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Characterization of the receptors of the endocannabinoid system in equine sperm: *Possible role of anandamide in sperm function*

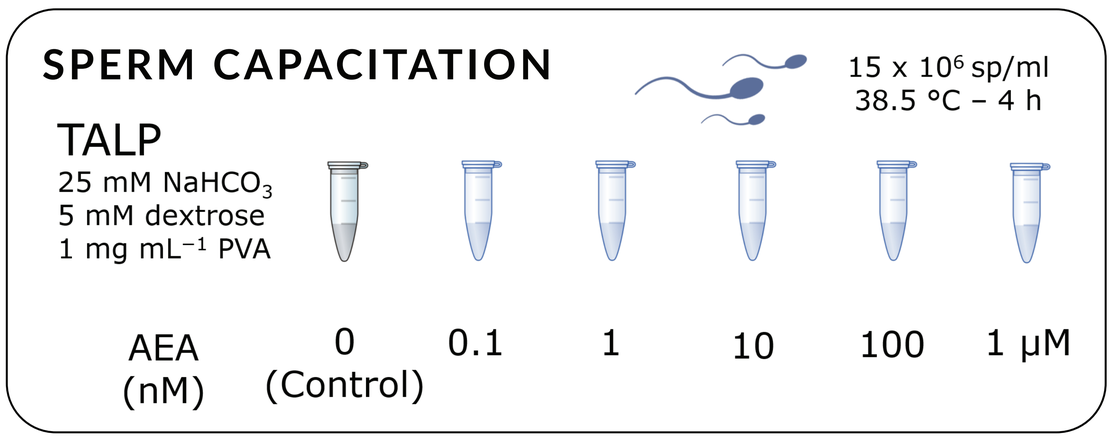
C. Arroyo-Salvo*, R. Lottero, A. Gambini,[1] S. Perez Martinez;
Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina
Producción Equina, Facultad de Agronomía, Universidad de Buenos Aires, Argentina[1]

INTRODUCTION

Conventional IVF in horses remains challenging. In particular, stallion sperm fails to penetrate the zona pellucida, possibly due to incomplete in vitro sperm capacitation. Therefore, there is a need to elucidate, in horses, molecules with a proven role during capacitation in other mammals. Our laboratory has reported that anandamide (AEA), an endocannabinoid present in follicular and oviducal fluids, induced capacitation-associated events in bovine and murine sperm. The aims of this work were to characterize the localization of cannabinoid receptors in equine sperm and to evaluate the effects of AEA on levels of tyrosine-phosphorylated proteins (pY) and substrates phosphorylated by protein kinase A (pPKA).

PROCEDURES

- Cryopreserved sperm were thawed and selected by glass wool columns.
- Cannabinoid receptors (CB1, CB2, TRPV1) and pPKA and pY were localised in sperm by indirect immunofluorescence.



- Western blot was used to determine levels of pY and pPKA in 4.5×10^6 sperm.

RESULTS

1. Immunolocalization of endocannabinoid system receptors

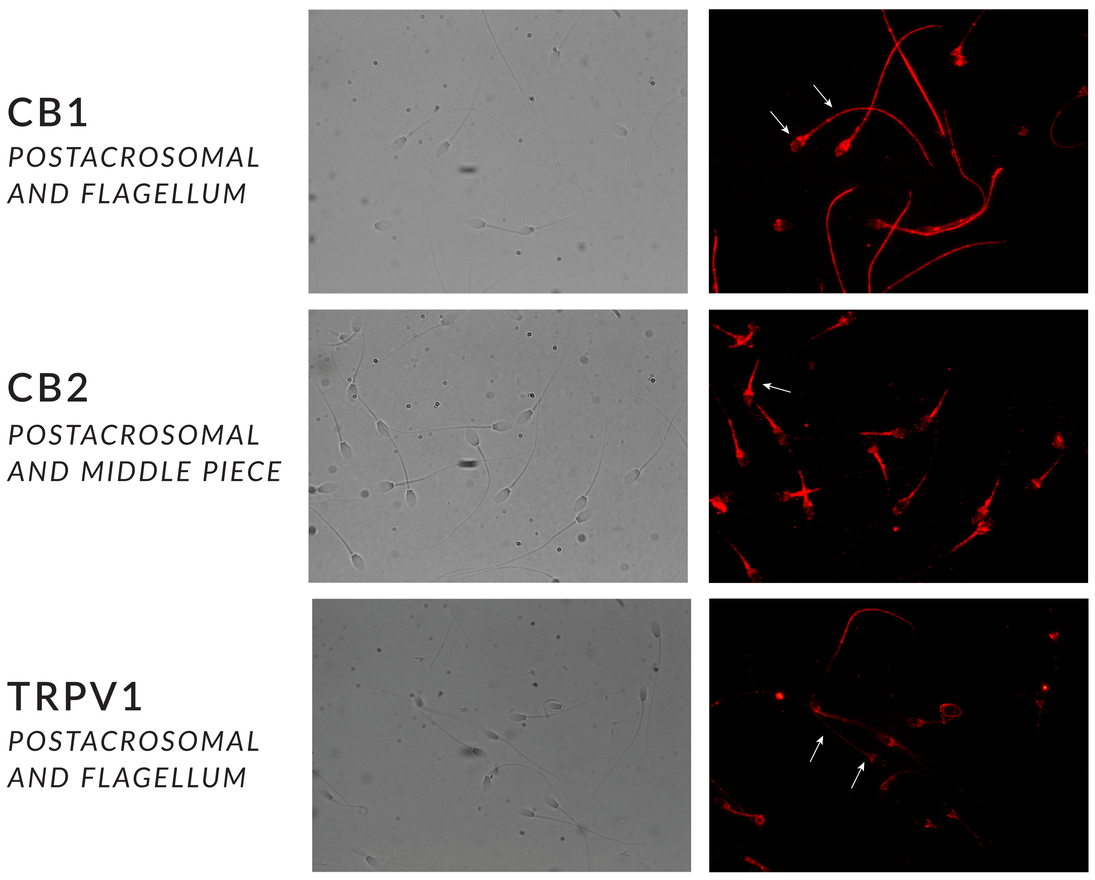


Fig.1. Thawed cryopreserved equine sperm were selected by glass wool columns and incubated with primary CB1 (1:50), CB2 (1:200) or TRPV1 (1:200) antibodies. Afterwards, samples were treated with Alexa555-conjugated goat anti-rabbit IgG (1:500). Arrows indicate receptors localization.

2. AEA (1 nM) induced an increase in PKA activity

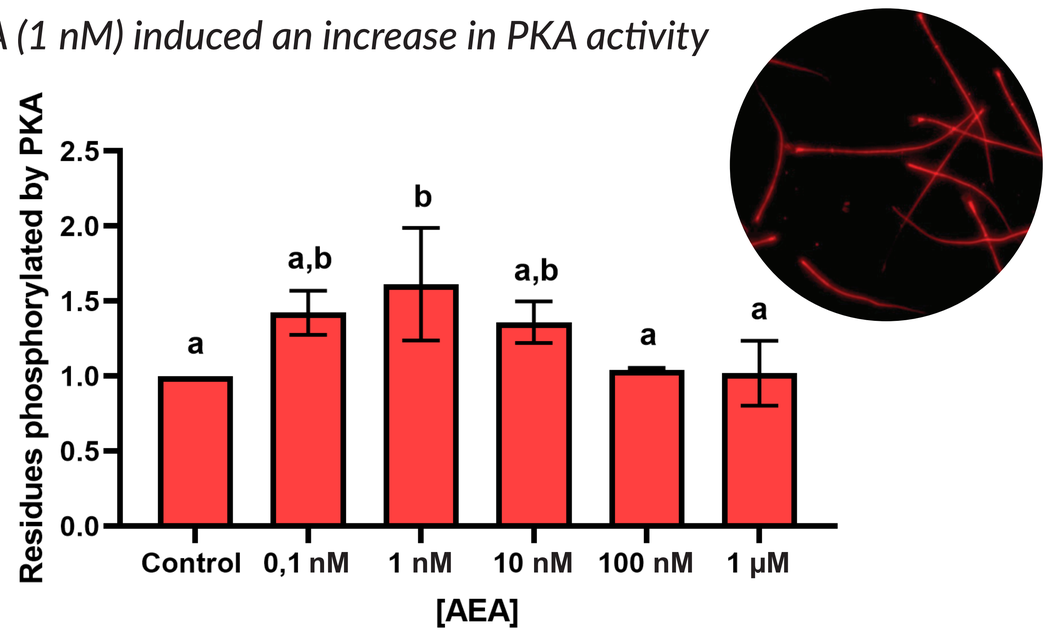


Fig. 2. Substrates phosphorylated by protein kinase A (pPKA) levels were measured in sperm incubated for 4h with 0, 0.1, 1, 10, 100 nM and 1 µM of AEA. Western blot was performed incubating with α-pPKA and the corresponding HRP-conjugated secondary antibodies. Densitometry of pPKA, relativized to control is shown. Data are expressed as mean ± SEM. Letters indicate significant differences ($p<0.05$). Immunolocalization of pPKA (1:250) is shown.

3. AEA had no effect in tyrosine phosphorylation levels

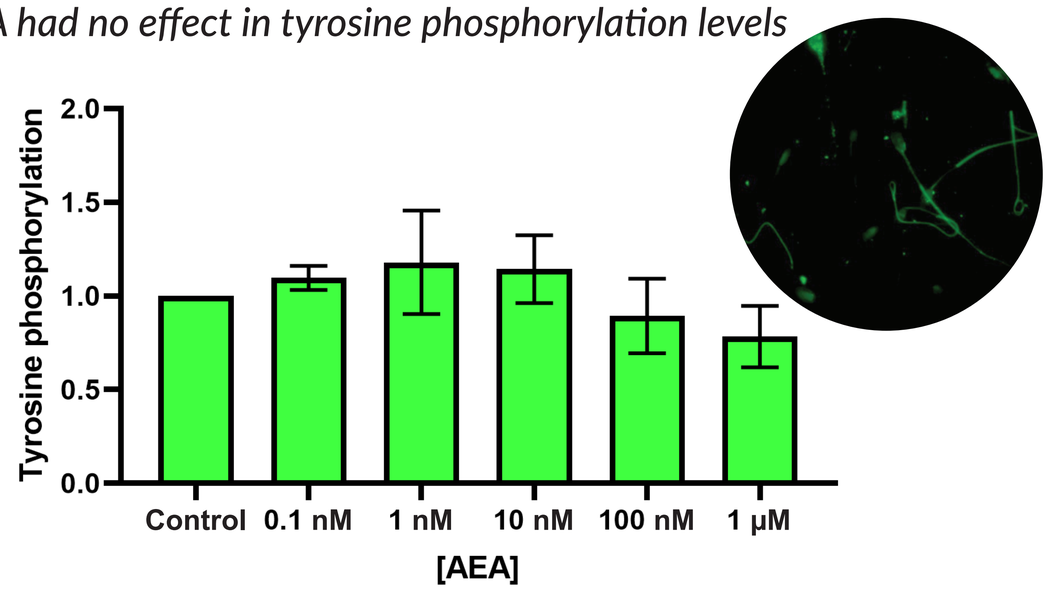


Fig. 3. Tyrosine phosphorylation (pY) levels were measured in sperm incubated for 4h with 0, 0.1, 1, 10, 100 nM and 1 µM of AEA. Western blot was performed incubating with α-pY and the corresponding HRP-conjugated secondary antibodies. Densitometry of pY, relativized to control is shown. No significant differences were observed. Immunolocalization of pY (1:250) is shown.

CONCLUSION

Cannabinoid receptors were present in equine sperm, and the incubation with AEA induced an increase in PKA activity, an essential event associated with sperm capacitation.