10.1071/RD18175_AC

Supplementary Material: *Reproduction, Fertility and Development*, 2019, 31(3), 433–442.

Supplementary Material

Tissue plasminogen activator (tPA) of paternal origin is necessary for the success of *in vitro* but not of *in vivo* fertilisation in the mouse

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(a)

Gen (GenBank accession number)	Forward	Reverse	Amplified region (pb)
tPA (NM_008872)	CGCTGTGACATAGATACCAAG	GGAGACCTCTTGTTCTTGAC	659
β-actin (NM_007393)	CAGATCATGTTTGAGACC	CTTCATGAGGTAGTCTGTC	213

(b)

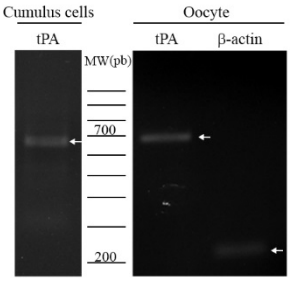

Fig. S1. (a) Primers used in the amplification of mouse tPA and β–actin. (b) Amplicons corresponding with tPA (659 bp) and beta-actin (214 bp) in mouse cumulus cells and oocytes. The experiment was performed in 3 animals ($n=3$).

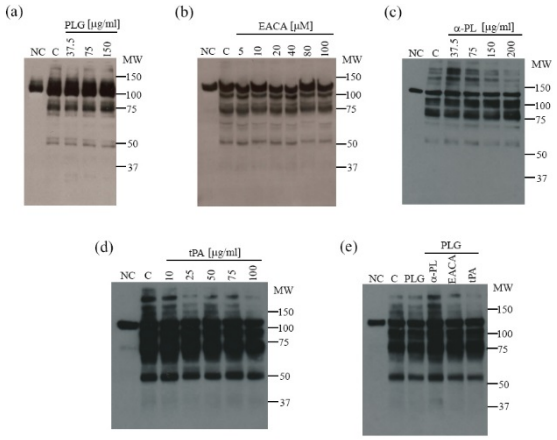

Fig. S2. Analysis of the effects of the PLG-PLA system in sperm tyrosine phosphorylation. Increased concentrations of (a) PLG (from 37.5 to 150 µg mL^{-1}), (b) EACA (from 5 to 100 µM), (c) α-PL (from 37.5 to 150 µg mL^{-1}), (d) tPA (from 10 to 100 µg mL^{-1}) and (e) different combinations [PLG (150 µg mL^{-1}), α-PL (150 µg mL^{-1}), EACA (20 µM), tPA (50 µg mL^{-1})] were used to evaluate tyrosine phosphorylation. NC = sperm incubated in non-capacitating conditions (negative control); C = sperm incubated in capacitating conditions (positive control). All images are representative of experiments repeated at least twice.

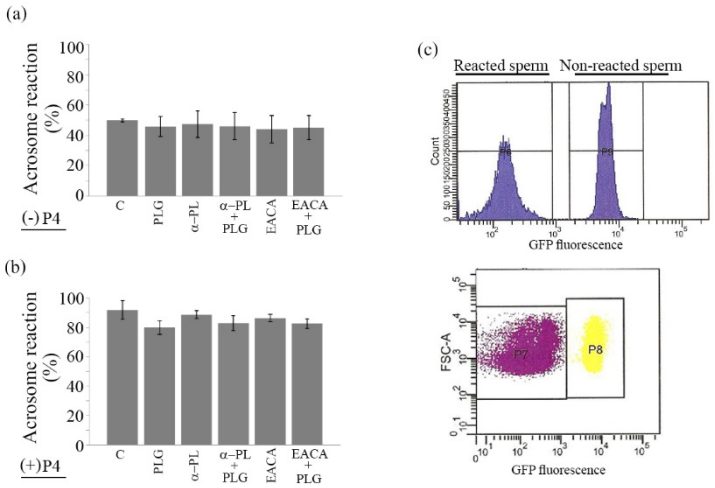

Fig. S3. PLG and inhibitors of the PLG-PLA system (α-PL and EACA) does not increase the sperm acrosome reaction. Epididymal sperm from Acr-GFP mice (containing GFP in their acrosome) were incubated in capacitating conditions and without (a) or with (b) P4 in combination with PLG and (or) α-PL and EACA. Values are means ± SEM of 5 different experiments. (c) Flow cytometry images in which two populations of spermatozoa are distinguished: intact acrosome sperm (non-reacted, right population) and reacted acrosome sperm (left population).