Instituto de Investigación en Recursos Cinegéticos
CSIC - UCLM - JCCM
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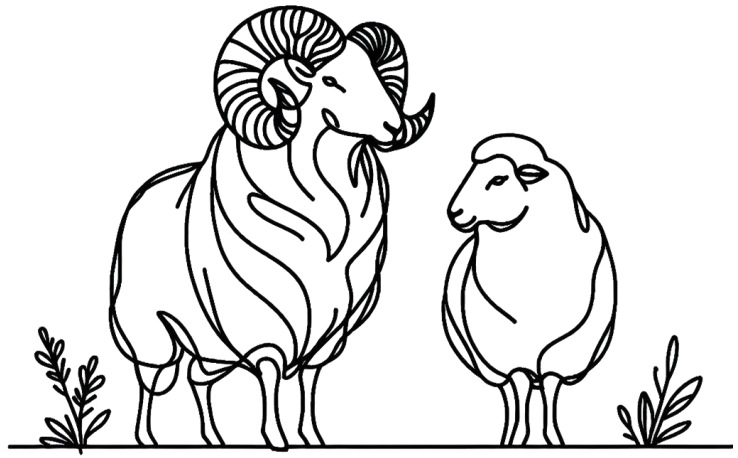
Sanidad y Biotecnología
Health and Biotechnology

UCLM Universidad de Castilla-La Mancha

Use of H_2S to improve ram sperm cryopreservation: preliminary results



The 3rd CZU Prague hybrid seminar

Biotechnology in small ruminant reproduction:

an international experience

Czech University of Life Sciences Prague

avantor delivered by VWR

María de Sousa Blanco, PhD Student

maria.sousa@uclm.es

María de Sousa Blanco

@mariaasou_

1. INTRODUCTION

2. AIM

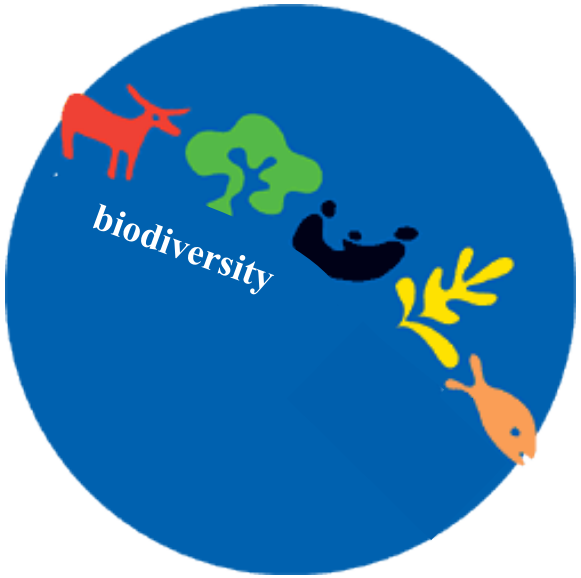
3. MATERIALS & METHODS

4. RESULTS

5. CONCLUSIONS

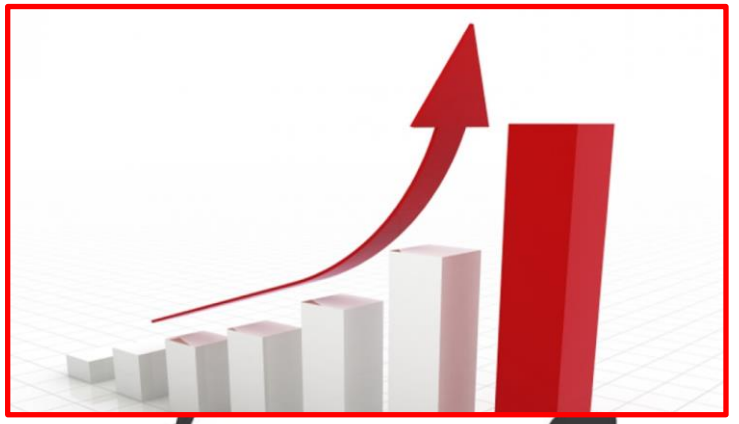
INTRODUCTION

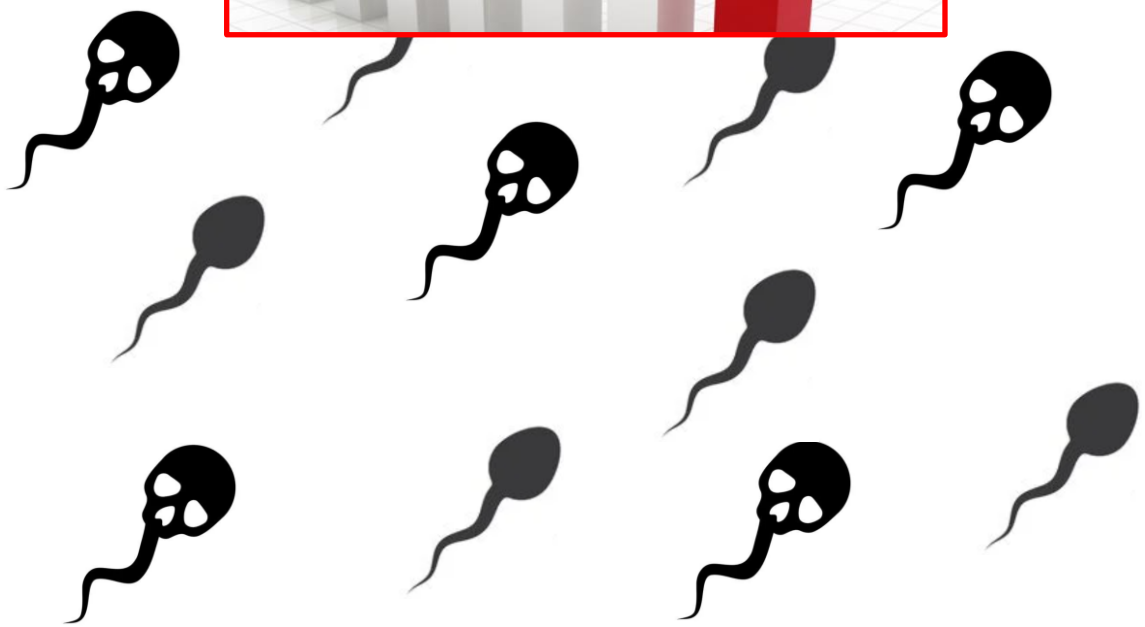
CRYOPRESERVATION



INTRODUCTION

CRYOPRESERVATION





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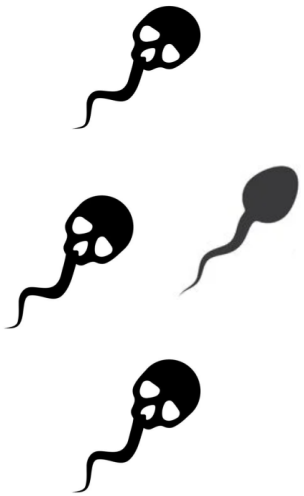
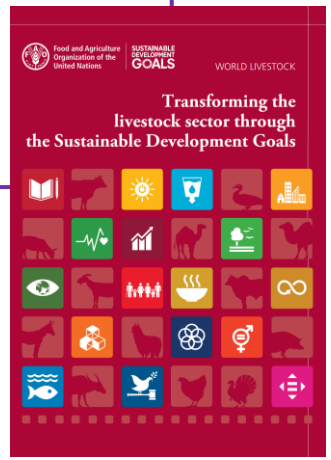
CRYOPRESERVATION



INTRODUCTION



INTRODUCTION

	Animals	Forages and feed crops	Labour force and capital
Variability in rainfall	Shortages of drinking and servicing water Diseases - Increased pathogens parasites and vectors - Changed distribution and transmission - New diseases	Decreased yields Decreased forage quality Changes in pasture composition (species, communities) Changes in production systems (e.g. from mixed crop–livestock to rangelands)	Altered human health and resource allocation to livestock Decreased productivity Migration Conflict for resources
Temperature	Heat stress - Decreased feed intake and livestock yields - Decreased conception rates - Altered metabolism and increased mortality Diseases - Increased pathogens, parasites and vectors - Decreased resistance of livestock - New diseases	Decreased yields Decreased forage quality Changes in pasture composition	
CO_2 in the atmosphere	Domestic biodiversity loss	Partial stomata closure and reduced transpiration Change in pasture composition	

A range of climate change adaption solutions exist for livestock production

Animals	Forages and feed crops	Labour force and capital
Water management (e.g. boreholes) Breed for resistance to drought, heat and harsh environments Shifts in species, breeds and/or production systems (e.g. small ruminants, poultry) Disease control and animal health Cooling (indoor systems) or provide shade (e.g. trees)	Irrigation Purchase feed Breed feed crops and forage resistance to drought and heat Changes in cropping calendar Agroforestry Increase mobility for resources	On and off farm diversification Insurance Reconversion (in the context of national/ regional production zoning) Insitutional changes (e.g. trade conflict resolution, income stabilisation programs)

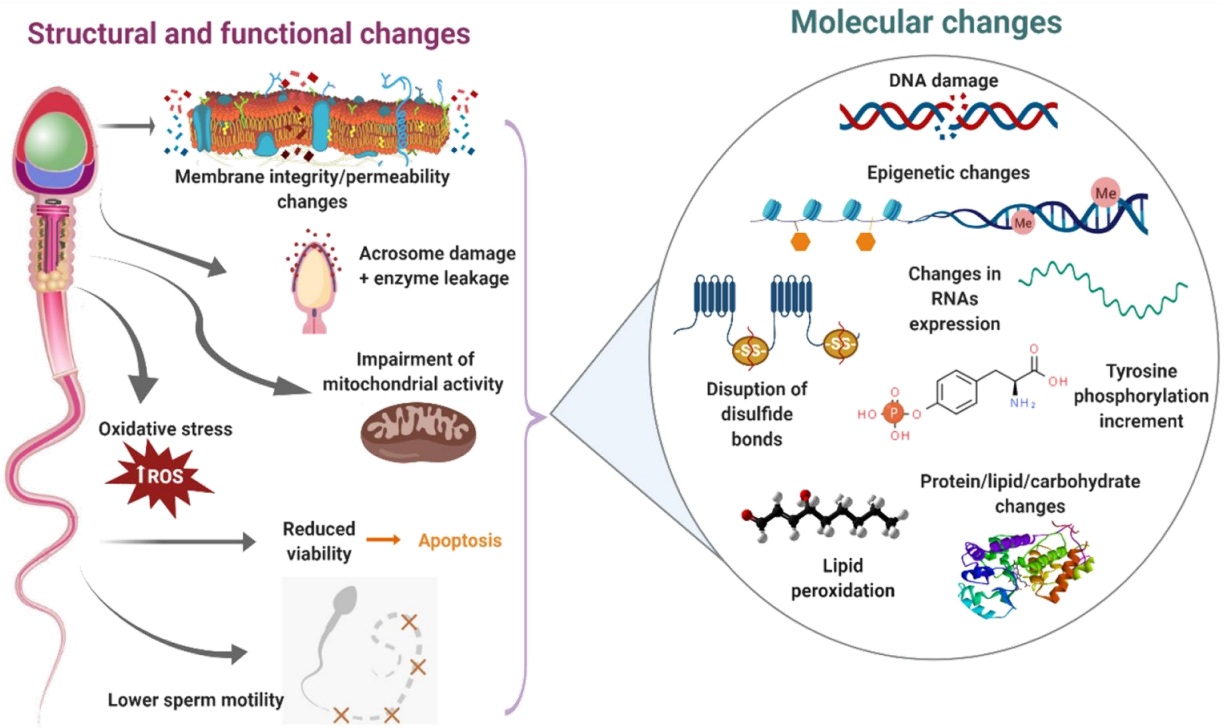
Source: Adapted from Thornton *et al.*, 2009; IUCN, 2010; Niang *et al.*, 2014.

SAFETY AND NUTRITION

Animals	Forages and feed crops	Labour force and capital
Water management (e.g. boreholes) Breed for resistance to drought heat and harsh environments Shifts in species, breeds and/or production systems (e.g. small ruminants, poultry) Disease control and animal health Cooling (indoor systems) or provide shade (e.g. trees)	Irrigation Purchase feed Breed feed crops and forage resistance to drought and heat Changes in cropping calendar Agroforestry Increase mobility for resources	On and off farm diversification Insurance Reconversion (in the context of national/ regional production zoning) Insitutional changes (e.g. trade conflict resolution, income stabilisation programs)

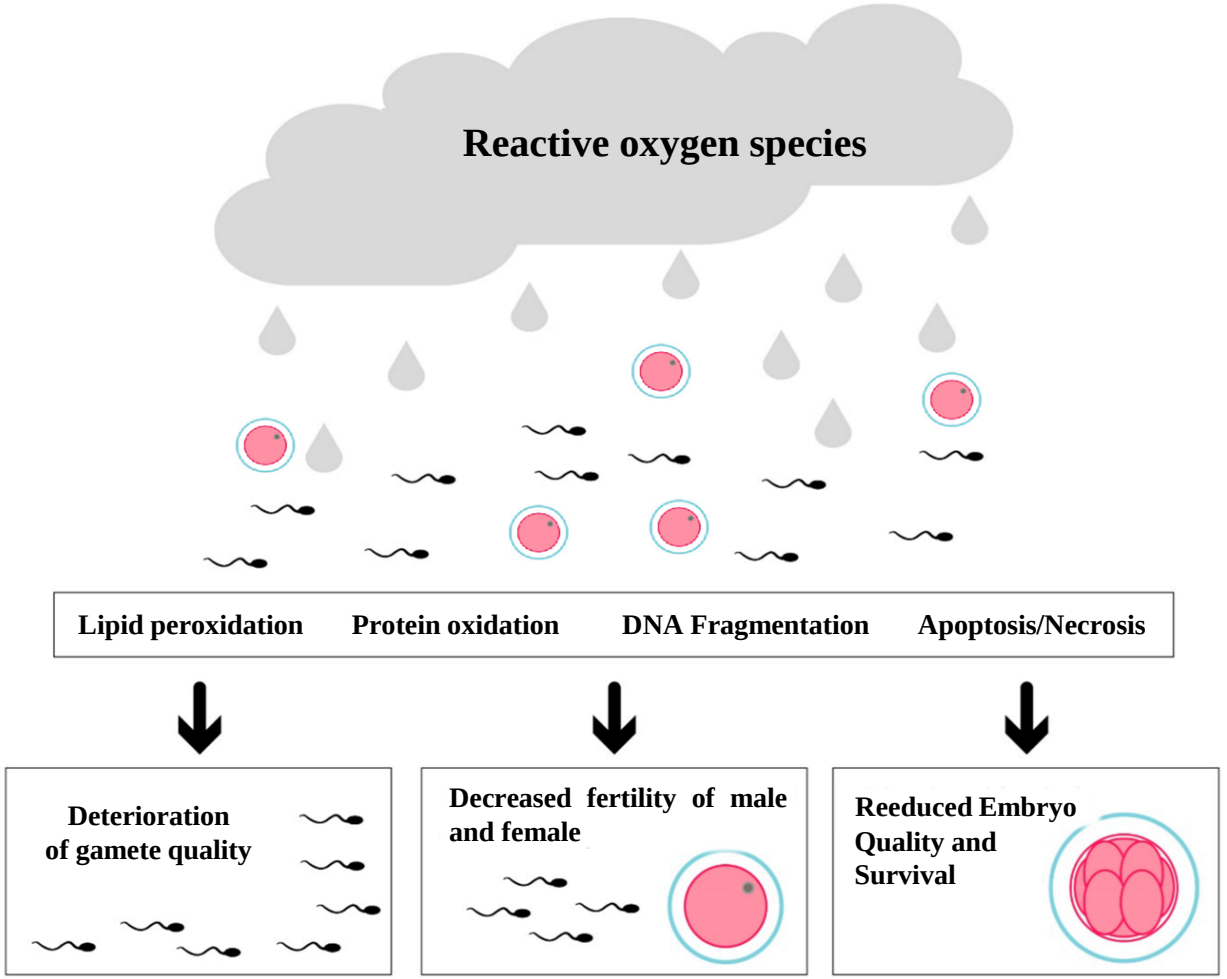
Food and Agriculture Organization of the United Nations

CRYOPRESERVATION



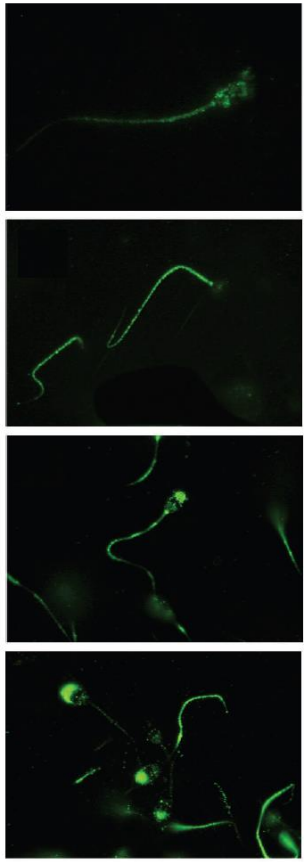
Peris-Frau *et al.* 2020

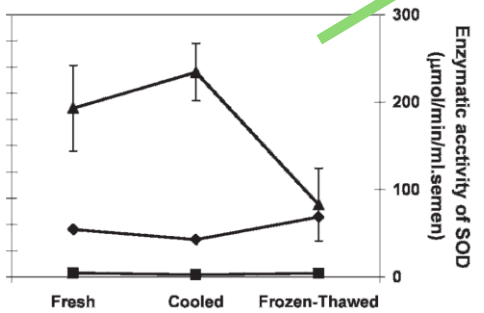
CRYOPRESERVATION



Pintus *et al.* 2021

CRYOPRESERVATION





Martí *et al.* 2008

INTRODUCTION

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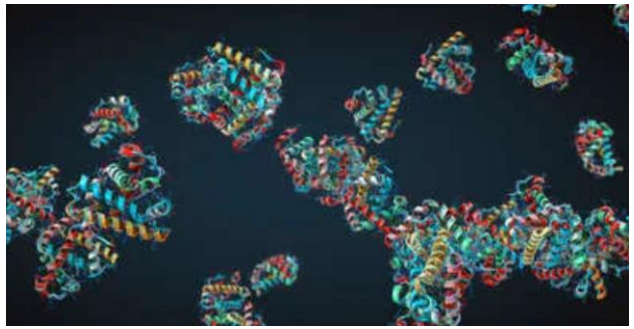
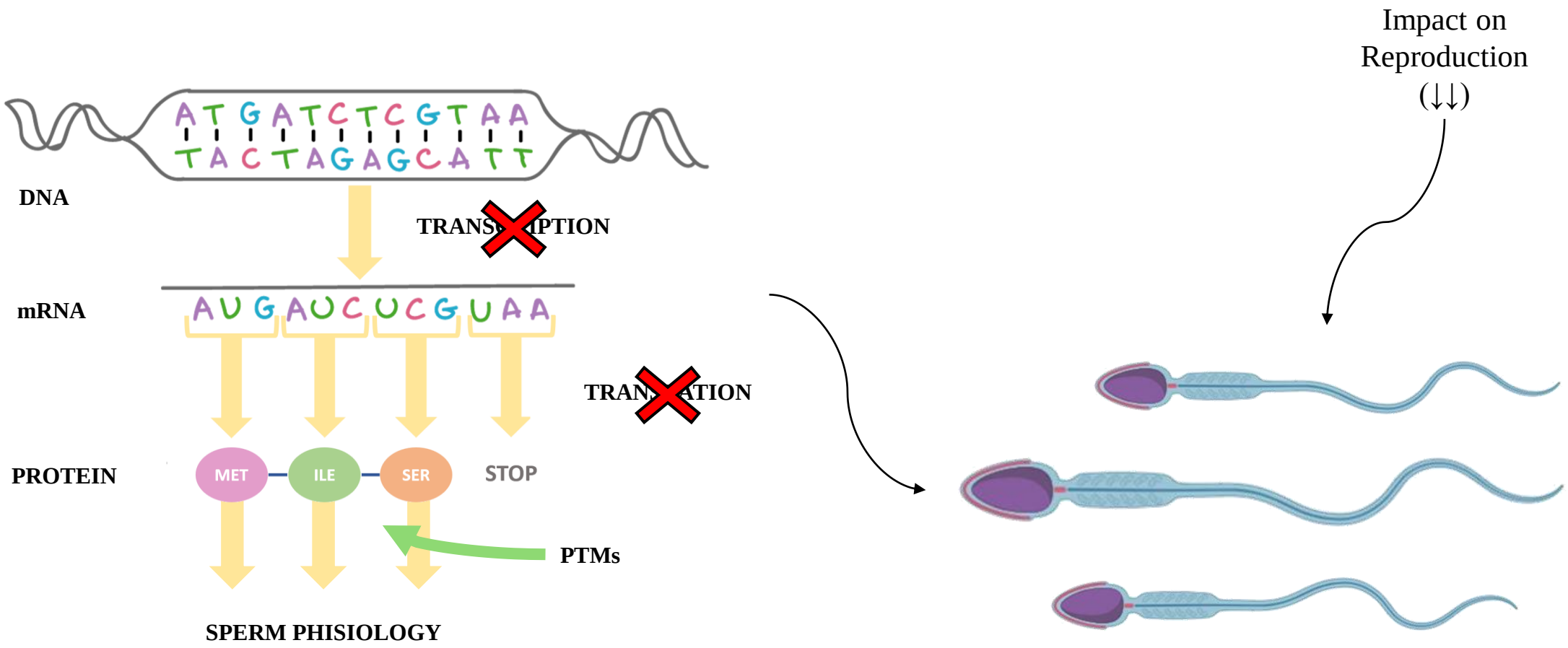
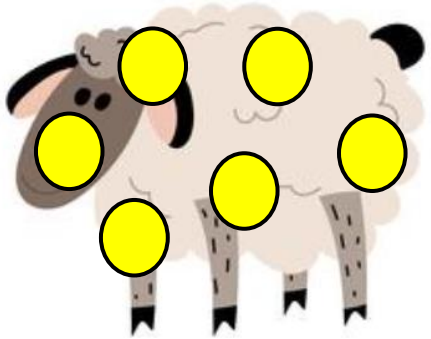
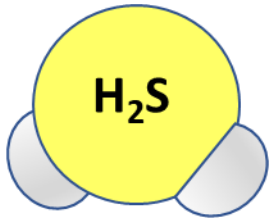


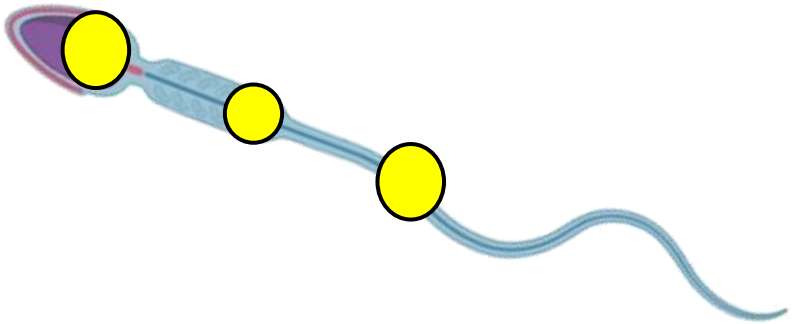
SPERM


Impact on Reproduction (↓↓)
DNA
ATGATCTCGTAA
TACTAGAGCATT
TRANSCRIPTION
mRNA
AUGAUCUCGUAA
TRANSLATION
PROTEIN
MET
ILE
SER
STOP
PTMs
SPERM PHISIOLOGY

HYDROGEN SULPHIDE (H_2S)



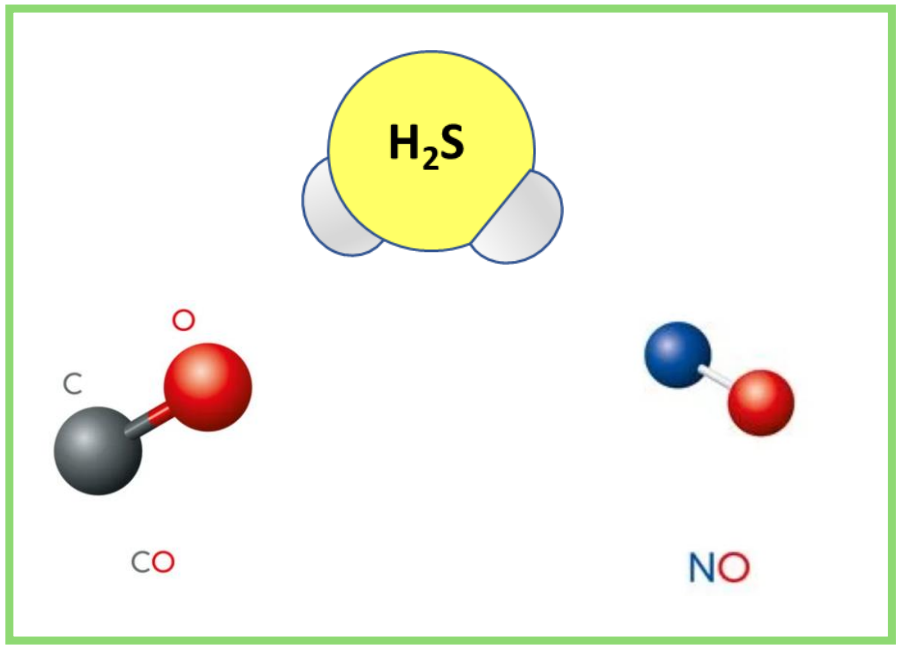




INTRODUCTION

HYDROGEN SULPHIDE (H_2S)

GASOTRANSMITTERS

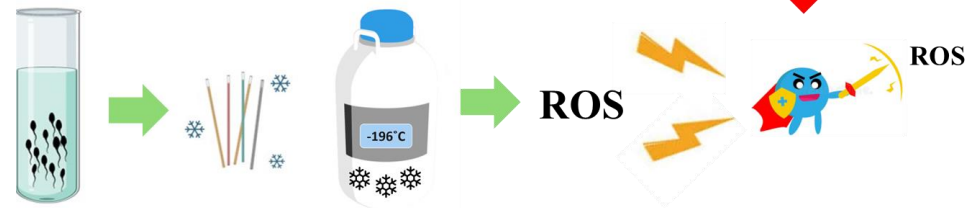


It is a simple compound to apply during the manipulation of sperm and can modulate the antioxidant defense.

It has a short half-life (a maximum of minutes), therefore the changes produced in the proteins are immediate, avoiding prolonged treatments that could compromise the viability of the sperm.

PTM

It is necessary for the persulfidation of the cysteines that make up proteins, which are key pieces in the antioxidant defense



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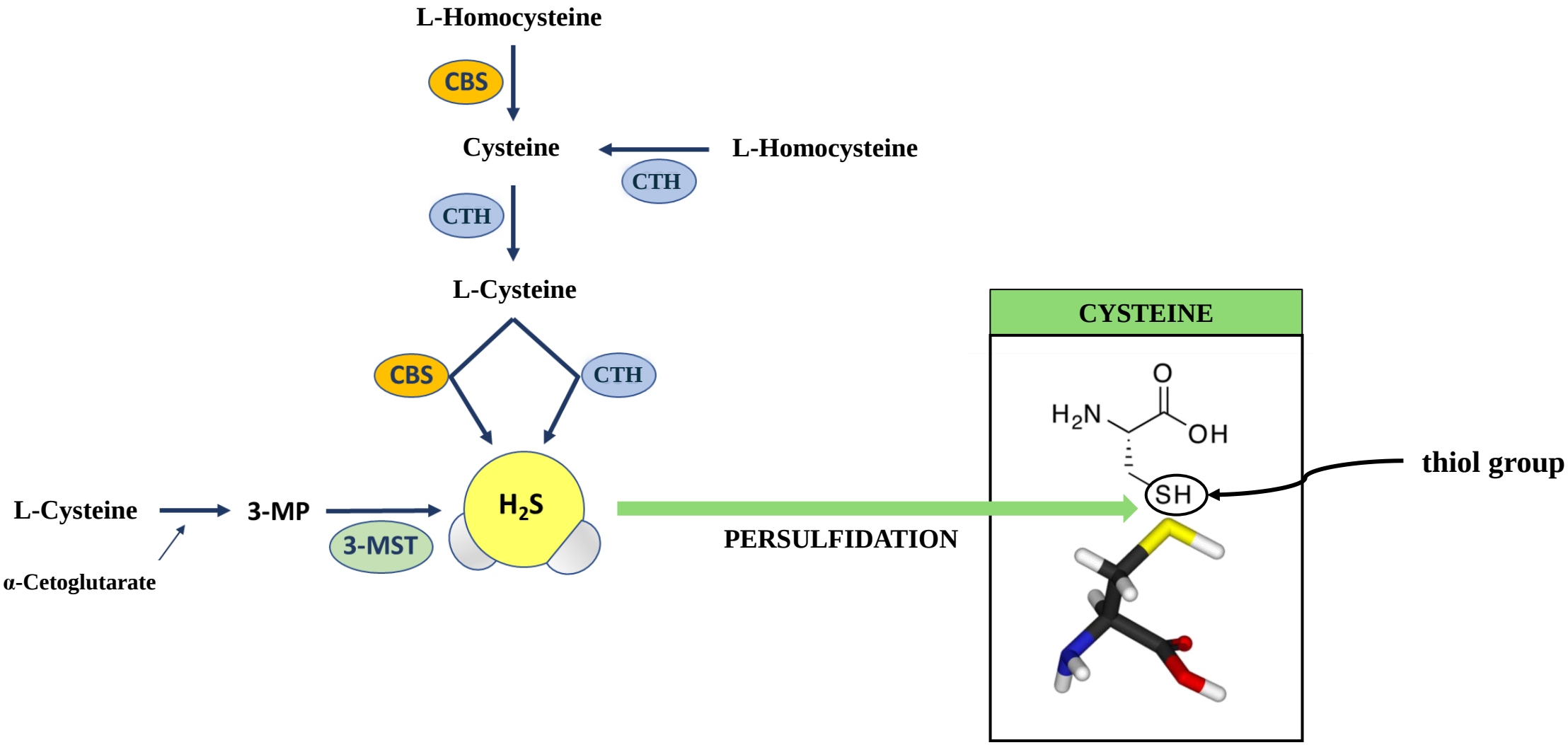

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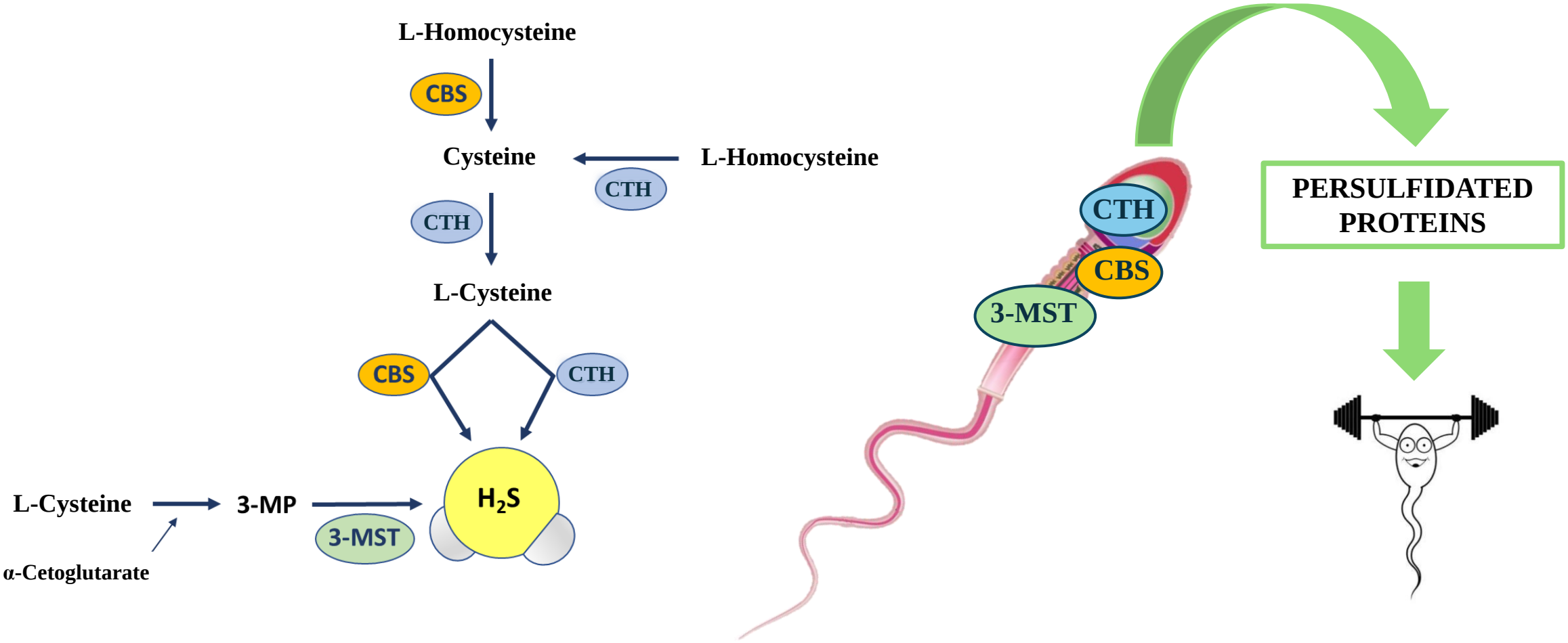

Řimnáčová *et al*. 2022

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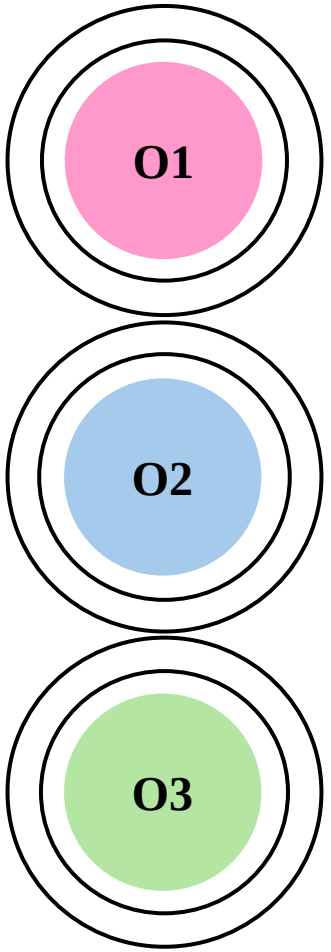
AIM

GENERAL AIM

H_2S plays a protective role against oxidative stress caused by cryopreservation in ram sperm, and its effect is mediated via persulfidation of the enzymes involved in the antioxidant response

3 SPECIFIC AIMS



To characterize the presence and location of H_2S-releasing enzymes (CBS, CTH and MST) in ram testes and spermatozoa.

To assess H_2S supplementation on fresh ram sperm parameters related to *in vitro* fertilization capacity

To assess H_2S supplementation after cryopreservation on ram sperm parameters related to *in vitro* fertilization capacity

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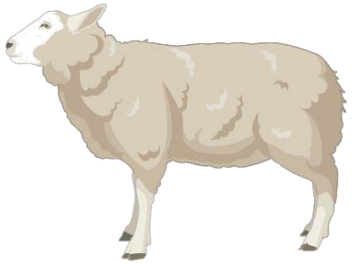
Samples:
testes + sperm samples

Manchego Ram

n = 9



Seminal contrast:
***post-mortem* epididymal origin**

Volume (ml) → Concentration ($x10^6$ spz/ml) → Individual motility → Movement quality

Assays

Testes Samples

Sperm Samples

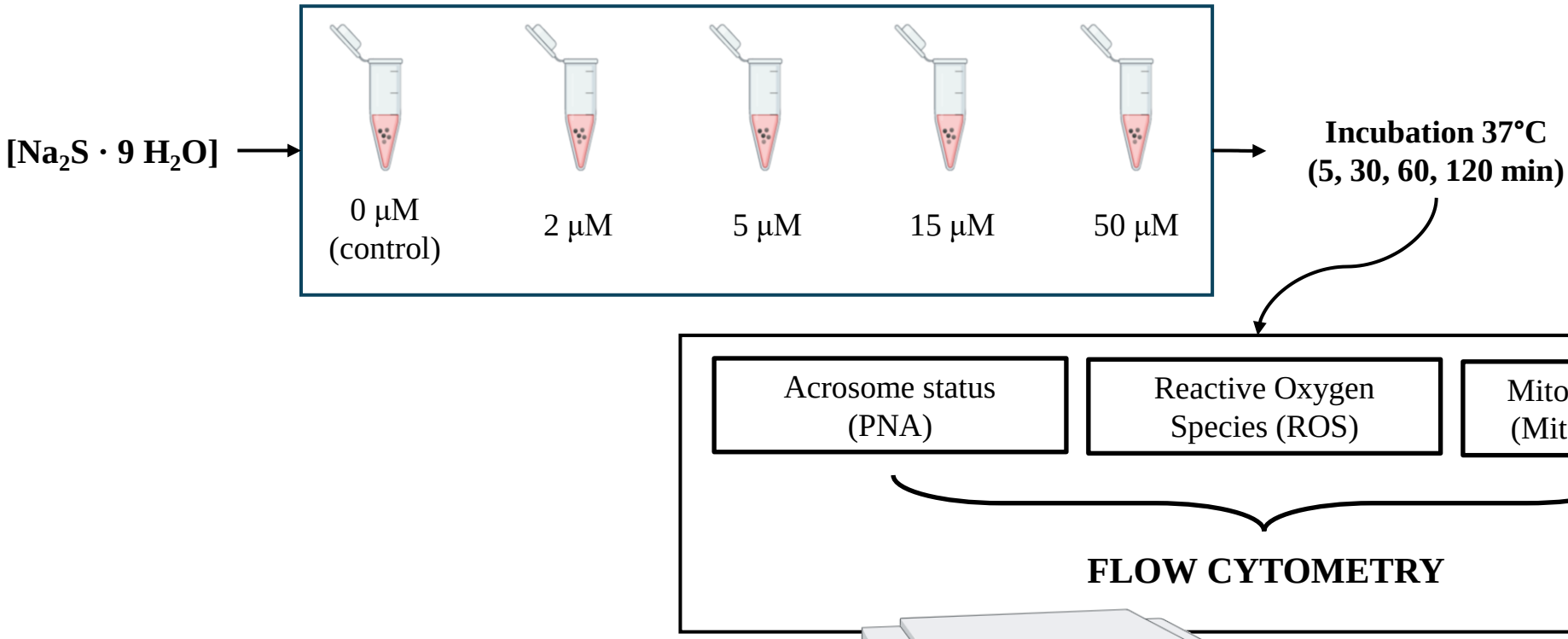
IF
Presence Location → H_2S Releasing Enzymes

IF
Fluorescence Intensity → Diferences Intraspecies

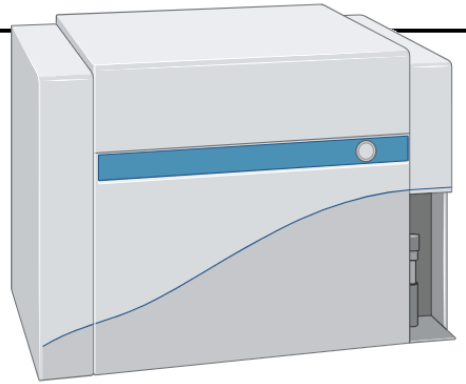
ICC
Presence Location → H_2S Releasing Enzymes

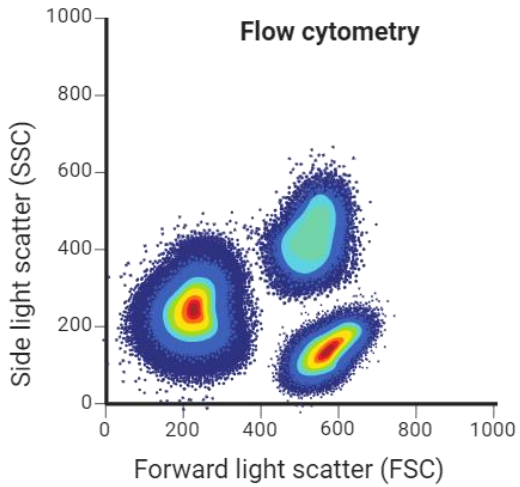
WB
Expression Cuantification → H_2S Releasing Enzymes

H_2S-releasing enzymes characterization



H_2S supplementation fresh sperm



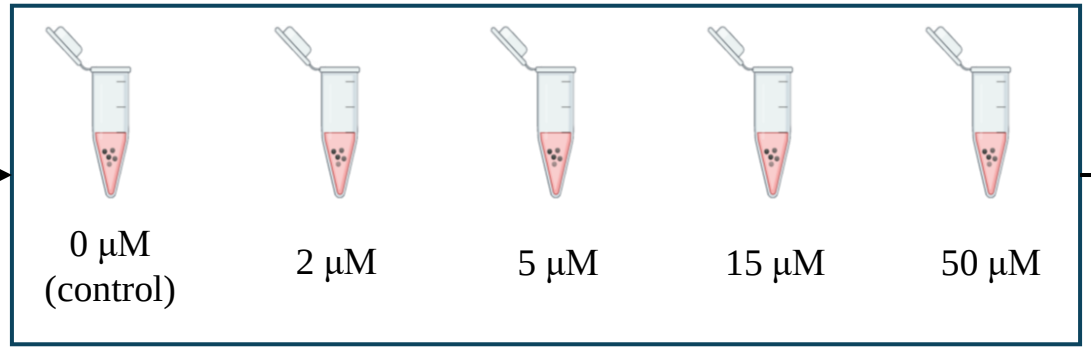


MATERIALS & METHODS

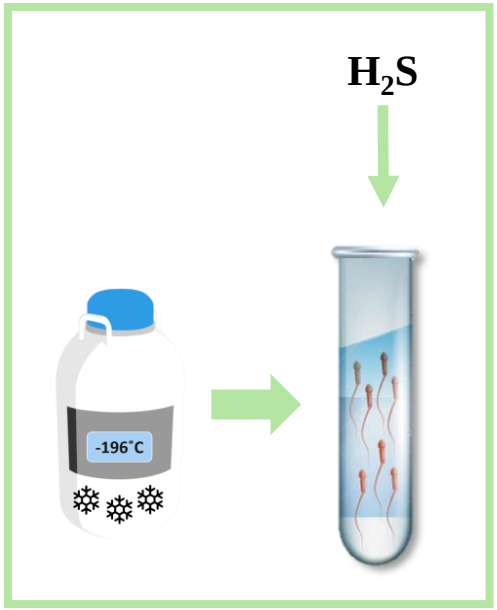
Freezing

Thawing

$[Na_2S \cdot 9\ H_2O]$



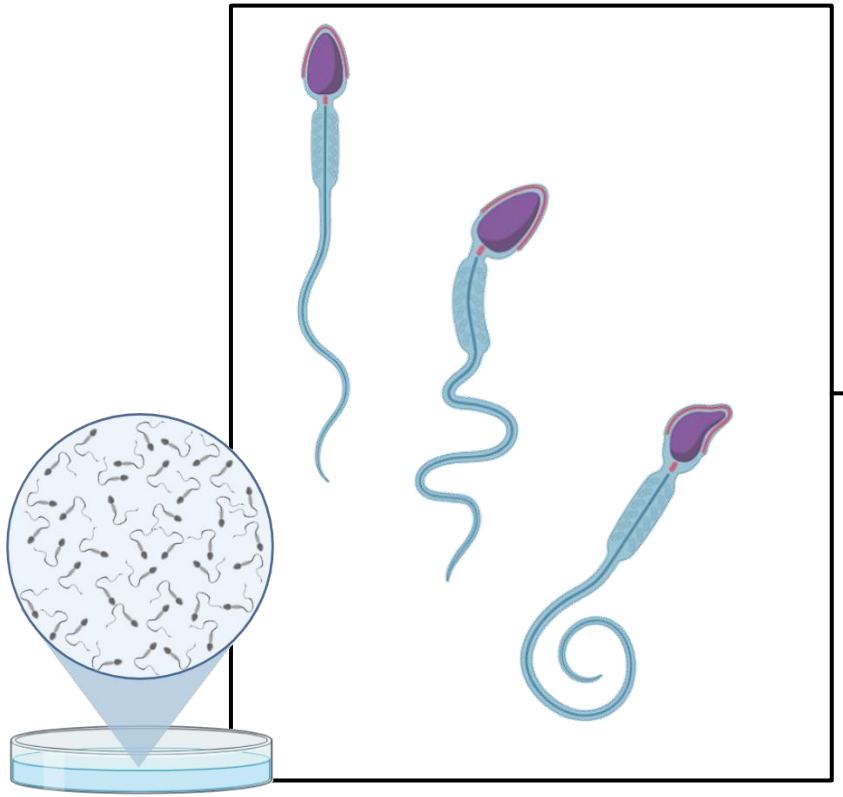
Incubation 37°C
(5, 30, 60, 120 min)

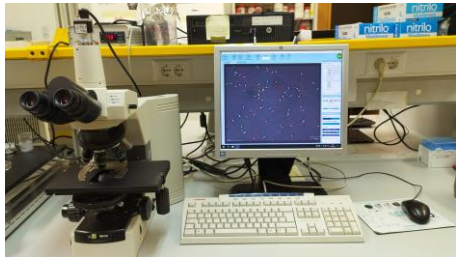


H_2S supplementation

after cryopreservation

Motility parameters





Sperm Subpopulations

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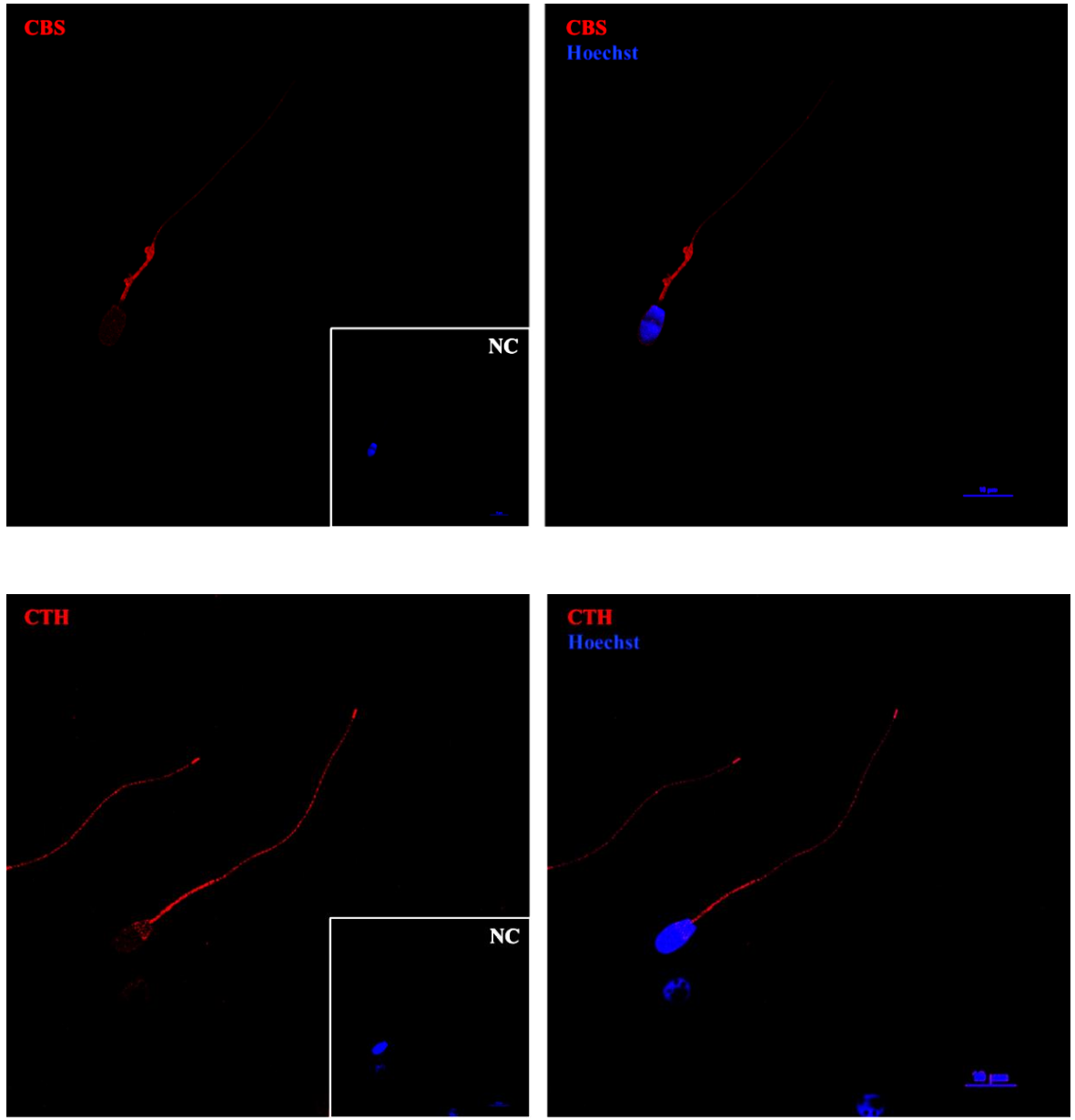
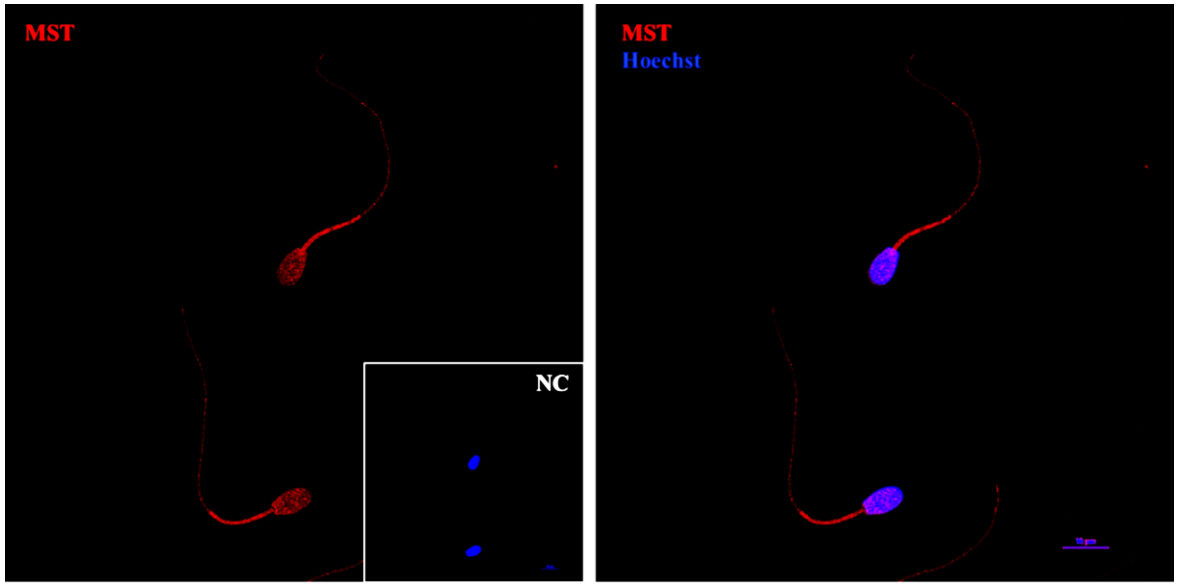
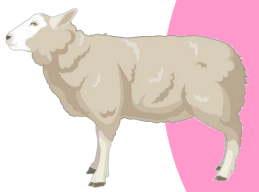
RESULTS

Unpublished data

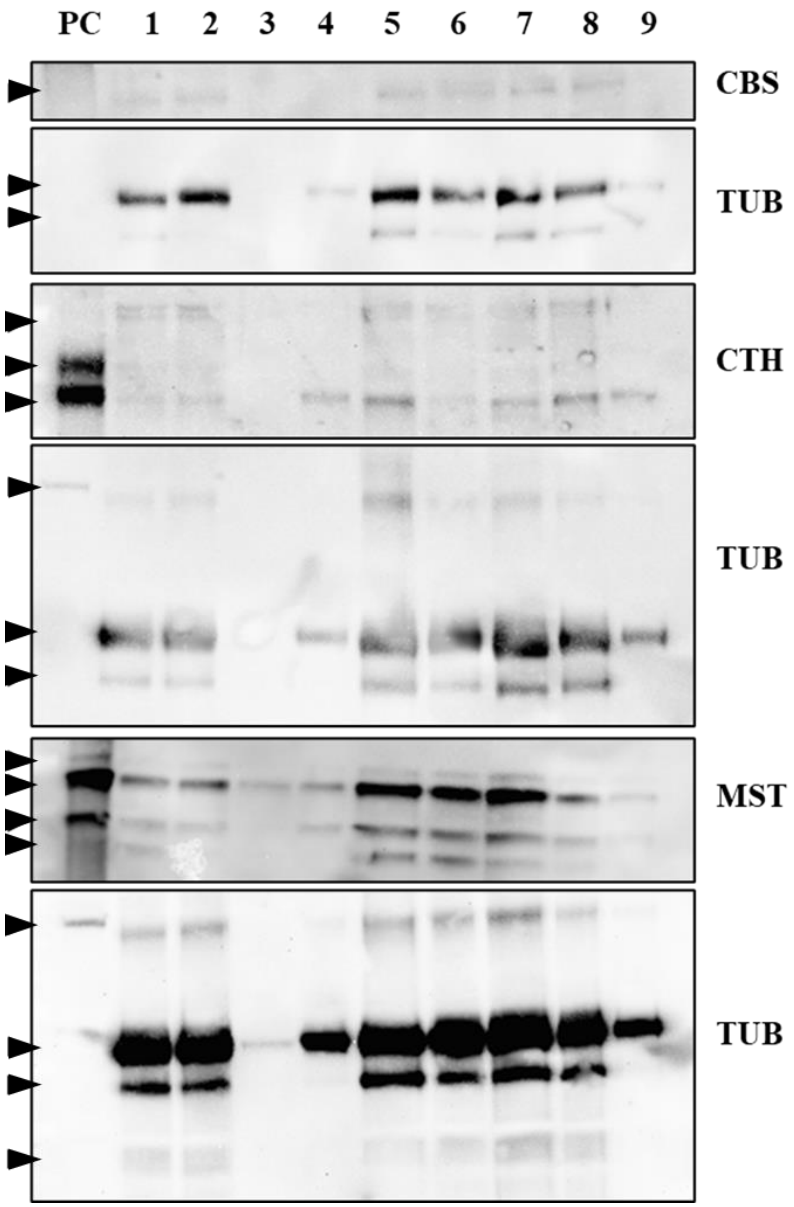




Presence and location of CBS, CTH and MST in ram epididymal sperm.

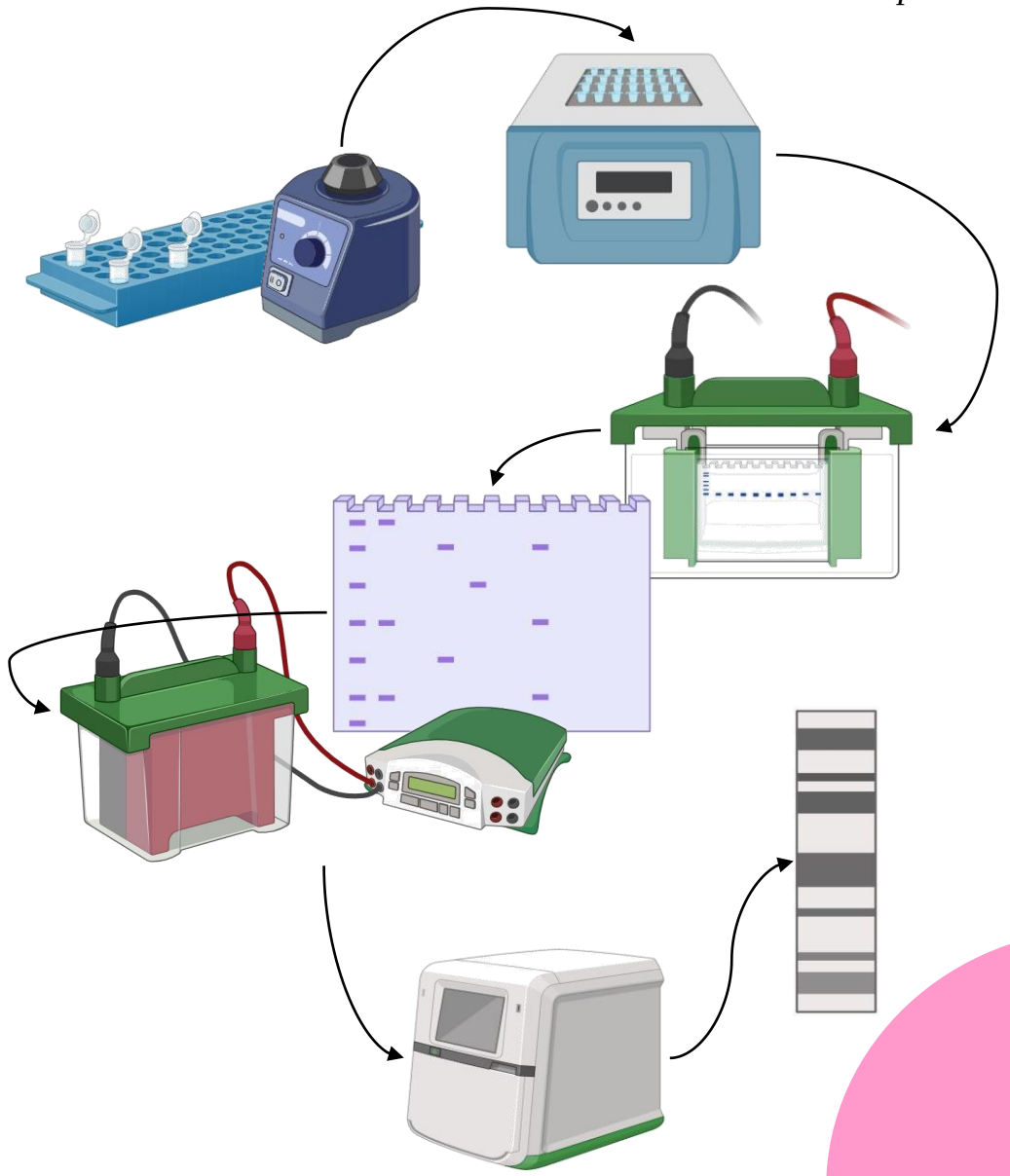


RESULTS



Expression patterns of CBS, CTH and MST enzymes in epididymal sperm from ram sperm samples.

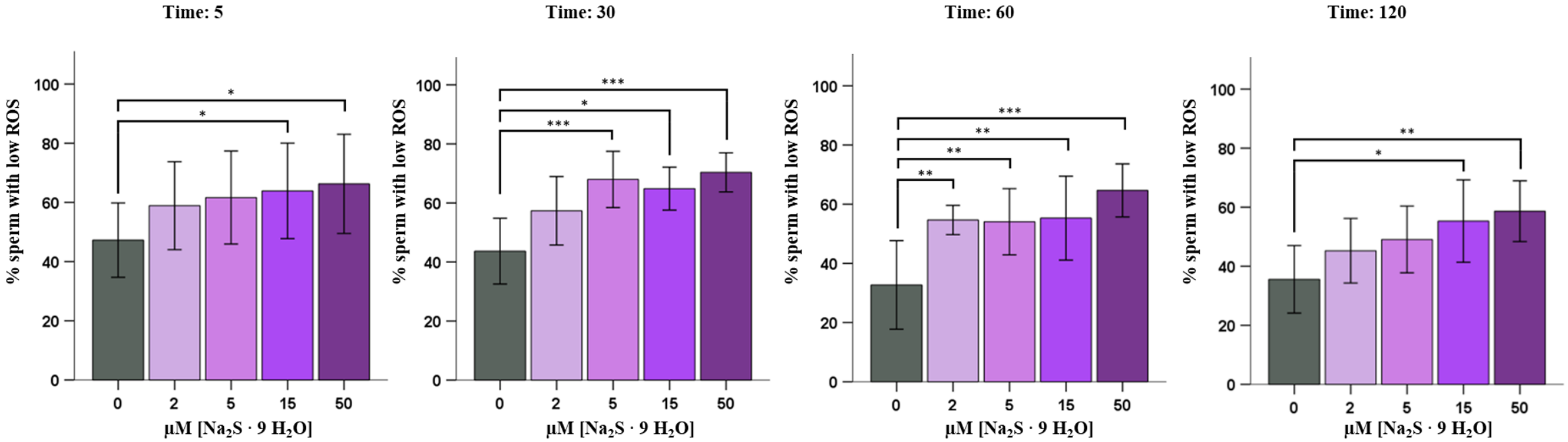
Unpublished data



O1

RESULTS

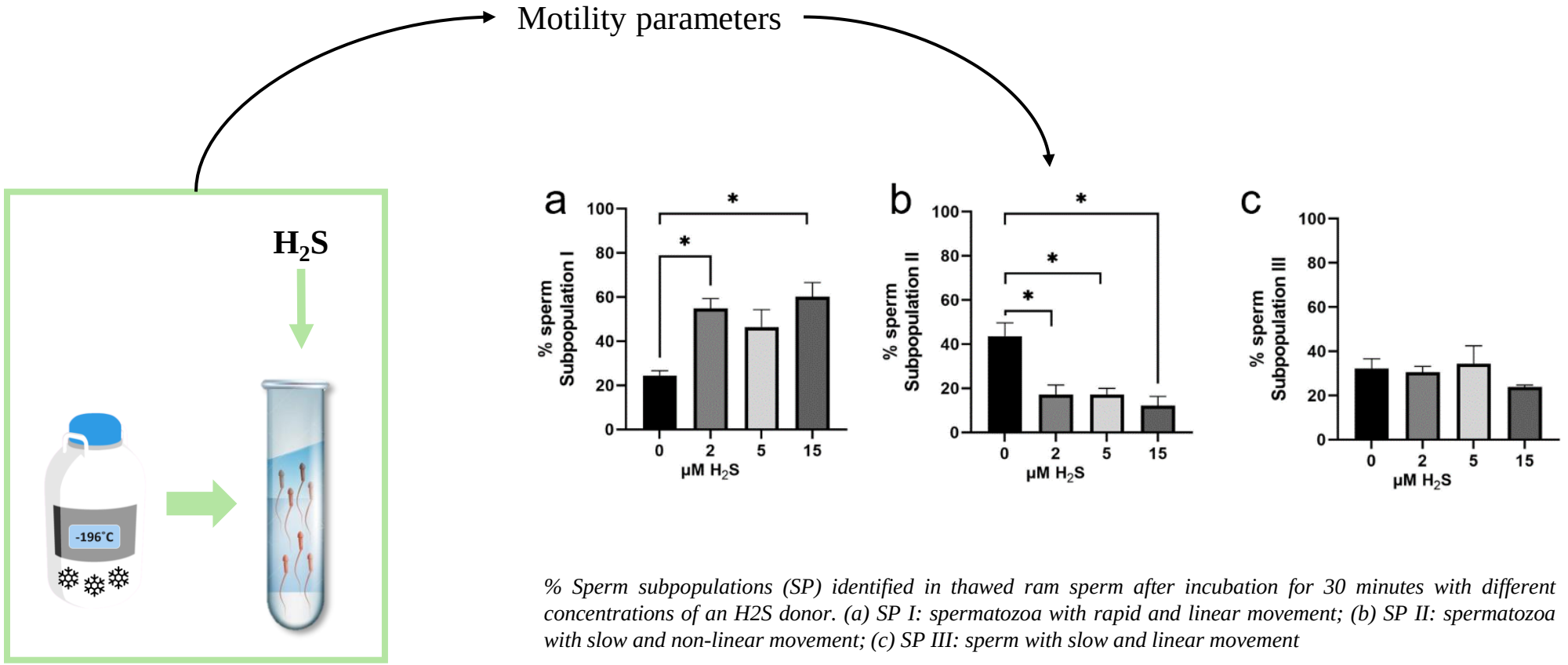
Unpublished data



Percentage of sperm population with low level of reactive oxygen species (ROS) at different incubation times (5, 30, 60, 120 min) with $Na_2S \cdot 9\ H_2O$.

O2

Unpublished data



% Sperm subpopulations (SP) identified in thawed ram sperm after incubation for 30 minutes with different concentrations of an H2S donor. (a) SP I: spermatozoa with rapid and linear movement; (b) SP II: spermatozoa with slow and non-linear movement; (c) SP III: sperm with slow and linear movement

ACKNOWLEDGMENTS

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MINISTERIO DE CIENCIA E INNOVACIÓN

Financiado por la Unión Europea NextGenerationEU

Plan de Recuperación, Transformación y Resiliencia

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CNS2022-135266

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FACULTY OF MEDICINE IN PILSEN

Charles University

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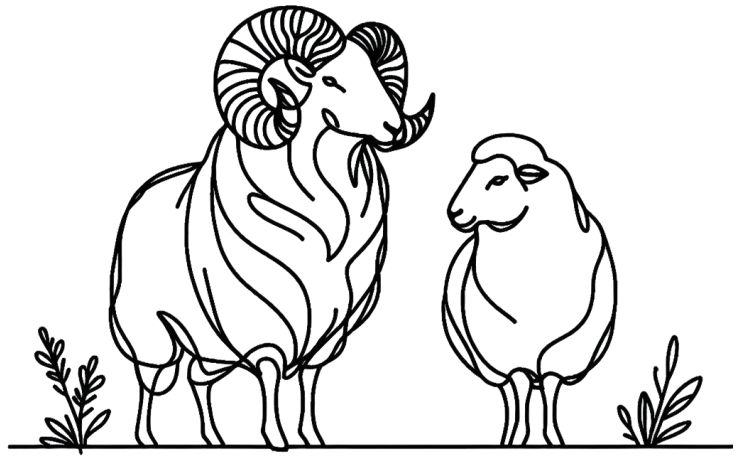
THANK YOU FOR YOUR ATTENTION



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Czech University of Life Sciences Prague

avantor delivered by VWR

María de Sousa Blanco, PhD Student

maria.sousa@uclm.es

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@mariaasou_