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Research Article

A sperm GPI-anchored protein elicits sperm-cumulus cross-talk leading to the acrosome reaction

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Abstract. The acrosome reaction has long been thought to be induced by the zona pellucida. Here we report the identification and function of a novel human sperm glycosylphosphatidylinositol (GPI)-anchored membrane protein, NYD-SP8. The release of the protein during sperm-egg interaction and its binding to the cumulus, the first layer of egg investment, elicits cross-talk between the gametes and produces calcium dependant release of progesterone, which lead to the acrosome reaction. An *in vivo* mouse model of NYD-SP8 immunization is also established showing **a** reduced fertility rate. Thus, contrary to accepted dogma, our study demonstrates for the first time that, prior to reaching the zona pellucida, sperm may release a surface protein that acts on the cumulus cells leading to the acrosome reaction, which may be important for determining the outcome of fertilization.

Keywords. Sperm, acrosome reaction, GPI-anchored protein, calcium, progesterone.

Introduction

Fertilization can be viewed as a series of interactions between the sperm and egg leading to the fusion of the gametes and generation of a new organism, the zygote. In order to enter the egg, sperm must penetrate the investment of the egg, first the cumulus, then the inner thick extracelluar matrix, the zona pellucida (ZP), which has long been believed to induce the acrosome reaction in sperm to release acrosomal hydrolytic enzymes to facilitate sperm penetration [1, 2]. However, remarkably little is known about the process by which sperm penetrate the cumulus and the physiological role of cumulus in sperm-egg interaction.

The ZP has traditionally been considered as a site to induce the acrosome reaction, since the release of hydrolytic enzymes from the sperm is thought to be required by sperm to penetrate through this thick extracellular matrix but is not necessary for the penetration through the relatively less dense cumulus layer [3, 4]. However, it has in fact been observed that

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some sperm have already undergone the membrane fusion step of the acrosome reaction within the cumulus in rabbit, man and hamster [5 – 7]. The cumulus has also been shown to induce acrosome reaction of bocine sperm [8]. Interestingly, a recent report has shown that human sperm exposed to the cumulus mass are also found to be acrosome reacted [9]. However, doubts persist as to whether the cumulus could indeed induce the acrosome reaction, since the underling mechanism responsible for the acrosome reaction-inducing ability of the cumulus remains elusive.

Materials and Methods

Ethical Approval. All animal work was approved by the Animal Ethics Committee of the Chinese University of Hong Kong and the Ethics Committee of Nanjing Medical University. Human sperm were obtained from healthy volunteers and oocytes were collected from women undergoing IVF, procedures for which were approved by the Ethics Committee of Nanjing Medical University with informed consent. The informed consent was verbal because, although most of the subjects coming for the IVF procedure were willing to give consent to the use of the discarded tissues (cumulus cells surrounding the oocytes) for research purposes, they were reluctant to sign a document, to avoid any other possible legal issue involved. The procedure used to obtain the verbal consent of the study was also approved by the Ethics Committee of Nanjing Medical University.

Cloning and characterization of NYD-SP8. NYD-SP8 was cloned from human testis cDNA microarray hybridization. Chromosomal mapping was carried out on Stanford TNG4 radiation hybrid (RH) panel (Research Genetics) using PCR primer P1 (5'-ATTGTCCAGCACTCTTCACC-3') and P2 (5'-AGACGACTCAATGCCACC-3'). The expression pattern of NYD-SP8 was determined by RT-PCR using Multiple Tissue cDNA (MTC; Clontech) panel with P1 and P2 mentioned above.

Preparation of recombinant NYD-SP8 protein and NYD-SP8 antibody. The cDNA fragment corresponding to amino acids 25 – 184 of the protein was subcloned into the pET28a vector (Novagen, Madison,WI) and expressed in *E.Coli* CodonPlus®DE3 RP (Stratagene,La Jolla, CA). The recombinant protein was affinity purified with a Ni-nitrilotriacetic acid (NTA) column and renatured by dialysis. Purified NYD-SP8 protein was used for mouse immunization and subsequent boosts (100 mg each). After serum titers and specificity were tested by ELISA and Western blot respectively, NYD-SP8 antibody was purified using Montage Antibody Purification Kit (Millipore, Billerica, MA) according to the manufacturer's instructions.

Mouse cumulus-oocyte complexes (COCs), and cumulus cells preparation. Female mice (six weeks old) were injected with 10 IU pregnant mare serum gonadotropin (PMSG) followed by 10 IU human chorionic gonadotropin (hCG) 48 h after PMSG injection. COCs were collected from mice oviducts 14 h after hCG injection. Cumulus cells were collected by gentle flushing of COCs using pre-warmed sperm washing medium (Irvine Scientific, Santa Ana,CA) and then were dispersed with 0.5 % hyaluronidase in mT medium.

Immunofluorescence analysis on sperm and cumulus cell. Human sperm were purified by percoll according to WHO standard and human cumulus cells were isolated from oocytes intended for IVF procedure using conventional clinical IVF medium before/after fertilization. Mouse sperm were diluted in modified Tyrode's (mT) medium and incubated with mouse cumulus cells for six h in modified human Tubal Fluid (mhTF) medium. Sperm and cumulus cells were washed in 1X PBS, air-dried on slides and fixed with 4 % paraformaldehyde for 45 min. Slides were blocked in 5 % goat serum in PBS for 2 h and then incubated with NYD-SP8 antibody (at dilution 1:100) at 4 °C overnight and FITC-conjugated anti-mouse IgG (dilution 1:1000) at room temperature for 1 h. Slides were coated with Slow-Fade® antifade reagent (Invitrogen, Carlsbad,CA) and viewed under a Zeiss Axioskop2® plus microscope.

Treatment of Sperm with PI-PLC. 2 x 10^8 sperm were incubated with 1 unit/ml of PI-PLC (Molecular Probes, Eugene, OR) for 30 min at 22 °C, with untreated sperm as control. Sperm pellet and its supernatants were then collected by centrifugation at 1000 g for 30 min at 4 °C. Proteins were reconstituted in lysis buffer (7M Urea, 2M Thiourea, 4 % CHAPS, 1X HaltTM protease inhibitor, 2 % DTT) for western blot analysis.

Fluorescence recordings of $[Ca^{2+}]I$ of oocyte in mouse COCs or isolated cumulus cells. COCs or isolated cumulus cells were incubated in 3 μM Fura-2-acetoxymethylester and 1.6 μM pluronic F127 (Molecular Probes, Eugene, OR) for 30 min at 37 °C. The incubation chamber was kept at 37 °C and images were taken using an Olympic IX70 microscope with magnification at 200X. Recombinant NYD-SP8 pro-

tein (5 – 12.5 μg/ml) was added to the chamber at indicated time point, the ratio of emission intensities and images acquired at 340 nm and 380 nm were recorded and processed using MetaFlour software (Universal Imaging Corp, Washington, DC).

Progesterone assay. Twenty COCs for each group were incubated in 50 μl mHTF medium (Irvine Scientific, Santa Ana) at 37 °C for 4 h before measurement. Recombinant NYD-SP8 and/or its antibody (15 μg/ml), or preimmune serum as control were added at the beginning of the incubation. After the incubation, media were collected for progesterone determination using Enzyme Immunoassay (EIA) Kit (Assay Designs, Ann Arbor, MI).

Induction of acrosome reaction by the cumulus cells or progesterone. $2.4x10^5$ sperm were capacitated in a 5 % CO_2 incubator at 37 °C for 90 min before co-incubation. $9 – 12\ x10^5$ cumulus cells were then added and incubated with capacitated sperm for another 30 min before CTC staining. To account for any potential effect of serum on the acrosome reaction, sperm were incubated in the culture medium containing preimmune serum for 15 min. NYD-SP8 antibody or BAPTA-AM (50 μM) was added to sperm or the cumulus cells.

***In-vitro* fertilization.** COCs were treated with different concentrations of NYD-SP8 antibody (3, 15, 30 μg/ml) for 30 min, or together with 15 μM of progesterone, with preimmune serum as control. Then, COCs were co-incubated with capacitated mouse sperm in a 5 % CO_2 incubator for 10 h at 37 °C. The numbers of two-cell stage embryo were counted under a phase-contrast microscope. Fertilization rate in each group was calculated by assigning the fertilization rate of the control as 100 %.

Immunization and fertility trial. Celiac immunization of NYD-SP8 protein was performed on eight-week old mice and followed by three boosts. Each immunization contained 100 μg of NYD-SP8 peptide. Control mice were performed only with PBS in complete Freund's adjuvant and incomplete Freund's adjuvant. Mating was performed seven days after the last injection when the serum titers exceed 1:1000 examined by ELISA assay. Mice were allowed to mate for four days. Successful mating were confirmed by the presence of vaginal plugs. Titers of IgA against NYD-SP8 in vaginal washes and IgG against NYD-SP8 in sera were examined by ELISA. The number of pups delivered by each mated animal was counted two weeks after mating.

Statistical Analysis. All percentage data were subjected to arc sine transformation before statistical analysis. Data were analyzed by ANOVA and shown mean ±SEM in tables. A probability of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Identification of GPI-anchored NYD-SP8 in human and mouse sperm. In the present study, NYD-SP8 was identified from the differential hybridization of human testis cDNA microarray for its **seven**-fold difference in expression level between embryo and adult testis [10]. The sequence of NYD-SP8 (GenBank Accession No.AY014285) was found to consist of 1123 nucleotides and contain an open reading frame of 249 amino acids (M_r27kDa). NYD-SP8 appeared to be the human homologue of a mouse testis specific protein, TES101 [11], which has been shown to be a surface protein on developing male germ cells with no definitive function demonstrated. Tissue distribution studies using RT-PCR showed that the gene was expressed predominantly in the testis, but not in 15 other tissues examined (Fig. 1A).

For further characterization and functional studies, antibody against NYD-SP8 was raised and a single specific band in both human and mouse testes and sperm was detected by the antibody (Fig. 1B), confirming a high homology of human NYD-SP8 to its mouse homologue, as predicted by their protein sequences (Fig. 1C). Immunolocalization results showed that NYD-SP8 was localized to the posterior head of both human and mouse sperm (Fig. 2A), a region primarily involved in sperm-egg interaction [12].

Bioinformatic analysis revealed that NYD-SP8 protein has 30 % structural homology to urokinase Plasminogen Activator Receptor (uPAR). Both of them contain a potential glycosylated phosphatidylinositol(GPI)-modification site. In mammals, more than 200 glycoproteins have been identified that are linked to the plasma membrane via a GPI-anchor[13 – 15]. GPI-anchored proteins possess a covalently linked GPI moiety that serves to attach the protein portion of the molecule to the lipid bilayer of the cell surface. We tested whether NYD-SP8 could be susceptible to PI-PLC, which removes GPI-anchored proteins from the membrane [16]. NYD-SP8 was detected in the supernatant of both human and mouse sperm treated with PI-PLC (Fig. 2B), indicating its anchoring to sperm membrane via a GPI-lipid linkage. Therefore, NYD-SP8 could be a new sperm-specific GPI-anchored protein.

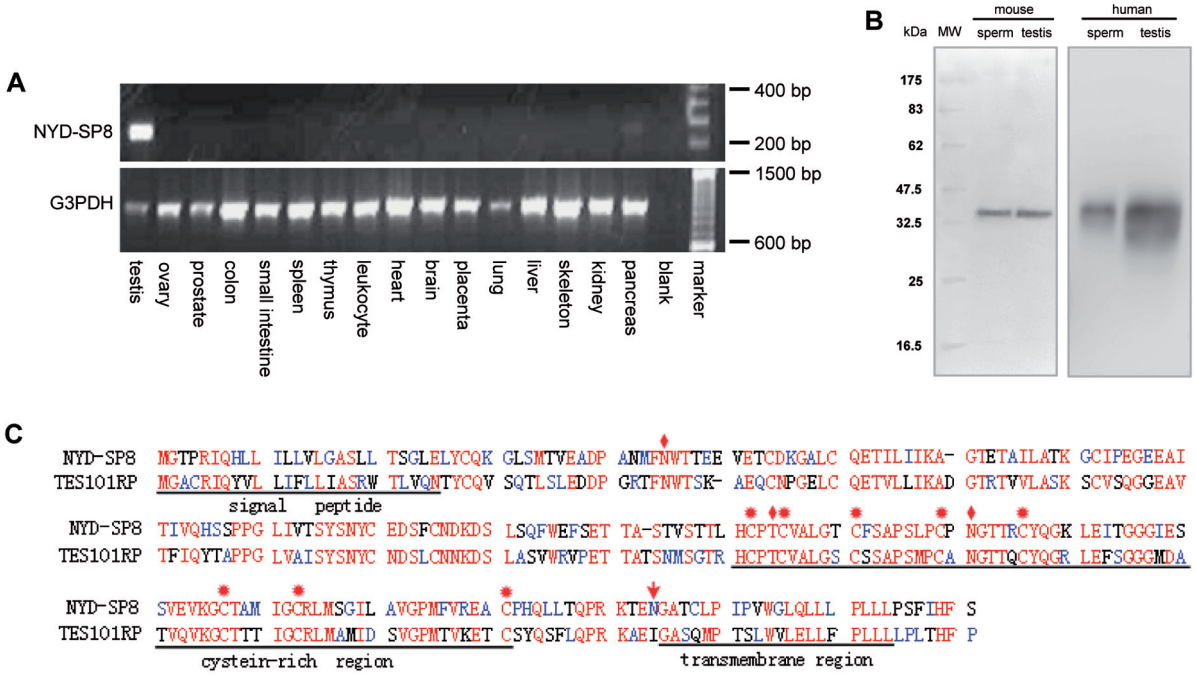

Figure 1. Characterization of NYD-SP8. (*A*) Testis-specific tissue distribution of NYD-SP8 tested by RT-PCR, with G3PDH as control. (*B*) Western blot analysis of mouse and human sperm and testes showing the specificity of NYD-SP8 antibody. (*C*) Protein sequence alignment of human NYD-SP8 and its mouse homologue. High consensus residues are in red, and similar residues are in blue. Potential glycosylated sites are marked with rhombus, and predicted GPI-link site is marked with an arrow. Conserved cysteines were indicated by asterisks.

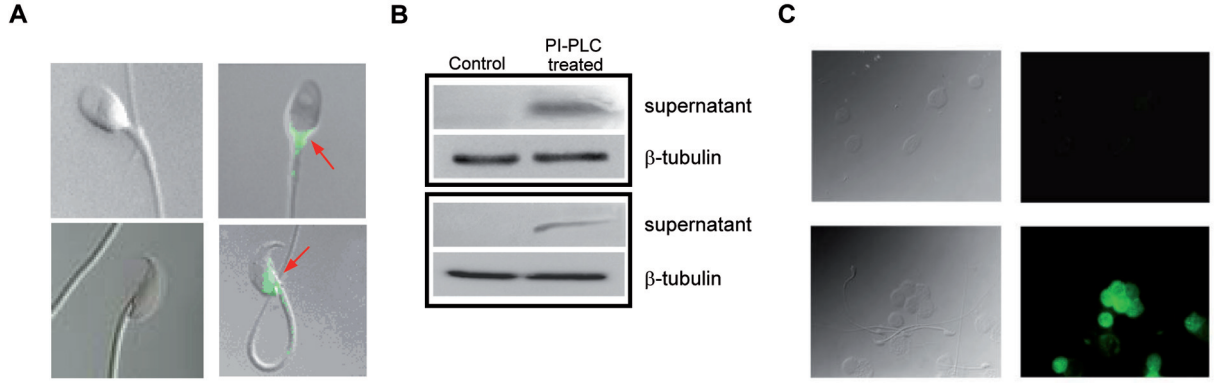

Figure 2. NYD-SP8 localization and release from sperm. (*A*) NYD-SP8 (arrows) labeled with FITC was visualized by overlaid DIC image. NYD-SP8 is localized to the posterior head of human (upper) and mouse (lower) sperm with corresponding controls treated with preimmune serum (left). (*B*) Western Blot analysis of NYD-SP8 in the supernatant of human (upper) and mouse (lower) sperm treated with PI-PLC (PI-PLC-treated), with β-tubulin as the control for the amount of sperm used in the assay. (*C*) Immunostaining of mouse cumulus cells to detect NYD-SP8 before (upper) and after (lower) mouse sperm incubation for six h, with corresponding bright-field micrographs shown on the left.

Binding of NYD-SP8 to the cumulus cells and triggering intracellular Ca^{2+} mobilization. In sperm, the cleavage of GPI-anchored proteins has been shown to be important for fertilization [17]. The localization of NYD-SP8 to a region primarily involved in sperm-egg interaction also suggests its possible involvement in the process. We then tested possible release of NYD-SP8 from sperm upon their encounter with the first layer of egg investment, the cumulus. When mouse sperm were incubated with mouse cumulus cells for six hours, the initially NYD-SP8-negative cumulus cells showed positive immunostaining of NYD-SP8 (Fig. 2C) after washing off the incubated sperm, indicating binding of NYD-SP8 to the cumulus cells upon gamete interaction.

Since GPI-anchored proteins, including the uPAR superfamily, are known to be mediators of cell signaling events including changes in cytosolic Ca^{2+}

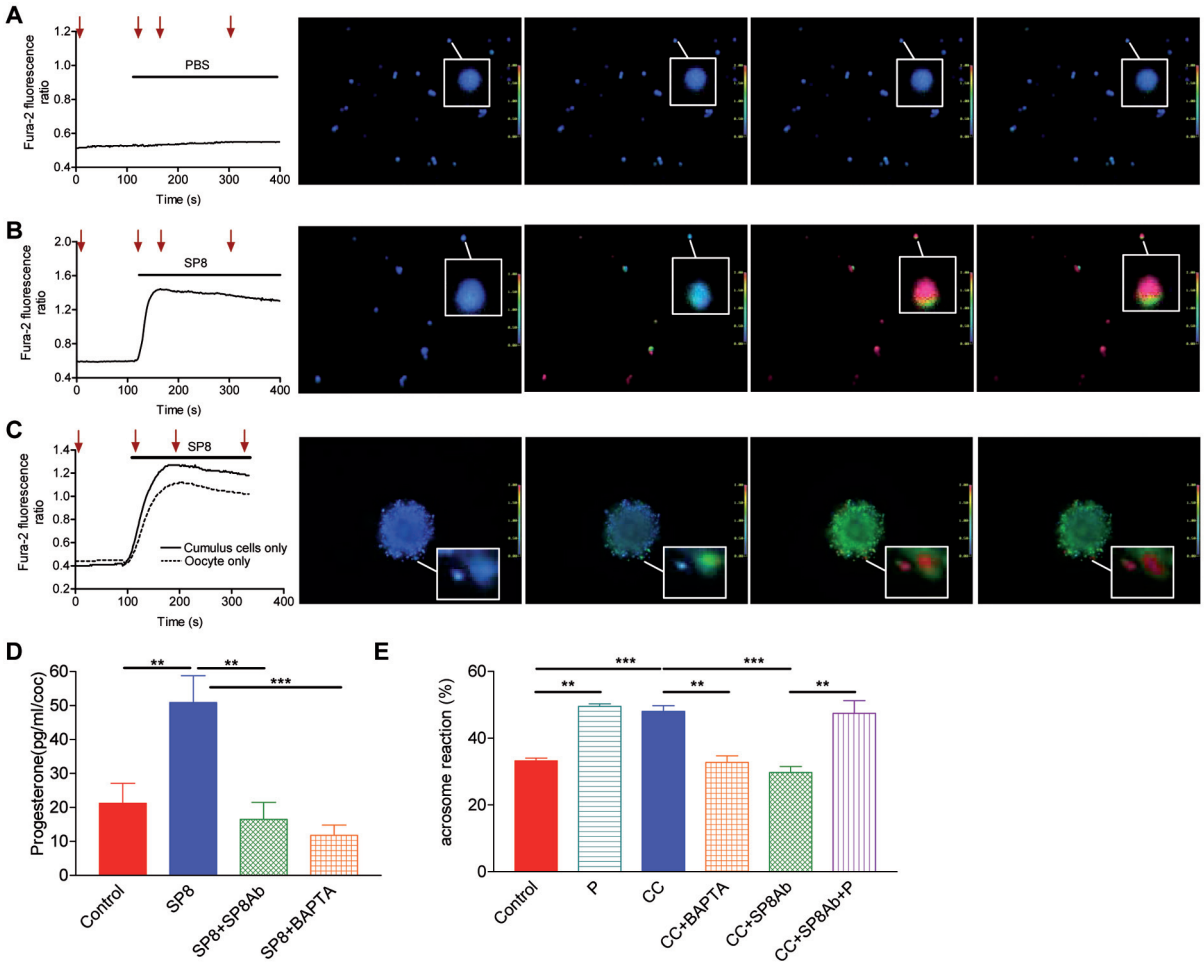

Figure 3. NYD-SP8-induced Ca^{2+} mobilization, progesterone release from cumulus /cumulus-oocyte complexes (COCs) and the cumulus-induced acrosome reaction. Time-dependent fluorescence ratio traces reflecting changes in intracellular Ca^{2+} concentrations in the cumulus cells treated with PBS (*A*); in cumulus cells treated with recombinant NYD-SP8 protein (*B*); and in COCs treated with recombinant NYD-SP8 protein (C). Arrows indicate the time points at which the corresponding photo images are shown (insets, 20X). Each trace represents the average intensity of all signals of interest, either from the cumulus cells (*A – C*, solid line) or the oocyte (*C*, dash line). (*D*) Compared to the control, recombinant NYD-SP8 protein enhanced progesterone release from COCs, which could be abolished by the presence of NYD-SP8 antibody (SP8Ab, 15 μg/ml) or a membrane permeable Ca^{2+} chelator, BAPTA-AM (10 μM). (*E*) Progesterone (P, 5 μM) and the cumulus cells (CC, treated with preimmune serum) enhanced acrosome reaction in mouse sperm as compared to the untreated control. CC-induced acrosome reaction could be significantly reduced by Ca^{2+} chelator BAPTA-AM (BAPTA, 50 μM) or NYD-SP8 antibody (SP8Ab, 15 μg/ml) and exogenous progesterone reversed the inhibitory effect of SP8Ab. The data represent the mean ±SEM obtained from at least three independent experiments. **, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.*

[18 – 20], we tested intracellular Ca^{2+} response of the cumulus cells to NYD-SP8. Fura-2 fluorescent dye was loaded into mouse cumulus-oocyte complexes, as well as isolated cumulus cells, for measurement of intracellular Ca^{2+}. Recombinant NYD-SP8 protein at concentrations between 5 – 12.5 μg/ml induced a Ca^{2+} increase in the cumulus cells as well as the cumulus-oocyte complexes (COCs, Fig. 3 – A, B, C), indicating that the Ca^{2+} signaling event may be associated with sperm penetration when they first encounter the cumulus layer of the egg.

Involvement of NYD-SP8 in the cumulus-induced acrosome reaction. What might be the physiological consequence to the NYD-SP8-induced Ca^{2+} mobilization in the cumulus? On the one hand, it has been reported that Ca^{2+} mobilization in the cumulus cells is associated with increased progesterone production [21, 22]. On the other hand, progesterone, in addition to ZP, has been shown to induce the acrosome reaction [23 – 25]. However, its exact role in a physiological context remains elusive. We therefore hypothesized that the binding of NYD-SP8 to the cumulus cells, upon sperm-cumulus encounter, activates Ca^{2+} mobilization leading to progesterone production and release from the cumulus cells, which in turns induces the acrosome reaction facilitating sperm penetration of the cumulus. To test this hypothesis, we first

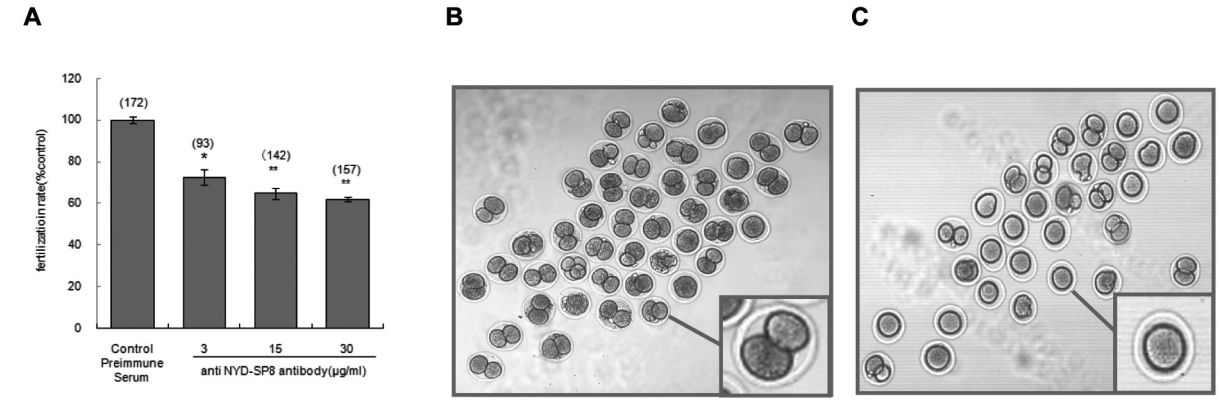

Figure 4. NYD-SP8 involvement in *in-vitro* fertilization. (*A*) Statistic results showing concentration-dependent inhibitory effect of NYD-SP8 antibody on *in vitro* fertilization ratio. Total number of egg counted in each group is marked in blanket. (mean values ±SEM, n = 3, *, $P < 0.05$ and **, $P < 0.01$ vs. preimmune serum control). (*B* – *C*) Corresponding micrographs showing reduced number of two-cell embryos in the NYD-SP8 antibody-treated group (30 μg/ml) (*C*) as compared to the preimmune serum-treated control (*B*).

examined possible effect of NYD-SP8 on progesterone release from COCs. ELISA measurement showed that recombinant NYD-SP8 at concentrations similar to that used to trigger Ca^{2+} mobilization in the cumulus cells could enhance progesterone release (50.9 ± 7.9 ρg/ml) as compared to the untreated control (20.2 ± 5.9 ρg/ml) over a four hour incubation ($P < 0.01$). The NYD-SP8-induced progesterone release could be blocked by NYD-SP8 antibody or treatment with a selective membrane permeable Ca^{2+} chelator, BAPTA-AM (Fig. 3D), indicating that the NYD-SP8-induced progesterone release was dependent on Ca^{2+} mobilization. Therefore, it is expected that the NYD-SP8-induced progesterone release should enable the cumulus to induce the acrosome reaction and that the cumulus-induced acrosome reaction should be mimicked by progesterone and inhibited by chelating intracellular Ca^{2+}. Indeed, as shown in Fig. 3E, incubation of sperm with the cumulus cells resulted in a significant increase in the acrosome reaction, which could be mimicked by progesterone but prevented by pretreatment of the cumulus cells with the Ca^{2+} chelator BAPTA-AM. NYD-SP8 antibody also prevented the cumulus-induced acrosome reaction; however, exogenously added progesterone could restore the acrosome reaction to a level similar to that observed in the absence of NYD-SP8 antibody (Fig. 3E). Taken together, it appears that the physiological consequence to the NYD-SP8-induced Ca^{2+} mobilization in the cumulus may be the triggering of the release of progesterone that induces the acrosome reaction in sperm.

Effect of NYD-SP8 immunization on fertility outcome. To demonstrate the importance of the NYD-SP8-mediated cumulus-induced acrosome reaction, we conducted subsequent experiments to examine the effect of NYD-SP8 on the outcome of fertilization, *in vitro* and *in vivo*. Mouse sperm were co-incubated with COCs in the presence of different concentrations of NYD-SP8 antibody. Significant inhibitory effect on the outcome of *in vitro* fertilization (IVF), as demonstrated by the reduced number of two-cell embryos, was observed in all NYD-SP8 antibody-treated groups, in a concentration-dependent manner, as compared to the control treated with preimmune serum (Fig. 4). Interestingly, progesterone was not able to completely reverse the effect of NYD-SP8 antibody on IVF rate (Fig. S2), indicating that apart from its involvement in the progesterone-dependent acrosome reaction, NYD-SP8 may also be involved in a progesterone-independent process during fertilization.

In another experiment, mice were immunized with recombinant NYD-SP8 protein and the effect of immunization on the fertility outcome was examined. All of the mice that received NYD-SP8 injections had IgG antibody responses to NYD-SP8 in their sera at dilutions of 1:1000, of which 98 % were above 1:10000. Of all the vaginal washes in immunized female mice, 68 % showed IgA, a cervical mucus associated immunoglobulin, positive signal against NYD-SP8 at a dilution of 1:100. This suggested that NYD-SP8 immunization did trigger antibody responses in mice and, more importantly, in the female reproductive tract. Furthermore, HE staining showed that morphological characteristics of the testis and ovary in NYD-SP8 immunized groups were as normal as the control groups (Fig. S1). In addition, motility and morphology of the sperm in the immunized group were not significantly different from that of the control group (Table S1). These analyses ruled out the possibility that NYD-SP8 immunization had an effect on gametogenesis. Seven days after the last injection, immu-

Table 1. Effect of NYD-SP8 immunization on fertility outcome in mice

Group	Mice (no.)	Pup born (no.)	Pups born /animal (mean± SEM)	% Pregnant animals	Fertility rate[#]
Control male +control female	14	80	5.71 ± 0.44	100	100 %
Immunized male +control female	12	34	2.83 ± 0.71**	66.7	49.6 %
Control male + immunized female	14	29	2.07 ± 0.82*	35.7	36.3 %
Immunized male +immunized female	14	12	0.86 ± 0.47*	21.4	15 %

[#] Defined as the percentage of average litter size in control group. (*P<0.05 vs. control group. **P<0.01 vs. control group)

nized males or females were allowed to mate with either the immunized or un-immunized opposite sex for four days (1 estrous cycle) and the mating was confirmed by the presence of a vaginal plug. As shown in Table 1, the pregnancy rate as well as the average litter size was significantly reduced in all groups of immunized animals, with immunization in both sexes having the most dramatic effect, 21.4 % of pregnancy rate and 0.86 of average litter size as compared to 100 % and 5.71 in the controls treated with PBS and adjuvant. In other words, immunization of NYD-SP8 in both sexes resulted in complete prevention of fertilization in nearly 80 % of the mice and also reduced the number of successful fertilization events in mice that did become pregnant, i. e. from six new pups to one per animal, an 85 % reduction in fertility rate. Interestingly, immunization of NYD-SP8 in male mice alone had less effect on the fertility outcome than the immunization in female mice alone, 33.3 % as compared to 64.3 % reduction in pregnancy rate, respectively. These results may first appear to be strange, since NYD-SP8 is not present in the female reproductive tract. However, as it was demonstrated earlier that NYD-SP8 is involved in the sperm-cumulus interaction, a process taking place in the female reproductive tract, the best way to block the action of NYD-SP8 would be to have its antibody present in the female reproductive tract and unnecessary in the male tract. Thus, both the *in vitro* and *in vivo* studies have demonstrated an important role of NYD-SP8 in fertilization.

The present findings suggest that NYD-SP8 may be involved in mediating cross-talk between sperm and the cumulus during sperm-egg interaction, which could include the following sequence of events: 1) NYD-SP8 cleavage from sperm; 2) binding of NYD-SP8 to the cumulus; 3) triggering Ca^{2+} mobilization in the cumulus leading to; 4) progesterone production/ release; 5) induction of the acrosome reaction in sperm by progesterone with; 6) release of hydrolytic enzymes acting on the cumulus to facilitate sperm penetration. While the acrosome reaction has long been believed to be induced by the egg's zona pellucida in order for sperm to penetrate through this thick extracellular matrix, it has not been considered for the penetration of the cumulus traditionally. Instead, hyperactivated sperm motility and sperm GPI-anchored surface hyaluronidases, such as PH-20 [26] and Hya15 [27], are thought to be sufficient for the sperm to get through the cumulus. The present findings have provided evidence indicating that the acrosome reaction may also be activated when sperm encounter the cumulus. Importantly, the present results indicate that the cumulus-dependent acrosome reaction is initiated by a sperm GPI-anchored protein, NYD-SP8, through sperm-cumulus cross-talk, involving both Ca^{2+} mobilization and progesterone release from the cumulus cells, which suggests that the penetration of the cumulus mass may require more enzymatic drill than previously thought. This notion is also consistent with the recent finding that human spermatozoa exposed to the cumulus mass, when compared with those spermatozoa cultured in medium alone, are more likely to be acrosome reacted in the absence of ZP [28]. These cumulus-induced acrosome reacted spermatozoa may not bind to the ZP of human oocytes [29] and may even die before reaching the ZP. However, their sacrifice may pave the way for other unreacted sperm to reach the ZP where they will undergo the conventional ZP-induced acrosome reaction to penetrate this second layer of egg investment. The current finding of the cumulus-induced acrosome reaction provides a better explanation as to why more than millions of sperm are required for successful fertilization in the human and mouse, since many of them will die along the way, including the penetration through the cumulus and not just the ZP. It should be noted that the application of recombinant NYD-SP8 protein in triggering Ca^{2+} mobilization in the cumulus cells in the present study represents only a stimulus equivalent in nature and magnitude to the presentation of the protein by sperm. It would be ideal if cumulus Ca^{2+} mobilization could be detected upon their direct interaction with sperm. The present results also suggest possible involvement of NYD-SP8 in other process of fertilization. The ability of recombinant NYD-SP8 to induce Ca^{2+} mobilization, not only in the cumulus cells but also the oocyte, suggests its possible direct effect on the oocyte. In addition, the observation that the effect of

NYD-SP8 antibody on the IVF rate could not be reversed completely by exogenous progesterone also suggests that the action of NYD-SP8 may extend beyond the cumulus layer on a progesterone-independent process. The signaling mechanism mediating the action of NYD-SP8 in this process requires further investigation.

Summary

The present study has demonstrated a molecular mechanism underlying the early process of sperm-egg interaction, which has long been neglected. The NYD-SP8-mediated cross-talk appears to be important for fertilization as evidenced by the impact of NYD-SP8 antibody and its immunization on fertilization outcome *in vitro* and *in vivo*. Better understanding of the mechanism by which NYD-SP8 affects sperm-egg interaction and fertilization may enable development of new methods for fertility regulation, diagnosis, and treatment of male infertility.

Electronic supplementary material. Supplementary material is available in the online version of this article at springerlink.com (DOI 10.1007/s00018-009-8482-2) and is accessible for authorized users.

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