# Glucosamine enhanced sperm-egg binding but inhibited sperm-egg fusion in mouse

M. Okabe, M. Yagasaki, S. Matzno, M. Nagira, Y. Kohama and T. Mimura

*Faculty of Pharmaceutical Sciences, Osaka University, Yamadaoka 1–6, Suita Osaka 565 (Japan)*
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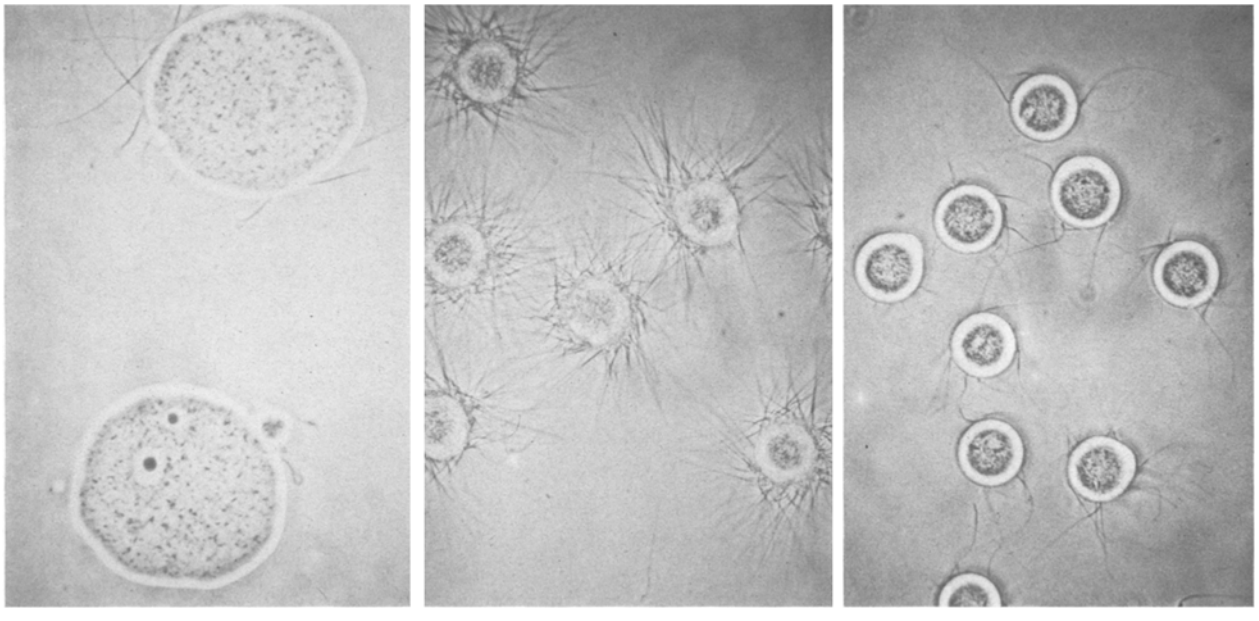
*Summary.* In order to study the sperm-egg recognition mechanism on the surface of the plasma membrane, zonae were removed from mouse eggs by exposure to acidic conditions. Sperm binding to denuded eggs was then observed in the presence of various sugars. Among several carbohydrates tested, only glucosamine (GlcN) was found to increase the number of sperm bound to eggs while inhibiting sperm-egg fusion. The inhibition was reversible; when denuded eggs were transferred to a GlcN free medium, a high rate of polyspermy was observed.
*Key words.* Mouse; zona-free egg; sperm-egg fusion; glucosamine.

Sperm-egg recognition on the surface of the zona pellucida has been studied intensively [1, 2]. However, little attention has been given to the interaction of sperm with egg plasma membrane. Since Yanagimachi [3] and Hanada and Chang [4] reported interspecies fusion of gametes, the recognition mechanism of sperm for egg plasma membrane seems to be considered to be less species-specific. However, this does not necessarily imply that there is no recognition mechanism in the fusion of gametes. Only acrosome reacted sperm can fuse with the egg [5]. Moreover, the possibility of species-specific binding of mouse sperm to egg plasma membrane was demonstrated using an anti-sperm monoclonal antibody (OBF 13) [6]. On the other hand, Wolf reported that the percentage of sperm-bound eggs decreased as fertilization proceeded [7]. Plasma membrane block seemed to be not only a mechanism preventing extra sperm from fusing with an egg, but there was also a release on extra sperm bound on the egg surface after initial sperm-egg fusion. These results may suggest the existence of a sperm receptor on egg plasma membrane. In the present article, zona-free eggs were prepared by exposing mouse egg to acidic saline, and sperm-egg interaction was studied from the viewpoint of gamete binding prior to fusion.

*Materials and methods.* In vitro fertilization of mouse eggs. The medium used was a modified Krebs-Ringer buffer (m-KRB) [8]. The sperm were obtained from the cauda epididymis of mature ddy mice (weighing 35–40 g) as previously reported [8]. 4–5-week-old ddy mice were injected with 5 IU of PMSG (Teikoku Zoki) 48 h before the administration of 5 IU hCG (Teikoku Zoki) i.p. 14–16 h after the hCG injection, the eggs in cumulus clots were collected by puncturing the ampullar portion of the oviduct with a needle. Eggs collected from 10 female mice were treated with 0.01% hyaluronidase (Sigma, Type I–S) for 5 min in the medium. Cumulus-free eggs were then washed by m-KRB and subjected to a further 60-s treatment with 0.01 M $H_3PO_4$-saline in a plastic petri dish to remove the zona pellucida. 10–20 zona-free eggs, washed 3 times with m-KRB, were placed in test tubes which contained 0.45 ml of the medium, and 10 µl of sperm suspension (approximately $1.5 \times 10^6$ sperm/ml) was added to the eggs with or without sperm preincubation. A stock solution of various extra pure grade carbohydrates, concentrated 25 times (all amino sugars were HCl type), from Nakarai or Sigma Chem., were made in sterilized saline. 18 µl of the solution was then added to the 0.45 ml of medium in the test tube the night before the experiment and equilibriated with 5% $CO_2$ in air at 37 °C.

Sperm and eggs were incubated for several hours, as indicated in the text, and were then removed to a watch glass. The eggs collected in a minimal volume (~0.3 µl) were placed between vaseline spots. The eggs were then gently pressed by a cover glass and observed for sperm binding and for the formation of pronuclei under phase contrast microscopy at ×320 magnification.



*A* Egg which formed pronuclei (bottom) had fewer sperm on its surface compared to unfertilized egg (top). Magnification ×400. *B* Eggs heavily covered by sperm in the existence of GlcN 1 h after the sperm addition. ×100. *C* Control eggs inseminated by the same sperm as shown in *B* without GlcN. ×100.

Table 1. Effect of various carbohydrates on the fusion of mouse sperm to mouse zona-free eggs in vitro

| Carbohydrate | Conc. (mM) | Pronuclear formation* (%) | Carbohydrate | Conc. (mM) | Pronuclear formation* (%) |
|---|---|---|---|---|---|
| None | – | 56.2 ± 4.4 | None | – | 69.3 ± 5.1 |
| L-Fuc | 20 | 54.1 ± 10.0 | GalN | 20 | 50.3 ± 22.4 |
| D-Man | 20 | 47.1 ± 15.1 | GlcN | 20 | 21.4 ± 11.5** |
| D-Gal | 20 | 51.7 ± 16.0 | ManN | 10 | 60.2 ± 5.9 |
| D-Glc | 20 | 52.9 ± 12.9 | | | |
| GalNAc | 20 | 55.0 ± 11.5 | | | |
| GlcNAc | 20 | 59.5 ± 9.2 | | | |
| ManNAc | 20 | 48.5 ± 8.7 | | | |

* Mean ± SE of 4 independent tests (more than 45 eggs were examined in each group) significantly different from control; ** $p < 0.01$.

Table 2. Reversible inhibition of pronuclear formation of mouse eggs by glucosamine in vitro

| | GlcN (mM) | No. of experiments | No. of eggs examined | Pronuclear formation[a] (%) | Sperm bound index[b] |
|---|---|---|---|---|---|
| Exp. 1: eggs were not transferred during the procedure | | | | | |
| Control | 0 | 4 | 95 | 56.5 ± 7.4 | 9.0 ± 1.9 |
| Test | 20 | 4 | 79 | 17.0 ± 5.7** | 16.5 ± 1.4* |
| Exp. 2: eggs were transferred after 1 h of incubation to a GlcN-free medium | | | | | |
| Control | 0 | 4 | 67 | 55.2 ± 6.7 | Not counted |
| Test | 20 | 4 | 59 | 86.6 ± 6.3* | Not counted |

[a] Mean ± SE of 4 independent tests. [b] The index was based on the number of sperm bound to eggs. Those eggs which had more than 20 sperm on their surface were scored as 20. The index was expressed as a mean ± SE of these scores. Significantly different from control; * $p < 0.05$, ** $p < 0.01$.

*Results and discussion.* When epididymal sperm were introduced to zona-free eggs, all the eggs were covered by sperm within 1 h. However, after 5 h of incubation, many sperm had detached from the fertilized eggs. Interestingly, eggs which remained unfertilized retained sperm on their plasma membrane (fig., A). The result indicated that extra sperm were no longer able to remain on the surface of a fertilized egg. In order to avoid the various carbohydrates tested affecting sperm capacitation (and/or acrosome reaction), sperm preincubated in m-KRB for 60 min were used in the following experiments. When preincubated sperm were added to eggs in the presence of various sugars, binding to the egg membrane was not inhibited by any of the carbohydrates tested. However, sperm-egg fusion was significantly inhibited by the addition of GlcN, whereas GalN and ManN were not similarly effective (table 1). The effect was not based on the inhibition of sperm-egg binding. More sperm were observed on the egg surface when GlcN was added to the medium (fig., B) compared to control eggs (fig., C). (The number of bound sperm remained high even after 5 h of incubation in the GlcN-added group. The photo was taken after 1 h of incubation to clarify the effect of GlcN on binding only, because many sperm detached from the eggs after 5 h in the control group when fertilization had been accomplished.) These data clearly demonstrated that the effect of GlcN was due to the block of sperm-egg fusion. It should be noted that the inhibition of fusion by GlcN was a reversible effect. When eggs, heavily covered by sperm in the GlcN-added medium, were transferred after 1 h of incubation to a GlcN free medium and incubated another 4 h, fusion of the gametes occurred at an even higher rate than in the control group. Reflecting the number of sperm on the egg surface, a majority of the eggs became polyspermic in the GlcN-added group, while many eggs were monospermic in the control group (table 2).

The data shown in this article demonstrated that sperm-egg fusion is a two-step reaction; binding and fusion. Shur et al., claiming an involvement of GalTase in sperm-zona binding, recently postulated that the enzyme might also play a role in sperm-egg binding [9]. If we interpret our data to indicate that binding was enhanced so strongly by GlcN that fusion was inhibited, the result, together with Shur's model, may provide a deeper view of sperm-egg fusion.

Abbreviations. GlcN: glucosamine; GalN: galactosamine; ManN: mannosamine; GalTase: galactosyltransferase.

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## Sex linkage of malic enzyme in *Xenopus laevis*

J.-D. Graf

*Station de Zoologie expérimentale, Université de Genève, Route de Malagnou 154, CH-1224 Chêne-Bougeries (Switzerland)*
*Received 6 September 1988; accepted 26 October 1988*

*Summary.* Genetic analysis of mME variants (mitochondrial malic enzyme, E.C. 1.1.1.40) in *Xenopus laevis* revealed sex linkage of the mMe locus and indicated a WZ/ZZ type of sex determination. Codominant mMe alleles occur on both W and Z chromosomes, with a recombination frequency of 6.1 % ± 1.5 % between mMe and the sex-determining locus (or region).
*Key words. Xenopus laevis;* sex-linked genes; sex determination; amphibian genetics.

In contrast to mammals, a great majority of anuran amphibians analyzed cytologically do not show heteromorphic sex chromosomes. However, genetic analysis of several frog species (genus *Rana*) revealed sex linkage of certain enzyme loci [1–4]. The mode of inheritance of these loci indicated an XX/XY type of sex-determining mechanism (i.e. male het-