Supporting Information

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SI Materials and Methods

Sample Preparation for Fluorescence Microscopy. Glass coverslips were coated with poly-lysine by incubation for 10 min with 0.01% poly(L)-lysine (Sigma) at RT, washed with distilled water, and air-dried overnight. Sperm cells were seeded on the coated coverslips, incubated for 1 min at RT, rinsed in PBS, and fixed in 3% pfa for 20 min at RT. After three washes in PBS, coverslips were blocked with gelatin from coldwater fish skin (Sigma) diluted to 0.5% in PBS for 1 h. They were then rinsed, and then incubated with affinity-purified polyclonal chicken-anti-Neu5Gc IgY or unspecific chicken-IgY (1) diluted 1:1,000 in PBS for 1 h at RT. The coverslips were washed in PBS, incubated with DyLight488-conjugated affinity pure F(ab')2 fragment donkey-anti-chicken IgY (Jackson Immunoresearch Laboratories) for 30 min, washed again in PBS, and mounted in Elvanol (Mowiol 4-88, Calbiochem). Some sperm cells were treated with 25 mU sialidase (AUS) for 1 h at RT before seeding on the coverslips. Cells were examined in DeltaVision real-time fluorescence microscope (Applied Precision).

Sialic Acid Analysis. Sia content of sperm was analyzed by high-pressure liquid chromatograpy (HPLC) of derivatized total Sia extracts obtained from washed sperm after 2 M acetic acid hydrolysis with and without mild base treatment (for studying O-acetyl modifications of Sias). Released Sias were filtered through microcon 10 spin columns (Amicon Millipore, Billerica, MA) and derivatized in 1,2-diamino-4,5-methylenedoxybenzene (DMB) reagent for 3 h at 50 °C in the dark before HPLC analysis over Varian C18 reverse-phase column and isocratic elution in 83% water, 7% methanol, and 8% acetonitrile at 0.9 mL/min over 50 min (2). The Sia standards were from bovine submaxillary mucin. Membrane-bound Sias were prepared by exposing washed sperm cells to double-distilled H_2O for 15 min at 4 °C, followed by centrifugation at 10,000 × *g* for 15 min. The pellets were acid hydrolyzed in 2 M acetic acid at 80 °C for 3 h before being passed over microcon 10 columns by spinning at 13,000 × *g* for 20 min to collect the run through containing the released Sias. DMB derivatization was performed as described above.

1. Diaz SL, et al. (2009) Sensitive and specific detection of the non-human sialic Acid Nglycolylneuraminic acid in human tissues and biotherapeutic products. *PLoS ONE* 4: e4241.

2. Manzi AE, Diaz S, Varki A (1990) High-pressure liquid chromatography of sialic acids on a pellicular resin anion-exchange column with pulsed amperometric detection: A comparison with six other systems. *Anal Biochem* 188:20–32.