# NOS/NO MODULATE THE PROTEIN PHOSPHORYLATION ON SERINE AND THREONINE RESIDUES DURING BOAR SPERM CAPACITATION

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## INTRODUCTION

Sperm capacitation involves the early activation of protein kinases and the inactivation of protein phosphatases. Nitric Oxide (NO) can be generated by spermatozoa with the help of three different isoforms of Nitric Oxide Synthases (NOS) and it can modulate this process in different ways (Fig. 1).

## OBJECTIVE

The aim of this study was to further investigate NO's involvement in PKA activation during *in vitro* capacitation of boar spermatozoa.

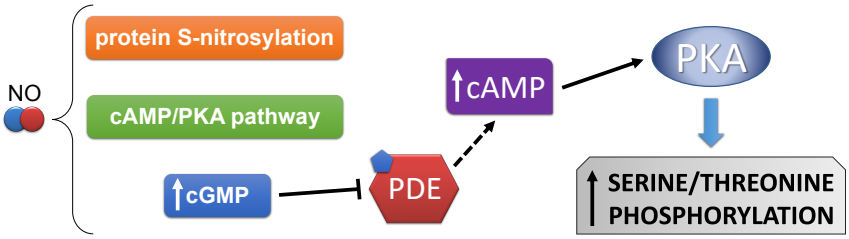
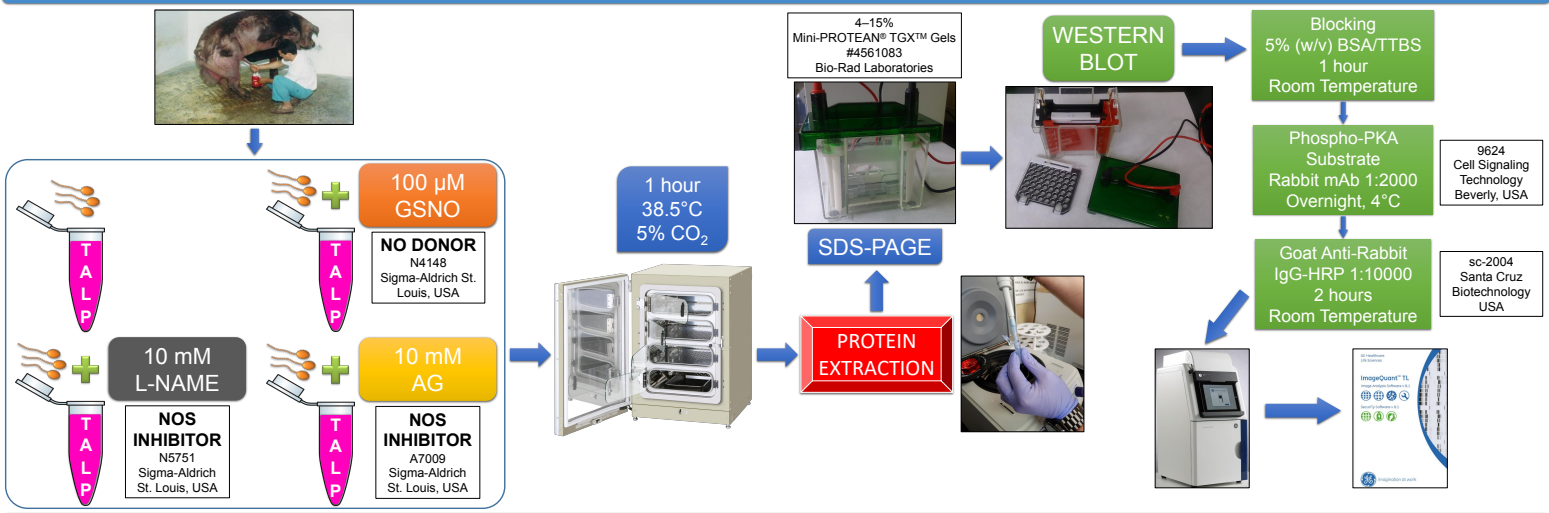



**Figure 1.** Representation of some of the mechanisms through which NO regulates sperm capacitation.

## MATERIALS AND METHODS




## RESULTS

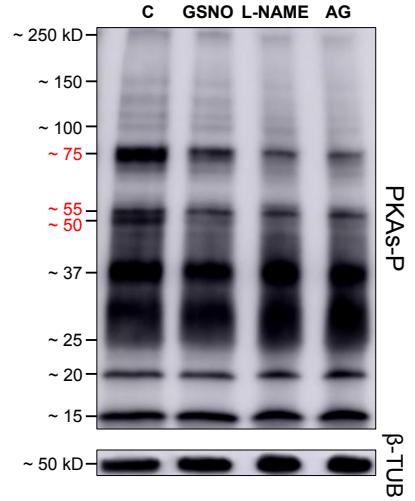



**Figure 2. Effect of GSNO, L-NAME and AG on PKA substrates phosphorylation (PKAs-P).** Phospho-PKA substrates were probed with an anti-phospho-PKA antibody.

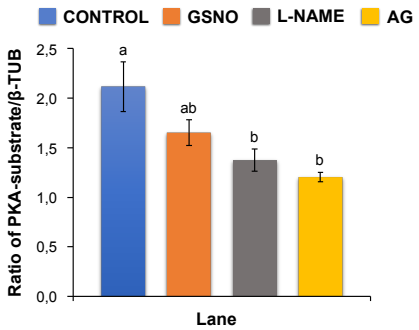



**Figure 3. Relative optical density of PKA substrates when considering the whole lane.** Data are shown as mean ± SEM. Different letters (a,b) indicate significant statistical differences between groups ($p < 0.05$). One-way ANOVA and Tukey's multiple comparisons test were performed.

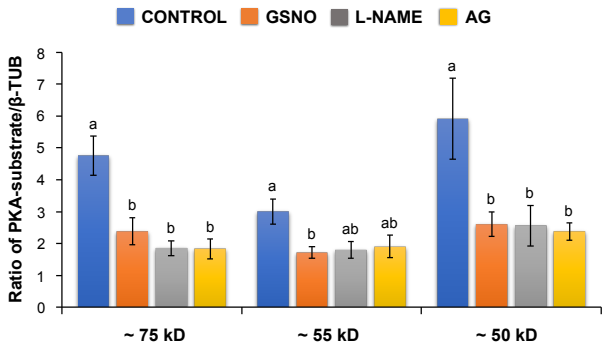



**Figure 4. Relative optical density of PKA substrates (~75 kD, ~55 kD and ~50 kD).** Data are shown as mean ± SEM. Different letters (a,b) within the same band indicate significant statistical differences between groups ($p < 0.05$). One-way ANOVA and Tukey's multiple comparisons test were performed.

When capacitated in the presence of a NO donor and NOS inhibitors, spermatozoa showed a lower Serine and Threonine phosphorylation pattern than the control (no treatment). This effect was more pronounced in the ~75 kD, ~55 kD and ~50 kD PKA substrates.

## CONCLUSION

This study provides additional evidence that NOS/NO plays a role in regulating the phosphorylation of Serine and Threonine residues during sperm capacitation in porcine, particularly in the ~75 kD, ~55 kD and ~50 kD species, suggesting that these three bands may include key proteins in modulating PKA-dependent events downstream of NO-mediated signaling.

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