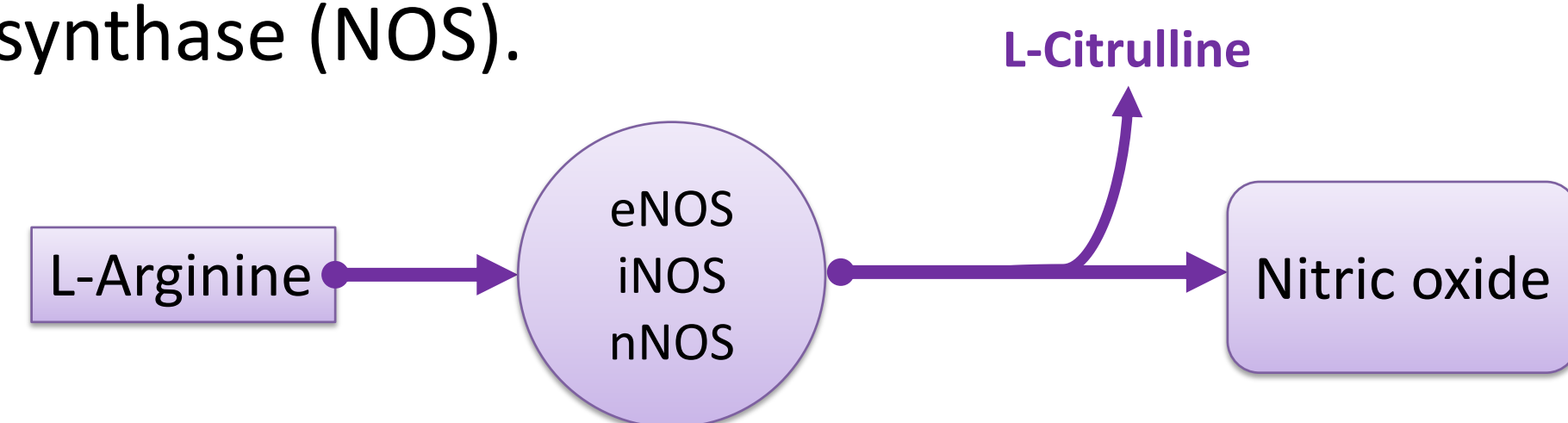


# Identification of intracellular nitric oxide synthase isoforms in ram sperm

Calvo, S.; Carvajal M.; Miguel-Jiménez, S.; Casao, A.; Cebrián-Pérez, J.A.; Muiño-Blanco, T. and Pérez-Pé, R.  
Department of Biochemistry and Molecular and Cellular Biology. Faculty of Veterinary, University of Zaragoza  
Environmental Sciences Institute of the University of Zaragoza (IUCA), Spain

## INTRODUCTION

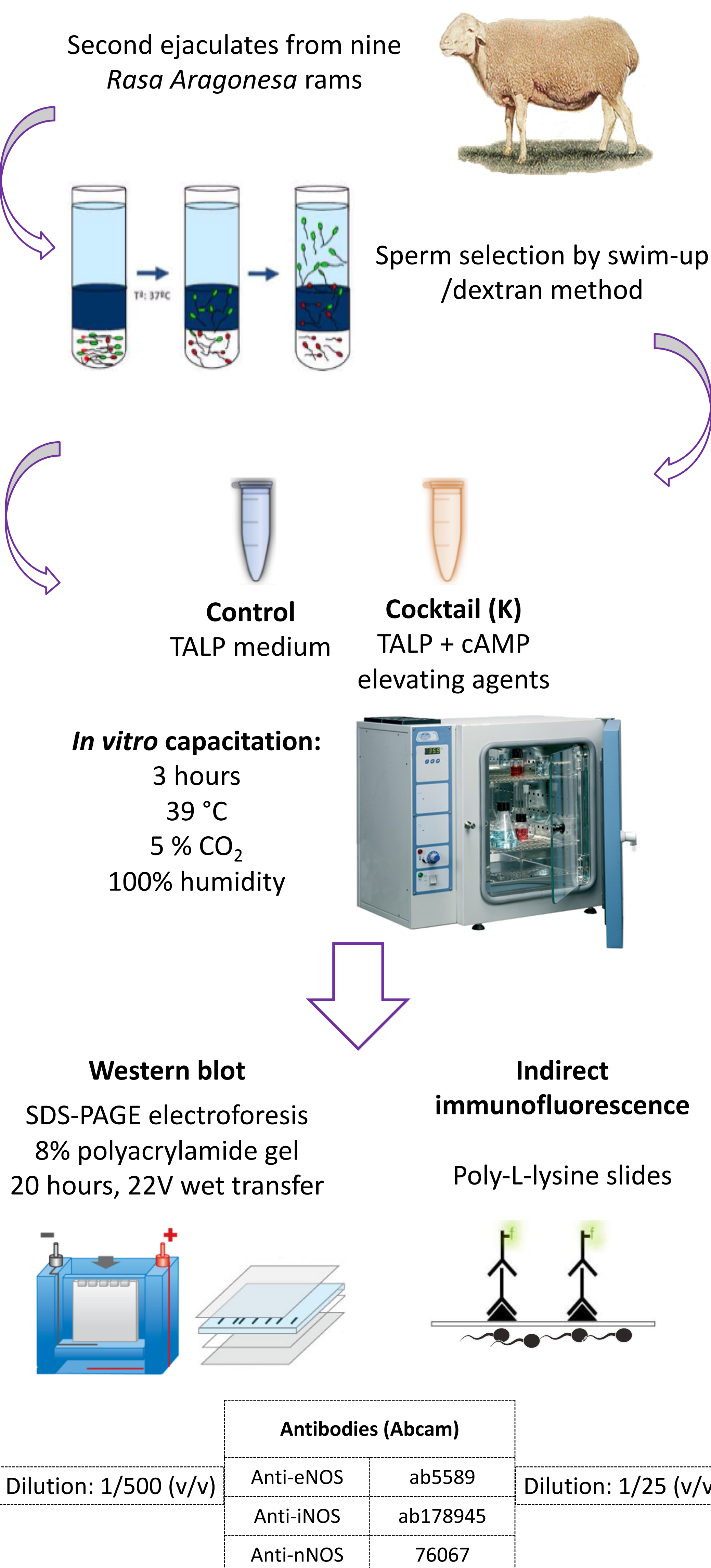
Nitric oxide (NO) plays a fundamental role in sperm functionality. It is synthesized by nitric oxide synthase (NOS).



Three isoforms of NOS (endothelial (eNOS), inducible (iNOS) and neuronal (nNOS)) have been identified in somatic cells and in the spermatozoa of several species. Also, it has been detected an increment in NOS activity during *in vitro* sperm capacitation.

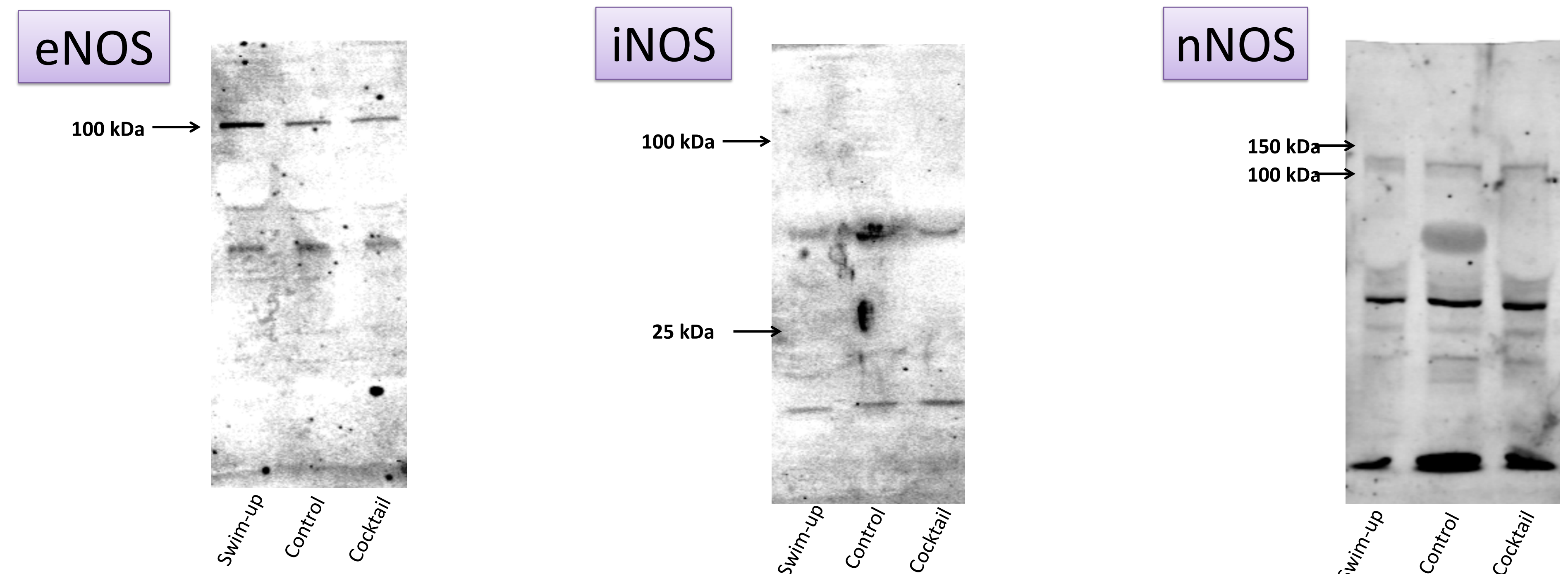
Thus, the objective of this work was to determine the presence and localization of the NOS isoforms in ram spermatozoa and their possible changes during *in vitro* capacitation.

## MATERIALS AND METHODS



## RESULTS

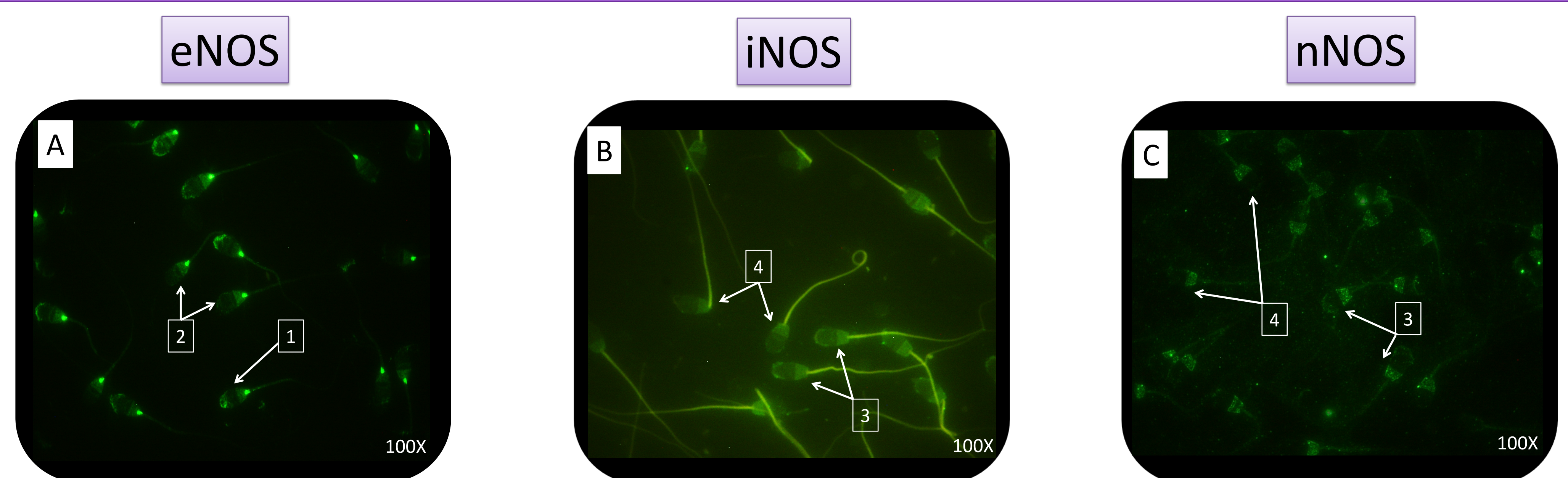
### Western blot



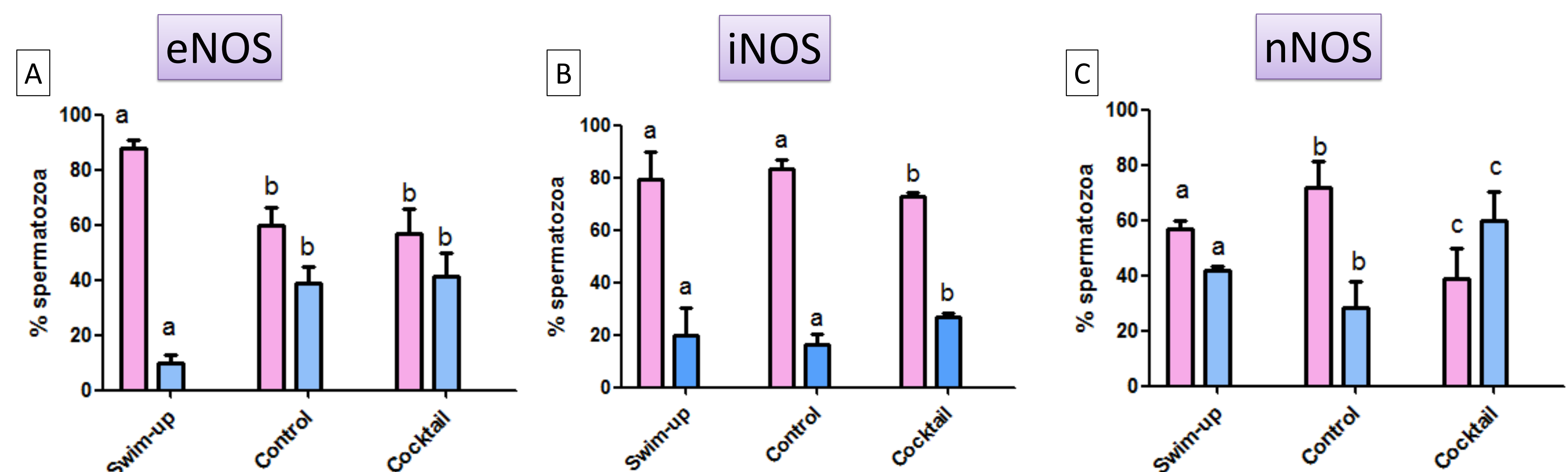
**Fig. 1:** Identification of nitric oxide synthase isoforms eNOS, iNOS and nNOS by Western Blot in swim-up selected and *in vitro* capacitated (incubated in capacitating conditions in TALP without (control) or with cAMP elevating agents (cocktail)) ram sperm proteins.

The antibody against the eNOS revealed a 100 kDa band, and the antibody against nNOS recognized proteins of approximately 120 kDa. No bands corresponding to iNOS isoform were detected.

### Indirect immunofluorescence



**Fig. 2:** Identification by indirect immunofluorescence of nitric oxide synthase isoforms eNOS (A), iNOS (B) and nNOS (C) evaluated by fluorescence microscopy. Two immunotypes can be seen in each isoform: labelling in post-acrosomal region + neck + apical edge (1) or post-acrosomal region + neck (2) in eNOS; post-acrosomal region + apical edge (3) or post-acrosomal region (4) in iNOS and nNOS.



**Fig. 3:** Percentages of the immunotypes for eNOS (A), iNOS (B) and nNOS (C), detected by indirect immunofluorescence in swim-up selected and incubated in capacitating conditions in TALP without (control) or with cAMP elevating agents (cocktail) in ram spermatozoa. Pink bars represent immunotype 1 for eNOS (Fig. 1A) and immunotype 3 for iNOS and nNOS (Fig. 1B and C), whereas blue bars represent immunotype 2 for eNOS (Fig. 1A) and immunotype 4 for iNOS and nNOS (Fig. 1B and C). Results are shown as mean  $\pm$  SEM (A: n = 6; B, C: n = 3). Different letters (a-b) indicate statistical differences between treatments ( $p < 0.05$ ).

## CONCLUSIONS

- Nitric oxide synthase isoforms, eNOS, iNOS and nNOS, are present in ram spermatozoa.
- The changes in immunotypes percentages after *in vitro* capacitation detected by indirect immunofluorescence in eNOS and nNOS suggests a possible role of these isoforms in ram sperm capacitation.