociation constant; B_{max}: maximal binding capacity. Association/dissociation half-time were determined at equilibrium concentration.

2005). This demonstrates that the carbohydrate moieties of glycodelin-S are important for the binding of the molecule to spermatozoa.

7. Role of glycodelin-A

The first evidence indicating that glycodelin modulates sperm function came from results of a hemizona binding assay (Oehninger et al., 1995). Human oocytes were bisected into equal halves (hemizonae). One hemizona was incubated with spermatozoa treated with glycodelin-A while the matching hemizona was place together with control spermatozoa without prior treatment with glycodelin-A. The numbers of spermatozoa bound on these hemizonae were counted and their ratio reflected the zona binding ability of glycodelin-treated spermatozoa relative to the control. The study demonstrated that glycodelin-A potently and dose-dependently inhibited spermatozoa-zona pellucida binding (Oehninger et al., 1995). Glycodelin-A was the first endogenous glycoprotein that was found to exhibit such potent anti-fertilization activity.

Similar to glycodelin-S, the binding of glycodelin-A to spermatozoa depends on the glycosylation of the molecule. Deglycosylation of glycodelin-A also abolishes the spermatozoa-zona pellucida binding inhibitory activity of glycodelin-A (Chiu et al., 2003b). Because there are significant differences in the carbohydrate sequences of the glycans of glycodelin-A and -S (Dell et al., 1995; Morris et al., 1996), the difference is likely to explain why glycodelin-S does not inhibit spermatozoa-zona pellucida binding (Morris et al., 1996). This is in keeping with the observation that all known effects of glycodelin isoforms on sperm function are glycosylation dependent.

Glycodelin-A may also have a role in feto-maternal defence mechanisms (Clark et al., 1996). This is suggested by a high glycodelin-A concentration in the endometrium at the time of implantation (Seppala et al., 1988b) and early placentation (Julkunen et al., 1985), when large granular lymphocytes and natural killer (NK) cells are present in the decidua (Gurka and Rocklin, 1987; Weetman, 1999). The reason why decidual NK cells are less aggressive is incompletely understood (Dosiou and Giudice, 2005). Glycodelin-A is immunosuppressive (Bolton et al., 1987). It inhibits the activity of natural killer cells from peripheral blood (Okamoto et al., 1991), whereas its role in reduced aggressiveness of the uterine NK cells remains to be clarified. Glycodelin-A also suppresses T cell proliferation (Rachmilewitz et al., 1999) and enhances T cell apoptosis (Mukhopadhyay et al., 2001). Pregnancy zone protein acts synergistically with glycodelin-A to modulate T-cell activation (Skornicka et al., 2005).

Unlike its action on sperm–zona binding, the immunosuppressive activity of glycodelin-A is mainly mediated via its protein backbone. Experiments using site directed mutagenesis at the glycosylation sites showed that removal of the glycosylation side chain did not affect the anti-proliferative property of glycodelin on Jurkat (JR4) T cell line (Jayachandran et al., 2004). Instead, the data suggested that glycosylation affected the rate of secretion of glycodelin.

Seminal plasma has immunosuppressive properties (Gonzales, 2001). It is believed that glycodelin-S contributes to this activity (Morris et al., 1996; Bolton et al., 1987) because the protein back bone in glycodelin-S and -A is the same (Koistinen et al., 1999) and the suppressive activity of seminal plasma on the proliferation of lymphocytes can be partially removed by immunoadsorption for glycodelin (Bolton et al., 1987). However, unlike glycodelin-A, glycodelin-S does not enhance apoptosis of the T lymphocytes (Mukhopadhyay et al., 2004). This difference between the two glycodelins has been attributed to the lack of sialic acid in glycodelin-S (Mukhopadhyay et al., 2004). It has been suggested that the apoptogenic region in the protein core of glycodelin-S is inaccessible to the T lymphocytes, but is exposed in glycodelin-A, due to charge repulsion of the negatively charged sialic acid in the carbohydrate side chains of glycodelin-A (Jayachandran et al., 2004).

Spermatozoa are immunologically foreign to the female recipient and, therefore, they should be immunogenic (Anderson and Tarter, 1982). Vital spermatozoa stimulate lymphocyte response in vitro (Gutierrez et al., 2003). Due to the immunosuppressive properties of glycodelin-A, it is possible that at the time of fertilization, its presence in the fallopian tube may protect the spermatozoa from the female immune system by binding on the spermatozoa with their carbohydrate moieties. The protein backbone of the bound glycodelin-A may then suppress the activity of the recipient's lymphocytes. However, direct evidence demonstrating such immunoprotective actions of glycodelin isoforms on spermatozoa is lacking.

8. Roles of glycodelin-F

The original finding that human follicular fluid inhibits the zona binding capacity of human spermatozoa was made almost a decade ago (Yao et al., 1996). This inhibitory activity is present in human follicular fluids from both hormone-stimulated and natural reproductive cycles (Yao et al., 1996; Qiao et al., 1998; Chiu et al., 2002). The molecule responsible for this activity in follicular fluid was first named as zona binding inhibitory factor-1 (Yao et al., 1998), which was later found to be an isoform of glycodelin. In accordance with the glycodelin nomenclature the major inhibitory molecule in follicular fluid was renamed as glycodelin-F (follicular fluid derived glycodelin) (Chiu et al., 2003a).

Binding kinetic studies demonstrated that human sperm membrane possesses two glycodelin-F receptors. The low affinity receptor also binds glycodelin-A (Chiu et al., 2003b). The identity of these glycodelin receptors in spermatozoa is under investigation in our laboratory. Available data show that the binding of glycodelin-A and -F to the sperm receptor involves different carbohydrate moieties. Mannose, fucose and possibly E-selectin ligand are important for glycodelin-A binding, while mannose, fucose and *N*-acetylglucosamine, but not selectin ligand play a part in glycodelin-F binding (Chiu et al., 2004). These binding sites are different from those binding glycodelin-S, as glycodelin-A and -F do not compete with glycodelin-S for its binding sites (Chiu et al., 2005).

8.1. Glycodelin-F suppresses progesterone-induced acrosome reaction

Although glycodelin-A and -F have no effect on spontaneous acrosome reaction, glycodelin-F, but not glycodelin-A, suppresses progesterone-induced acrosome reaction (Chiu et al., 2003b). The concentration of glycodelin-F required to elicit the activity is well below that found in the follicular fluid and cumulus cell mass, suggesting that the observation is physiologically relevant. Sperm-bound glycodelin-F appears to protect spermatozoa from undergoing premature acrosome reaction, and is likely to be important to fertilization because the cumulus cells produce progesterone (Chian et al., 1999), and acrosome reacted spermatozoa have reduced affinity to zona pellucida (Yanagimachi, 1994) and difficulty in penetrating the cumulus mass (Saling, 1989). Oviductal cells produce glycodelin-F. The observation that human oviductal fluid inhibits progesterone-induced acrosome reaction (Zhu et al., 1994) is compatible with the observed action of glycodelin-F.

The activities of glycodelin-A and -F are likely to be physiologically relevant. Both isoforms bind to the acrosome region of human spermatozoa (Chiu et al., 2003a). Interestingly, zona pellucida proteins displace sperm membrane bound glycodelin-F and -A in vitro (Chiu et al., 2003b; Chiu and Yeung, unpublished data), suggesting that the sperm glycodelin-F and -A receptors may be a part of zona protein receptor complex (Thaler and Cardullo, 1996), or molecule closely associated with the receptors.

8.2. Modification of glycodelin-F into a new isoform of glycodelin

Glycodelin-F in the follicular fluid is likely to originate from the cells in the ovarian follicles. Glycodelin immunoreactivity has been detected in the granulosa cells of late secondary follicles (Tse et al., 2002). Luteinized granulosa cells do actually synthesize glycodelin, shown by detection of glycodelin mRNA (Tse et al., 2002). In contrast, the cumulus cells do not synthesize glycodelin, but they take up glycodelin-A and -F from the surrounding medium and modify them into a molecule of smaller size (Tse et al., 2002; Chiu and Yeung, unpublished data). Furthermore, we have isolated from the cumulus cell matrix yet another glycodelin-like molecule with similar size and immunoreactivity (see below). Therefore, it is possible that the cumulus cells modify the uptaken glycodelin into this new isoform.

The uptake of glycodelin isoforms by the cumulus cells is specific as it is not affected by the presence of other lipocalin family members (Tse et al., 2002). Previous studies have suggested that some lipocalin proteins might be taken up by specific cell-surface receptor mediated endocytosis (Senoo et al., 1990; Malaba et al., 1995). The mechanism by which glycodelin enters the cumulus cells remains to be investigated.

9. Cumulus matrix contains a new isoform of glycodelin

We have used anti-glycodelin affinity and ion-exchange columns to study the glycodelin isoforms in the human cumulus cell matrix (Chiu and Yeung, unpublished data). A 23–25 kDa molecule with glycodelin immunoreactivity was identified (Fig. 1). Deglycosylation reduces the size of this molecule to that of the glycodelin protein core (calculated molecular mass of 18.8 kDa), suggesting that the molecule is a new isoform of glycodelin with the same protein core, but with less carbohydrate. Interestingly, unlike glycodelin-A and -F, this new glycodelin isoform stimulates spermatozoa-zona pellucida binding (Fig. 2). These observations demonstrate that the cumulus cells can change the biological activity of glycodelin by modifying its glycosylation.

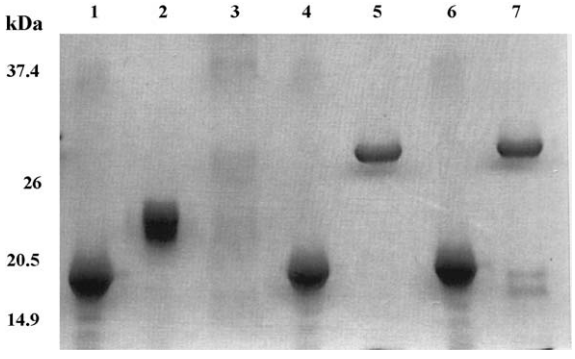

Fig. 1. Gel electrophoresis of cumulus-derived glycodelins. Cumulus matrix was obtained after hyaluronidase digestion of the cumulus oophorus from patients coming for treatment with intracytoplasmic sperm injection. The matrix was passed successively through an anti-glycodelin affinity column and an ion-exchange column. The purified glycodelins were analysed in 12% SDS-PAGE gel. Lane 1: deglycosylated cumulus glycodelin isoform; lane 2: native cumulus glycodelin isoform; lane 3: molecular weight marker; lane 4: deglycosylated glycodelin-A; lane 5: native glycodelin-A; lane 6: deglycosylated glycodelin-F; lane 7: native glycodelin-F.

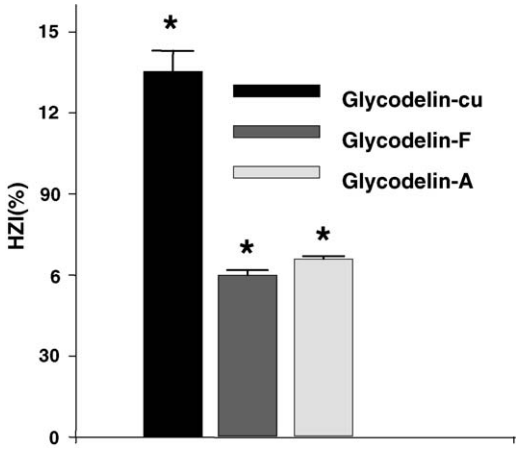

Fig. 2. Effects of 1 μg/ml of glycodelin-A, glycodelin-F and cumulus matrix derived glycodelin on the zona binding capacity of human spermatozoa. Each point represents mean of the results of five hemizona binding assays using five zona pellucida and five different sperm samples. One sperm sample and one zona pellucida were used in each hemizona binding assay. $^{*}P<0.05$ when compared with the control hemizona without glycodelin preincubation. The hemizona binding index (HZI) was defined as: HZI = (number of spermatozoa bound in test droplet/number of spermatozoa bound in control droplet) × 100.

Available data suggest that sperm-bound glycodelin-A and -F will be removed during the passage of the spermatozoa through the cumulus oophorus (Hong et al., 2003, 2004). How this is accomplished is not clear. The cumulus cells may do this in the same way as they take up glycodelin-A and -F from the follicular fluid. However, this must depend on fortuitous collision between the spermatozoa and the cumulus cells, and it is difficult to reconcile that all the sperm-bound glycodelin isoforms can be removed by such a mechanism. The more likely alternative is that molecules in the cumulus cell matrix displace the sperm-bound glycodelin-A and -F upon their passage through the cumulus/corona cell layer. Experiments are under way to test the hypothesis that the modified glycodelin isoform in the cumulus matrix participates in such a process.

10. Summary

Based on the available data we propose the following sequence of events regarding the role of glycodelin in fertilization. At ejaculation, spermatozoa come into contact with glycodelin-S that maintains them in an uncapacitated state in the seminal plasma. Removal of sperm-bound glycodelin-S during the passage of spermatozoa through the cervix would allow albumin in uterine fluid to initiate capacitation by enhancing cholesterol efflux. In the oviduct, oviductal glycodelin-A and -F bind to the spermatozoa and protect them against female immune system. At the same time, glycodelin-F protects the spermatozoa against premature acrosome reaction. This latter activity becomes critical after ovulation when the spermatozoa migrate towards the oocyte–cumulus mass for fertilization. This is important because progesterone that induces acrosome reaction is produced by the cumulus cells and by the luteinized granulosa cells in the ovary. To cope with the need for more protection, the spermatozoa are exposed to additional glycodelin-F in the follicular fluid that has entered with the ovulated oocyte–cumulus complex. Glycodelin-F, the main glycodelin isoform in the follicular fluid, also serves as the substrate for the cumulus cells to produce the cumulus matrix isoform of glycodelin, which stimulates spermatozoa-zona pellucida binding.

The abundance of glycodelin in the female reproductive tract and the diverse effects of glycodelin on sperm functions are consistent with an important role of the molecule in human fertility. Further studies will provide new information on the regulation of fertilization. The identification of the glycans responsible for the observed biological activities of glycodelin will be useful in designing new drugs for fertility regulation by simulating the part of glycodelin that stimulate/inhibits fertilization. Glycodelin provides an excellent model for functional glycomics as it demonstrates how expression of the same gene may lead to various post-translational changes in tissue/cell-dependent glycosylation affecting biological activity and function.

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