

Citrate Promotes Nitric Oxide Production during Human Sperm Capacitation

Diego Loggia^{1,2,3,4} and Cristian O'Flaherty^{1,2,3,4,*}

¹ Department of Pharmacology and Therapeutics, Faculty of Medicine and Health Sciences, McGill University, Montreal, QC H3G 1Y6, Canada

² Department of Surgery, Urology Division, Faculty of Medicine and Health Sciences, McGill University, Montreal, QC H4A 3J1, Canada

³ The Research Institute, McGill University Health Centre, Montreal, QC H4A 3J1, Canada

⁴ Department of Anatomy and Cell Biology, Faculty of Medicine and Health Sciences, McGill University, Montreal, QC H3A 0C7, Canada

* Correspondence: cristian.oflaherty@mcgill.ca; Tel.: +1-514-934-1934 (ext. 35410)

Supplemental Figures S1-S4

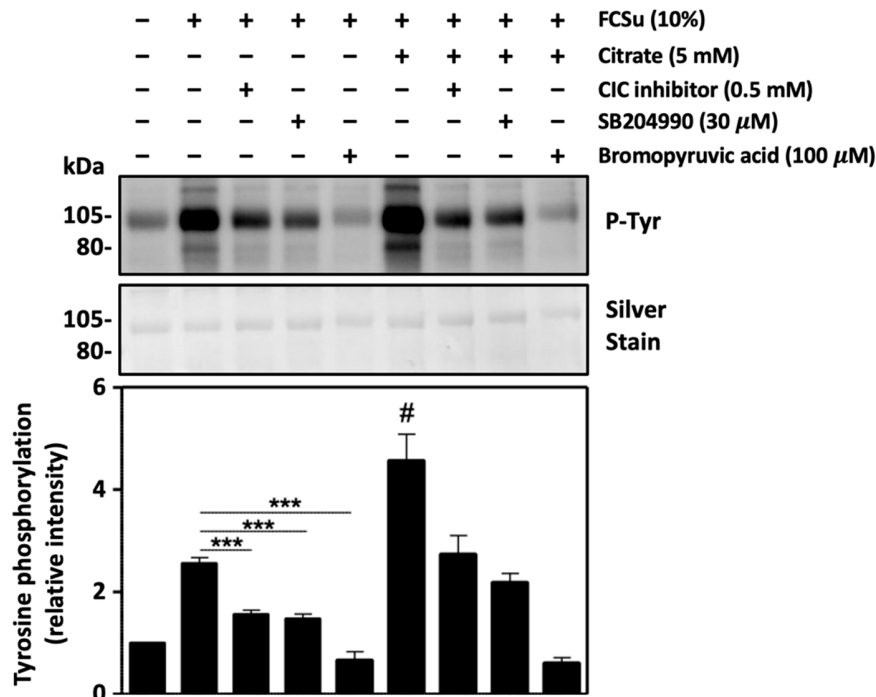


Figure S1. Inhibition of CIC, ACLY, and ME decreases sperm capacitation in BWW-NES. Human spermatozoa incubated in BWW medium with no energy substrates (BBW-NES) show decreased capacitation when incubated with inhibitors of the mitochondrial citrate transporter (CIC inhibitor), ATP-citrate lyase (SB204990) and the malic enzyme (bromopyruvic acid) in the presence and absence of 5 mM citrate, with FCSu as an inducer of capacitation. Relative intensities are expressed as mean $\pm$ SEM (# means higher than all other groups, *** $p \leq 0.005$, ANOVA and Tukey's test, $n = 4$).

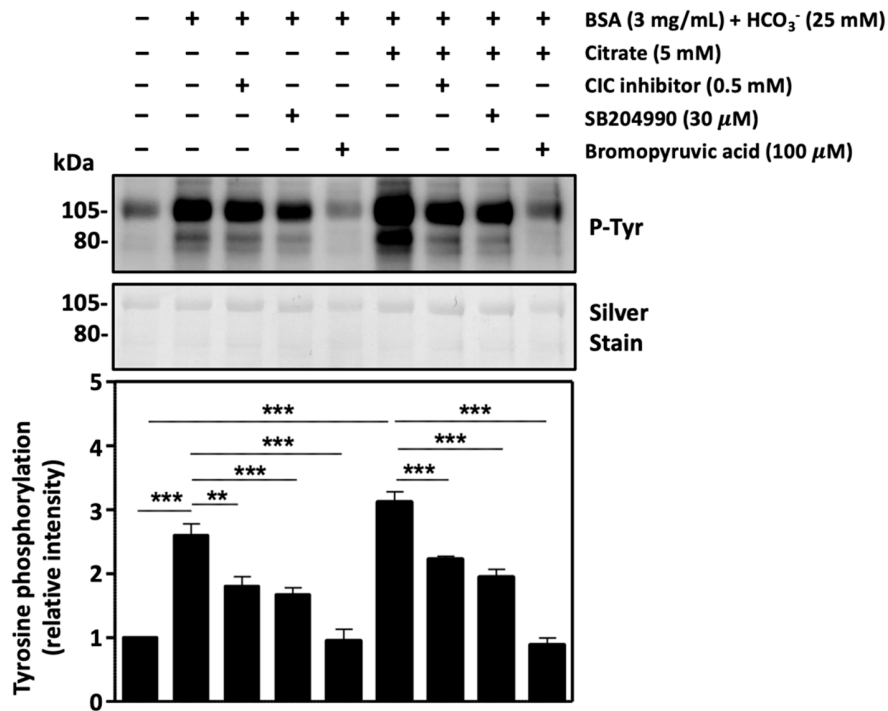


Figure S2. Inhibition of CIC, ACLY, and ME decreases BSA-bicarbonate-induced sperm capacitation. Human spermatozoa incubated in regular BWB medium (BWB-REG) show decreased capacitation when incubated with inhibitors of the mitochondrial citrate transporter (CIC inhibitor), ATP-citrate lyase (SB204990) and the malic enzyme (bromopyruvic acid) in the presence and absence of 5 mM citrate, with BSA and bicarbonate as an inducer of capacitation. Relative intensities are expressed as mean $\pm$ SEM (** $p \leq 0.01$, *** $p \leq 0.005$, ANOVA and Tukey's test, $n = 4$).

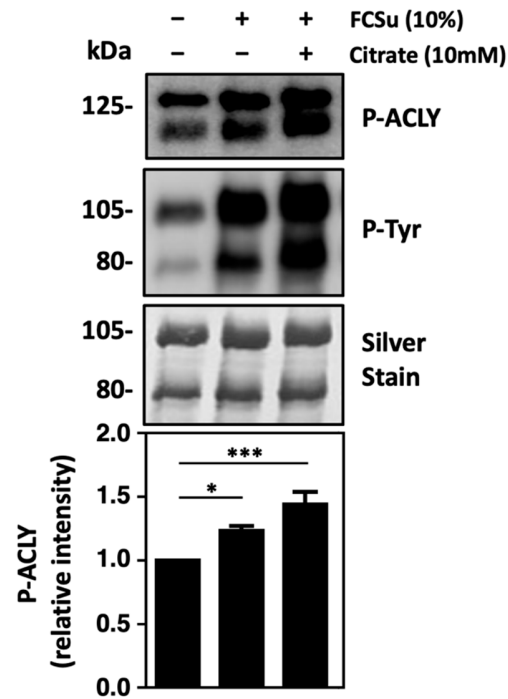


Figure S3. ATP-citrate lyase is activated during sperm capacitation in BWB-NES. Human spermatozoa incubated in BWB medium with no energy substrates (BWB-NES) show increased phosphorylation of ATP-citrate lyase when incubated with FCSu, and a further increase when supplemented with 10 mM citrate. Increased levels of P-ACLY are consistent with increased tyrosine phosphorylation and capacitation in the same samples. Relative intensities are expressed as mean $\pm$ SEM (* $p \leq 0.05$, *** $p \leq 0.005$, ANOVA and Tukey's test, $n = 6$).

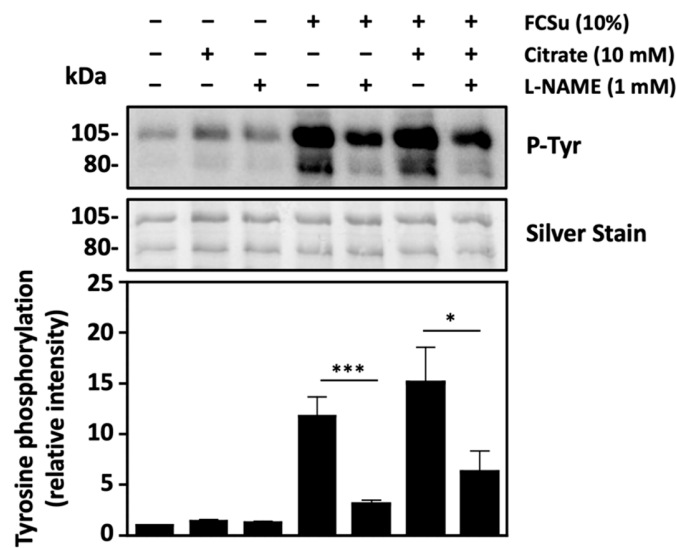


Figure S4. Inhibition of NOS decreases sperm capacitation in BWW-NES. Human spermatozoa incubated in BWW medium with no energy substrates (BBW-NES) show decreased capacitation when incubated with an inhibitors of nitric oxide synthase (L-NAME) in the presence and absence of 10 mM citrate, with FCSu as an inducer of capacitation. Relative intensities are expressed as mean $\pm$ SEM (* $p \leq 0.05$, *** $p \leq 0.005$, ANOVA and Tukey's test, $n = 4$).