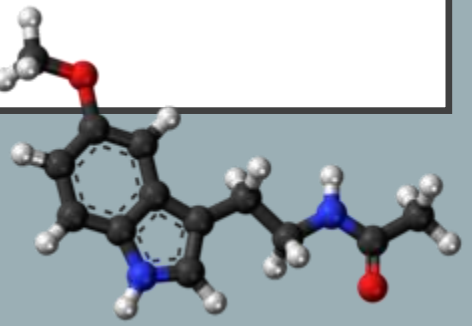
THE MELATONIN RECEPTOR MT1 IN RAMS AS A TOOL TO IMPROVE REPRODUCTIVE PARAMETERS OF THE SHEEP FLOCK



ROSAURA PÉREZ-PE

BIOFITER
BIOLOGÍA, FISIOLOGÍA Y TECNOLOGÍAS DE LA REPRODUCCIÓN

Instituto Universitario de Investigación en Ciencias Ambientales de Aragón
Universidad Zaragoza

INTRODUCTION

- MELATONIN
  - ORIGIN AND SYNTHESIS
  - FUNCTIONS
  - MECHANISMS OF ACTION
- MELATONIN RECEPTORS
- POLYMORPHISMS OF THE MT1 GENE

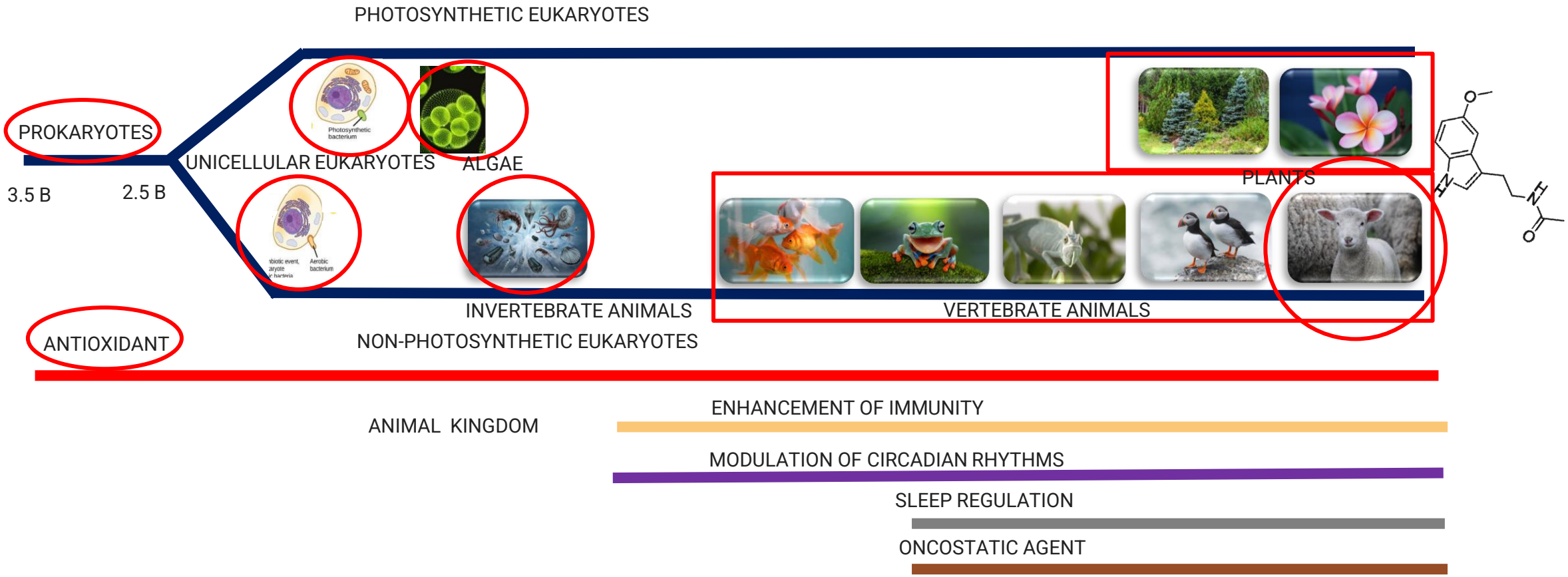
RELEVANT RESULTS

- RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS IN RAMS AND:
  - REPRODUCTIVE SEASONALITY
  - FERTILITY RATE
  - SPERM QUALITY

FINAL REMARKS

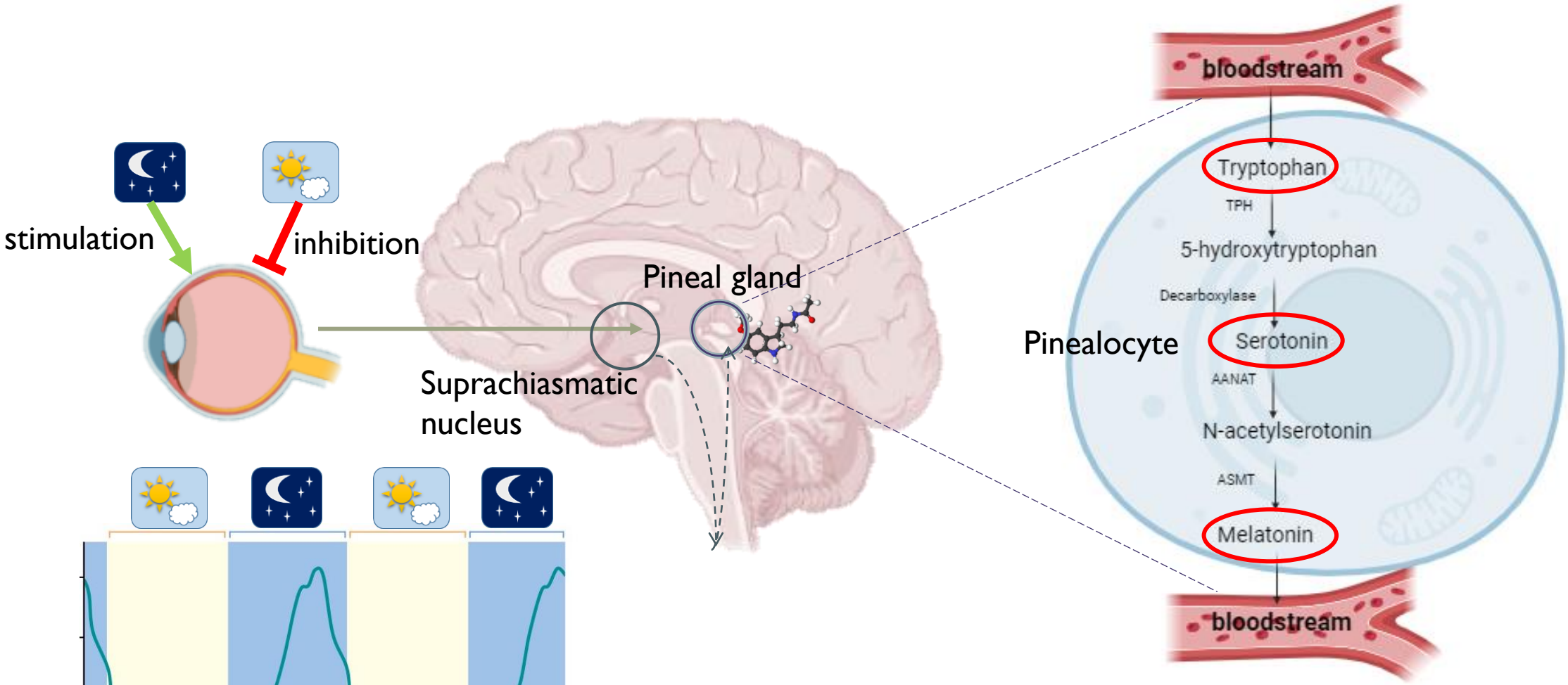
- GENERAL CONSIDERATIONS
- POTENTIAL APPLICATIONS

MELATONIN IN ORGANISMS: EVOLUTIONARY HISTORY

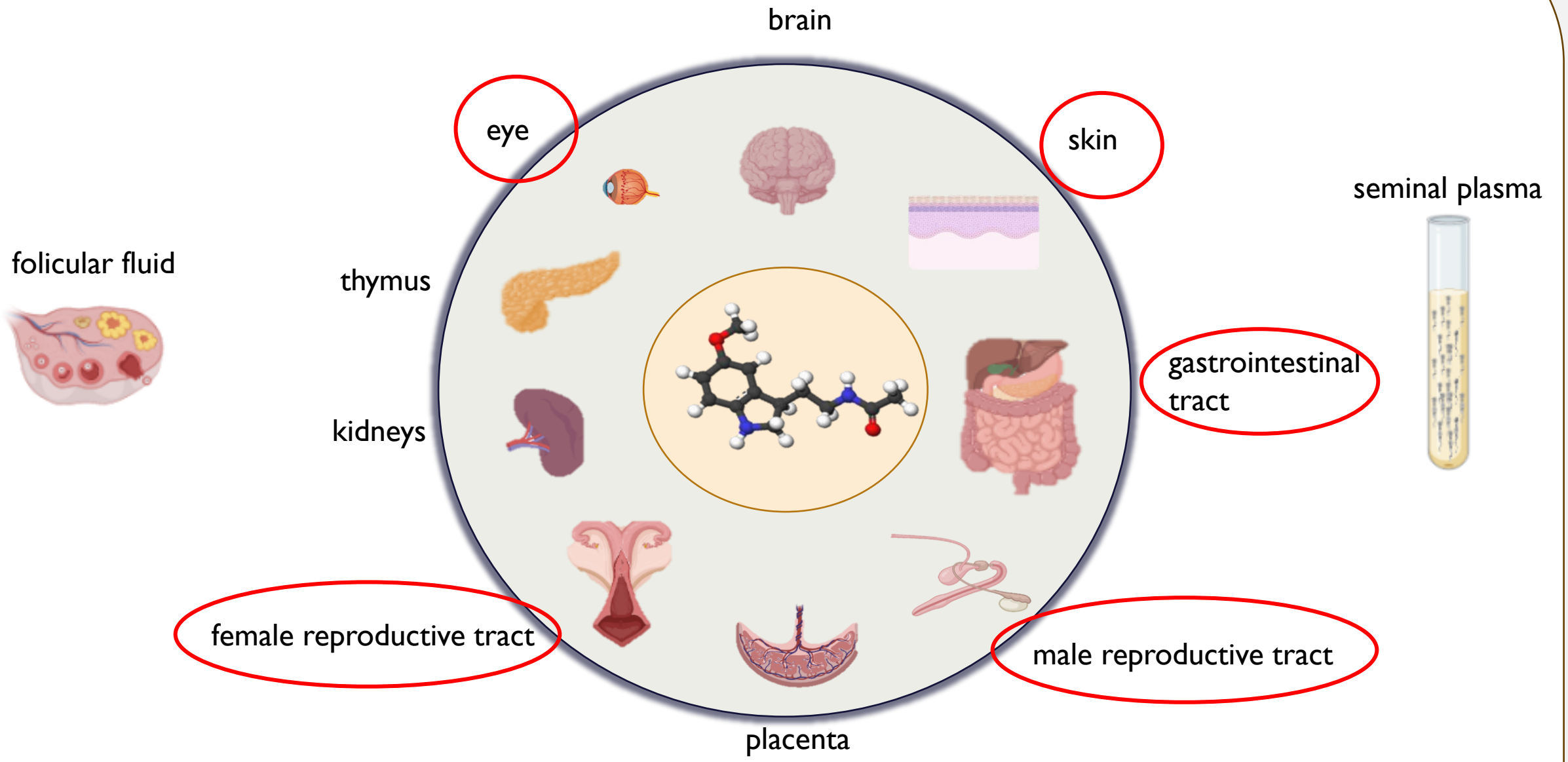


Modified from Zhao D et al. Melatonin Synthesis and Function: Evolutionary History in Animals and Plants. Front Endocrinol, 2019

MELATONIN SYNTHESIS: PINEAL GLAND



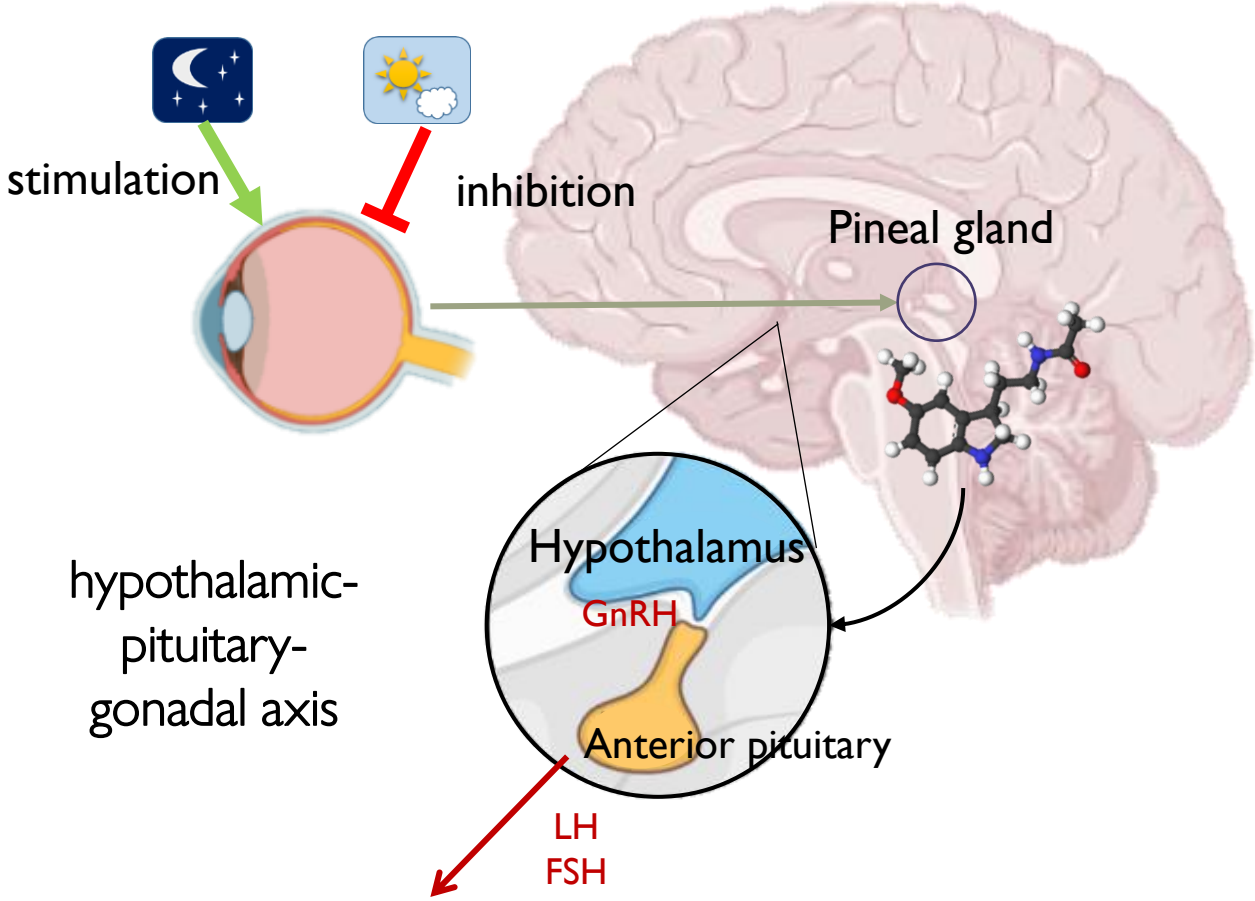
MELATONIN SYNTHESIS: EXTRAPINEAL

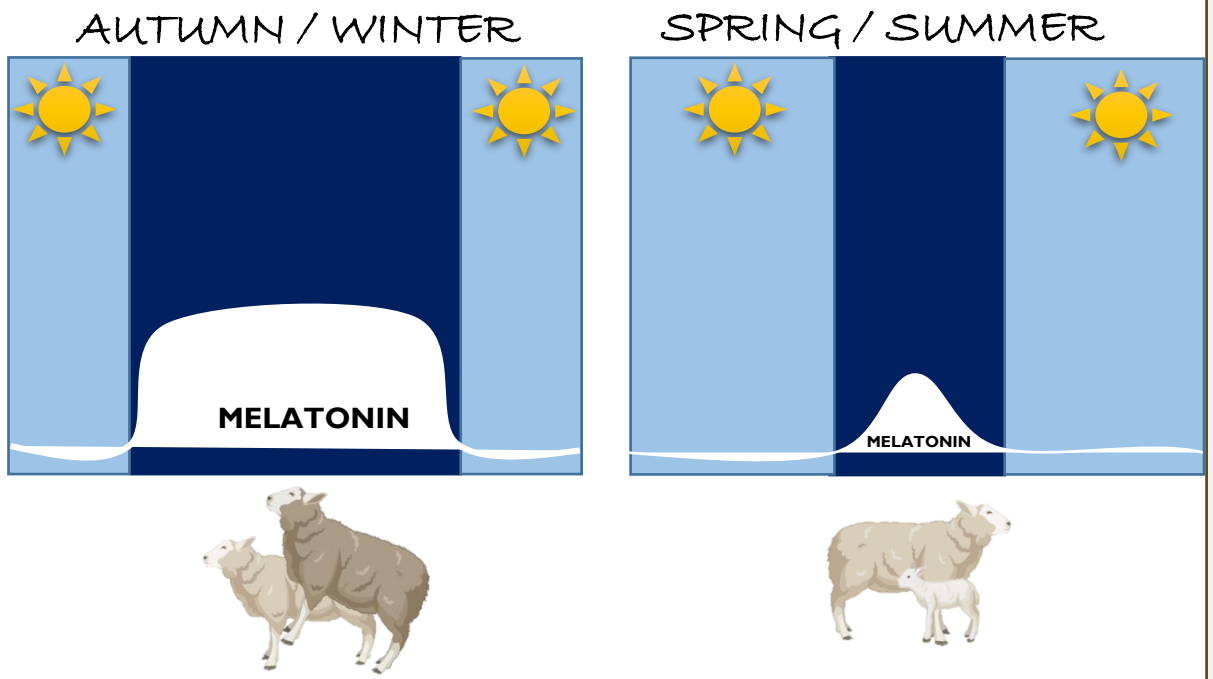


Modified from Kvetnoy, I. *et al.* Melatonin as the Cornerstone of Neuroimmunoendocrinology. *Int. J. Mol. Sci.* **2022**, *23*, 1835.

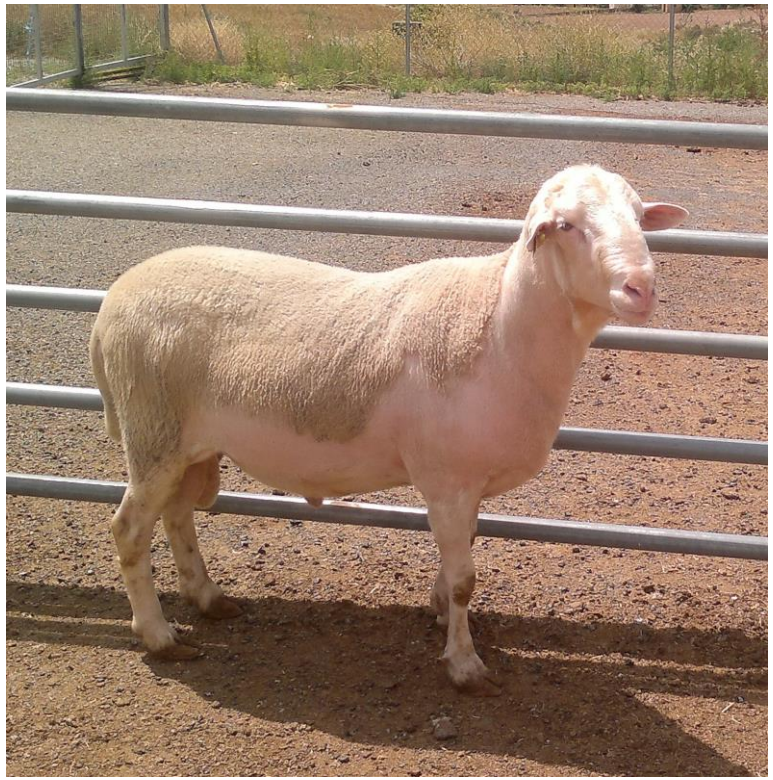
MELATONIN AND CONTROL OF REPRODUCTIVE SEASONALITY

MELATONIN AND CONTROL OF REPRODUCTIVE SEASONALITY





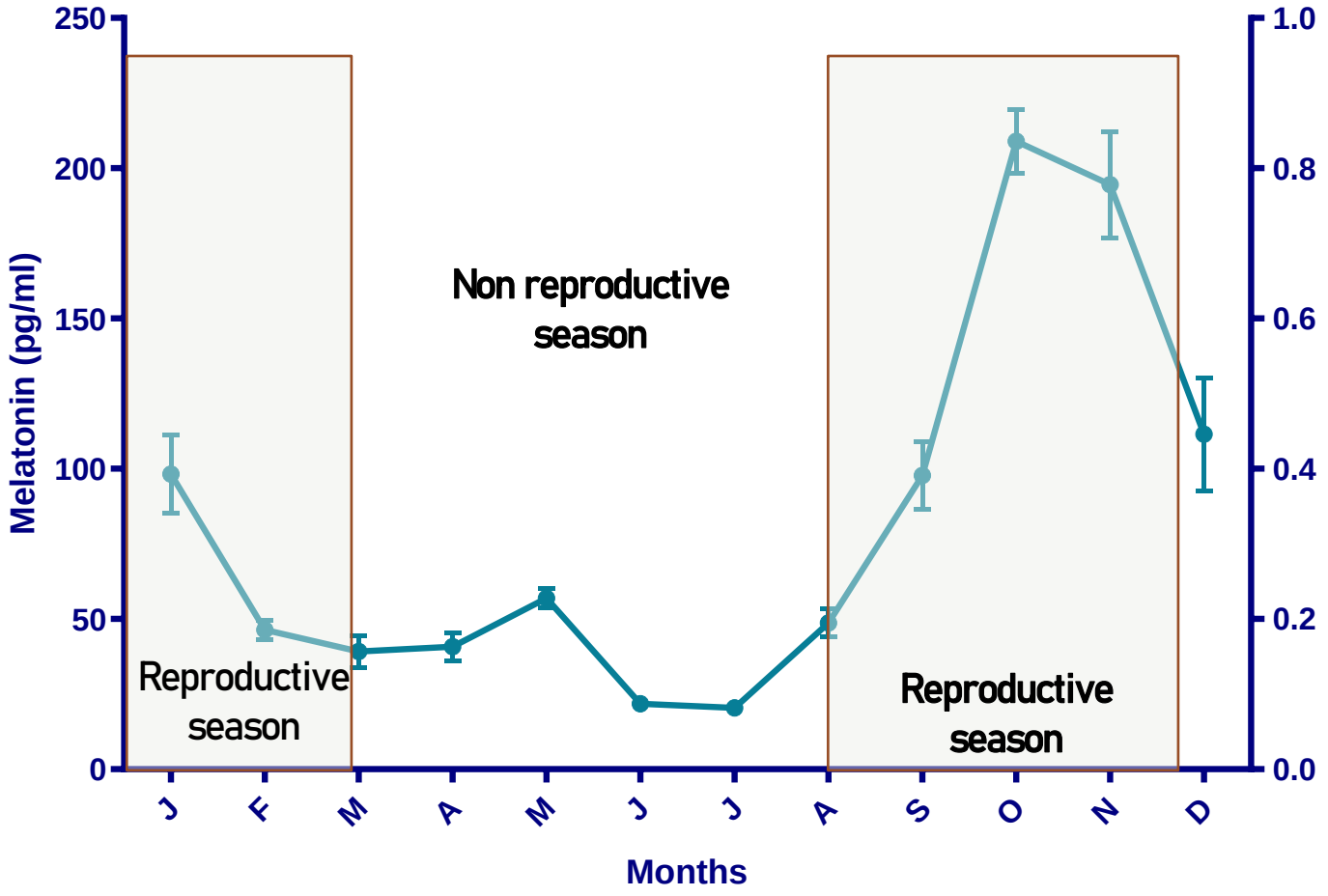
MELATONIN AND CONTROL OF REPRODUCTIVE SEASONALITY



IN NON-REPRODUCTIVE SEASON

- Reduction in sexual behavior
- Decrease in testicular weight and volume
- Decrease in sperm quality
- Changes in the seminal plasma composition

MELATONIN LEVELS IN RAM SEMINAL PLASMA



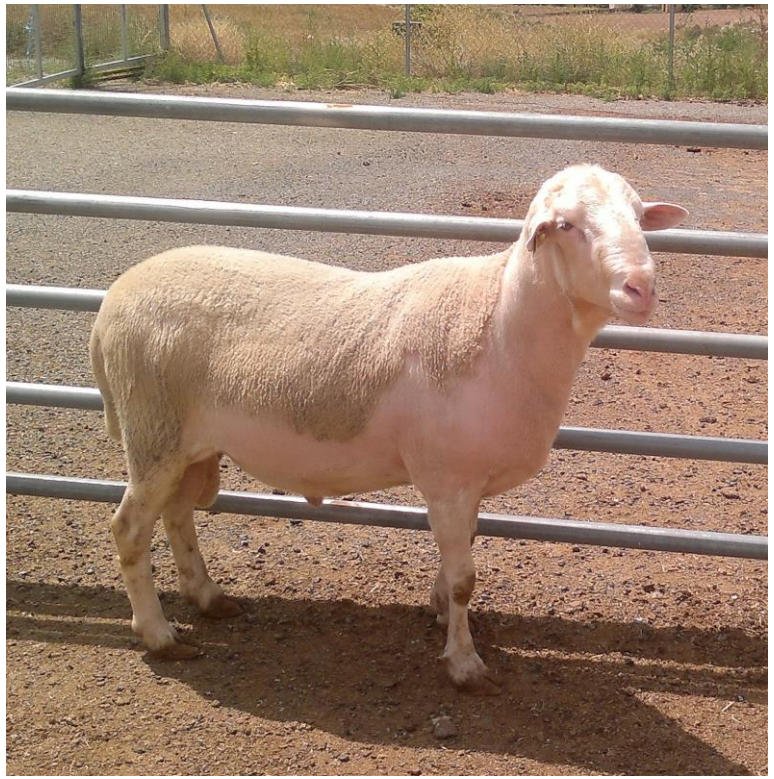
Casao et al. Reproductive Biology and Endocrinology 2010, 8:59
http://www.rbej.com/content/8/1/59

RB&E REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH Open Access

Seasonal variations of melatonin in ram seminal plasma are correlated to those of testosterone and antioxidant enzymes

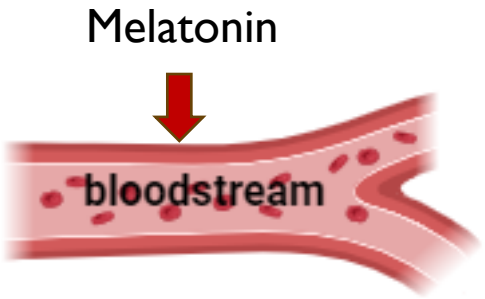
MELATONIN AND CONTROL OF REPRODUCTIVE SEASONALITY

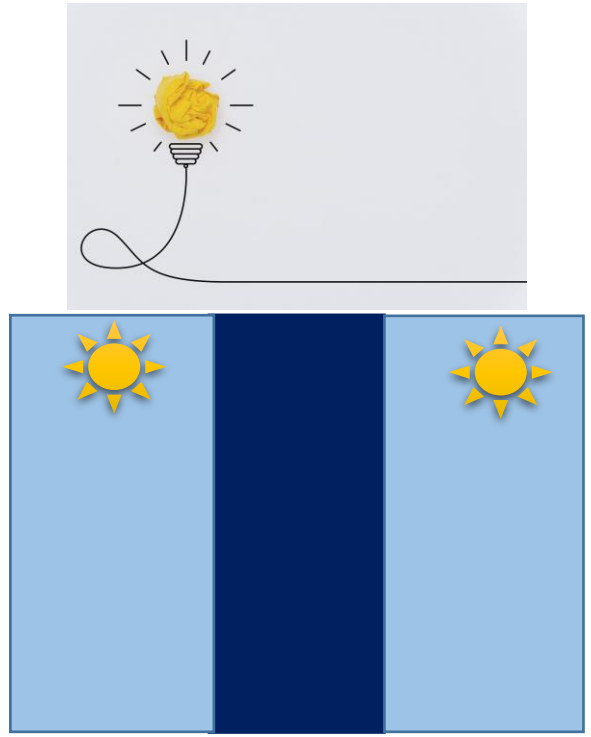


Reproductive seasonality is a limiting factor that affects the productive potential of most sheep breeds

MELATONIN AND CONTROL OF REPRODUCTIVE SEASONALITY

Melatonin implants





Management of light/dark cycles

Reprod Dom Anim doi: 10.1111/j.1439-0531.2008.01215.x
ISSN 0936-6768

Effects of Melatonin Implants During Non-Breeding Season on Sperm Motility and Reproductive Parameters in Rasa Aragonesa Rams

A Casao[1], S Vega[1], I Palacín[2], R Pérez-Pe[1], A Laviña[3], FJ Quintín[4], E Sevilla[4], JA Abecia[2], JA Cebrián-Pérez[1], F Forcada[2] and T Muiño-Blanco[1]

MELATONIN AND CONTROL OF REPRODUCTIVE SEASONALITY

Melatonin implants

Diet rich in phytomelatonin

Increase melatonin levels in seminal plasma

Improve sperm viability and morphology

Protect sperm against oxidative damage

Reprod Dom Anim doi: 10.1111/j.1439-0531.2008.01215.x
ISSN 0936-6768

Effects of Melatonin Implants During Non-Breeding Season on Sperm Motility and Reproductive Parameters in Rasa Aragonesa Rams

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animals MDPI

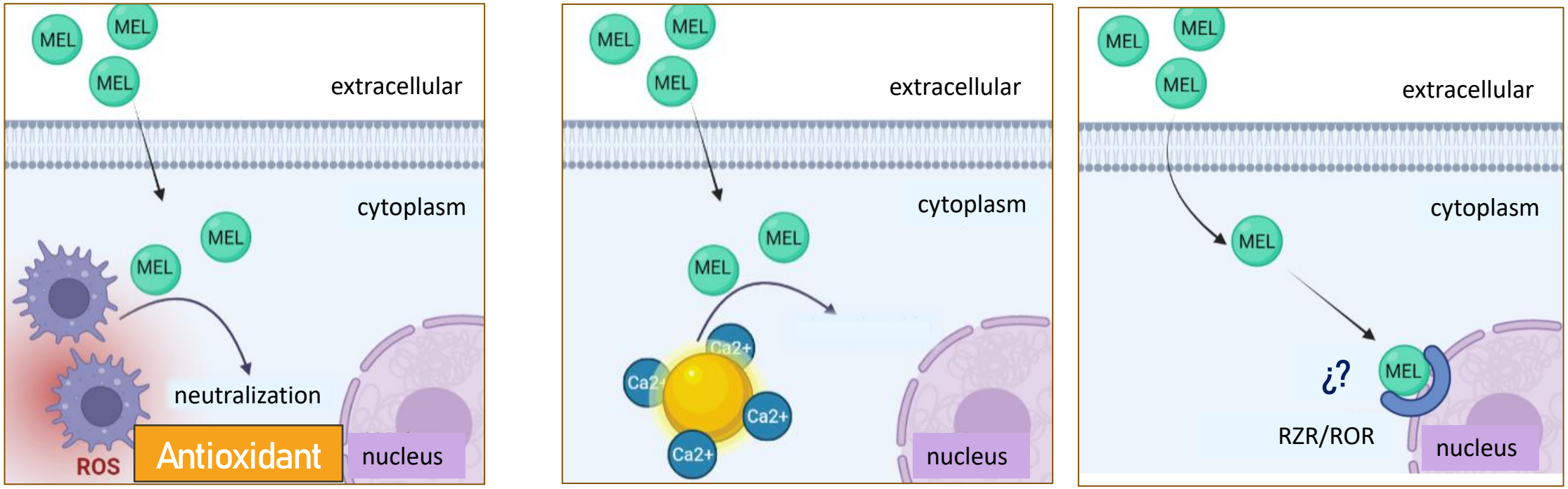
Article

Improvement of the Seminal Characteristics in Rams Using Agri-Food By-Products Rich in Phytomelatonin

Victoria Peña-Delgado [1,†], Melissa Carvajal-Serna [1,†], Manuel Fondevila [2], María A. Martín-Cabrejas [3,4], Yolanda Aguilera [3,4], Gerardo Álvarez-Rivera [4], José A. Abecia [1], Adriana Casao [1] and Rosaura Pérez-Pe [1,*]

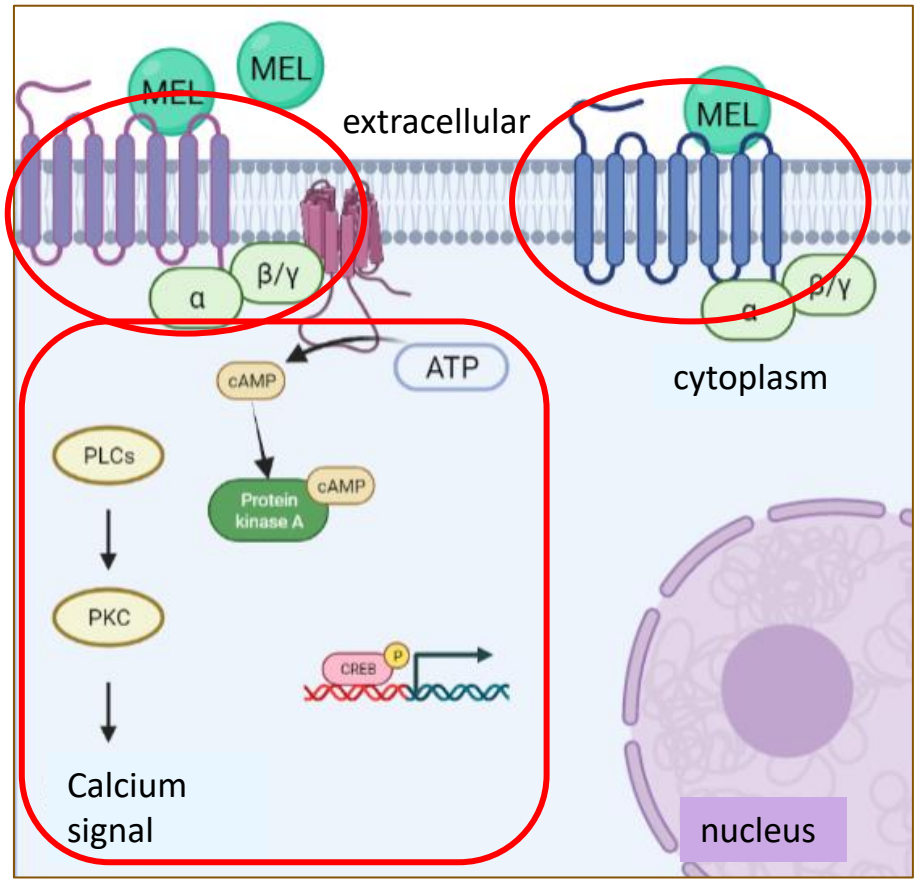
MECHANISMS OF ACTION OF MELATONIN

1. Directly passing through the cell membrane



MECHANISMS OF ACTION OF MELATONIN

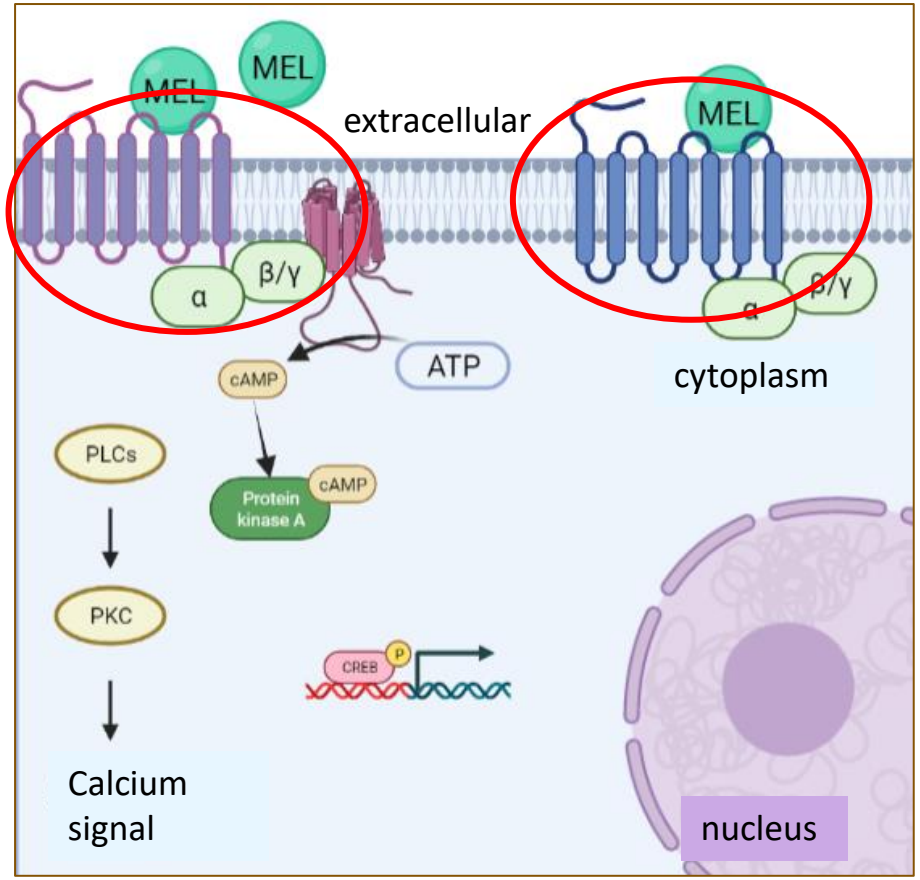
2. Through its binding to specific membrane receptors



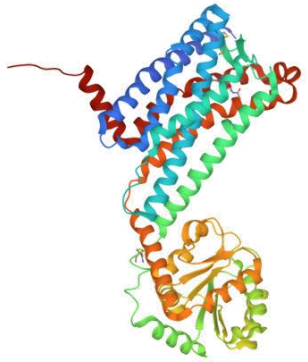
MT1

MT2

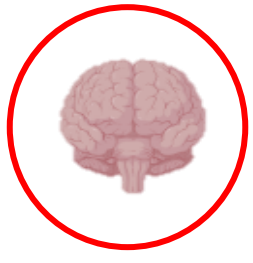
MELATONIN MEMBRANE RECEPTORS

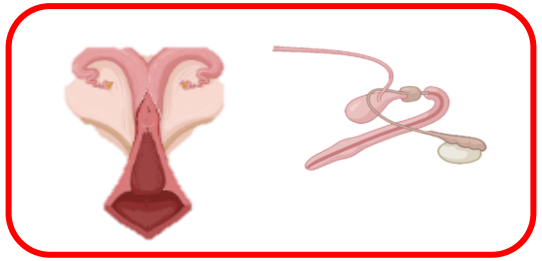


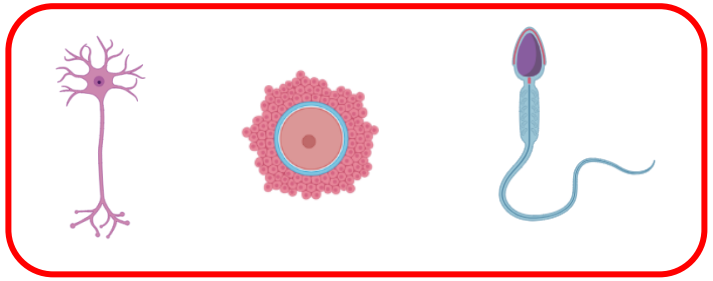
MT1



MT2

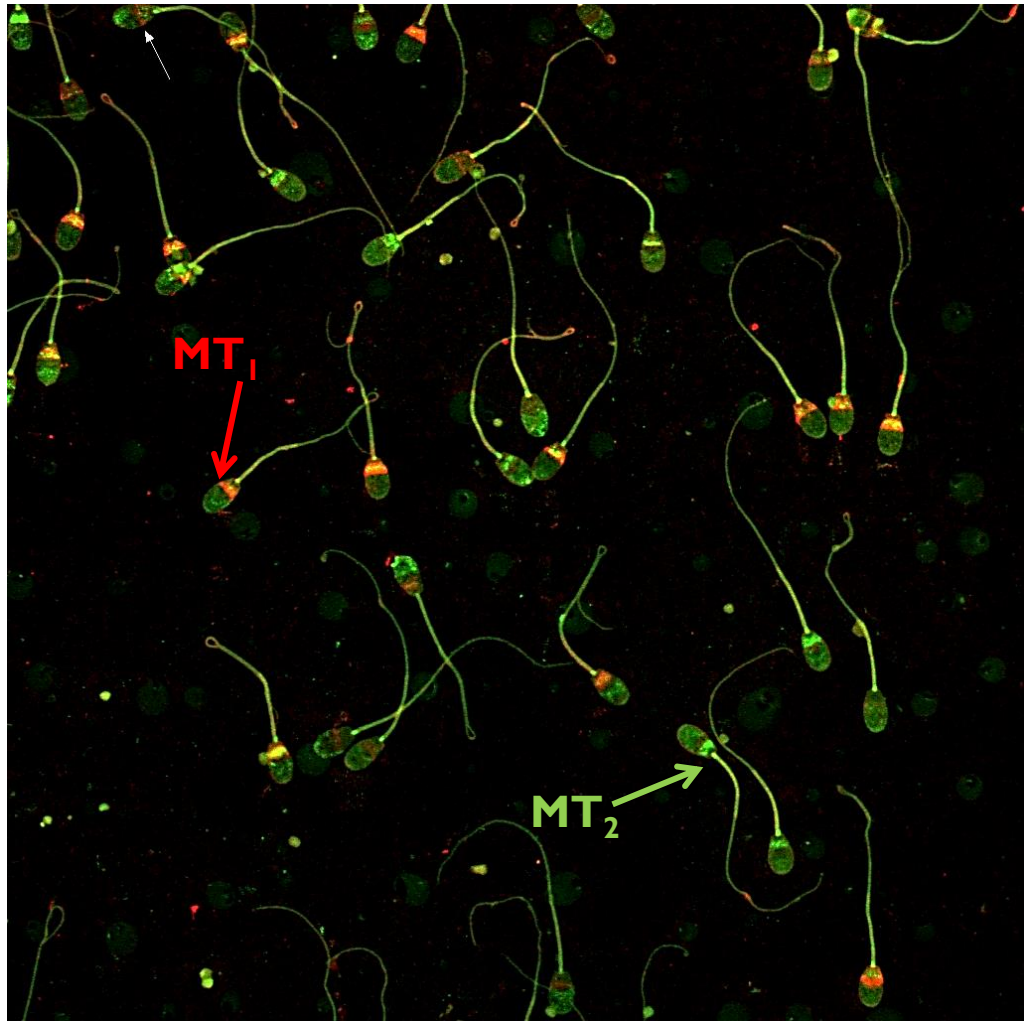






MELATONIN MEMBRANE RECEPTORS

Identification of melatonin MT_1 and MT_2 receptors in ram spermatozoa



CSIRO PUBLISHING

Reproduction, Fertility and Development, 2012, **24**, 953–961
http://dx.doi.org/10.1071/RD11242

Identification and immunolocalisation of melatonin MT_1 and MT_2 receptors in Rasa Aragonesa ram spermatozoa

Adriana Casao[A,C], *Margarita Gallego*[B], *José Alfonso Abecia*[A], *Fernando Forcada*[A], *Rosaura Pérez-Pé*[A], *Teresa Muiño-Blanco*[A] and *José Álvaro Cebrián-Pérez*[A]

CSIRO PUBLISHING

Reproduction, Fertility and Development
http://dx.doi.org/10.1071/RD14302

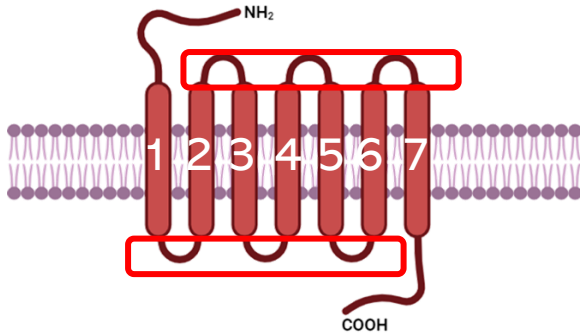
New evidence of melatonin receptor contribution to ram sperm functionality

Marta Gonzalez-Arto[A,*], *Carolina Luna*[A,*], *Rosaura Pérez-Pé*[A], *Teresa Muiño-Blanco*[A], *José A. Cebrián-Pérez*[A] and *Adriana Casao*[A,B]

[A]Grupo Biología y Fisiología de la Reproducción, Instituto de Investigación de Ciencias Ambientales de Aragón (IUCA), Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Veterinaria, Universidad de Zaragoza, C/Miguel Servet, 177, 50013, Zaragoza, Spain.
[B]Corresponding author. Email: adriana@unizar.es

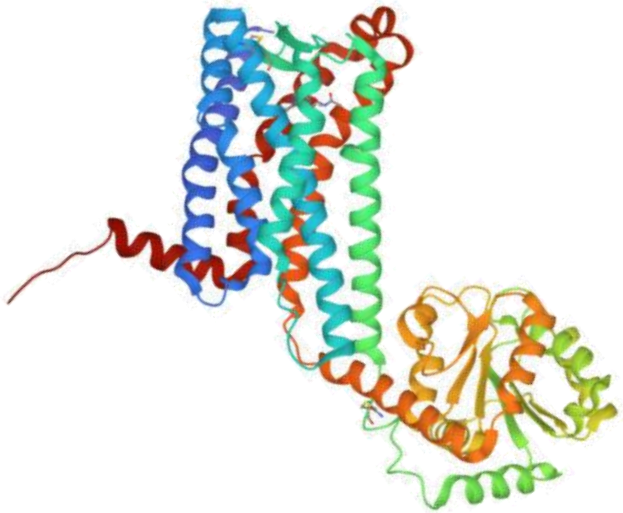
MELATONIN MEMBRANE RECEPTORS

G-protein-coupled receptors



schematic representation

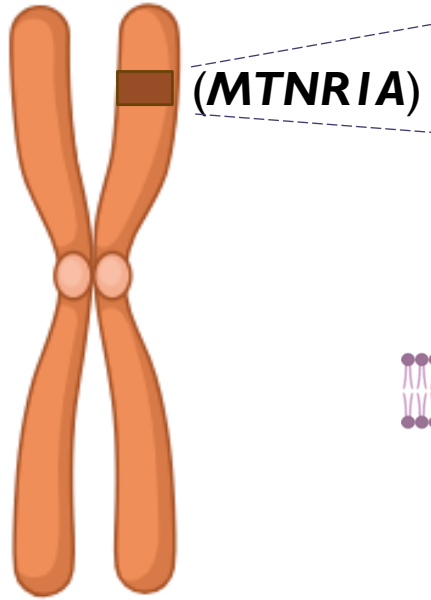
MT1



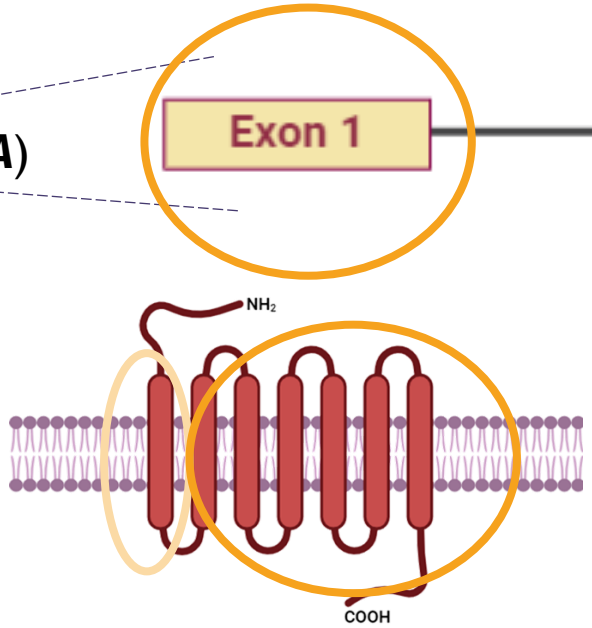
MT2

3-dimensional representation

THE MT1 RECEPTOR GENE (*MTNR1A*)

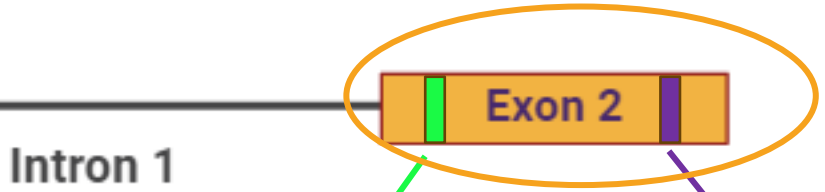


(***MTNR1A***)



chromosome 26 (Ovis aries)

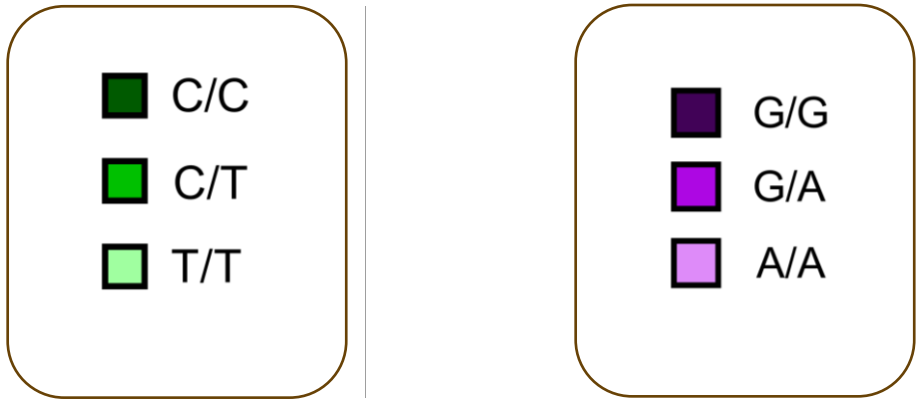
POLYMORPHIC SITES



RsaI (g.17355458 C>T)

MnlI (g. 17355452 G>A)

(Messer *et al.* 1997)



GENOTYPES

RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTION IN EWES

shorter anoestric period
(Pelletier *et al.*,2000)
(Chu *et al*, 2006)
(Carcangiu *et al.*, 2009)

higher fertility rates
(Mura *et al.*, 2019)

higher number of complete cycles per year
(Calvo *et al.*, 2018)

different responses to melatonin treatments
(Luridiana *et al.*, 2016)

lower number of days between the introduction of the males in the flock and the parturition
(Mura *et al.*, 2019)

RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTION IN OVINE

IN EWES

IN RAMS???

OBJECTIVE: TO STUDY THE INFLUENCE OF *MTNR1A* POLYMORPHISMS ON RAM REPRODUCTION

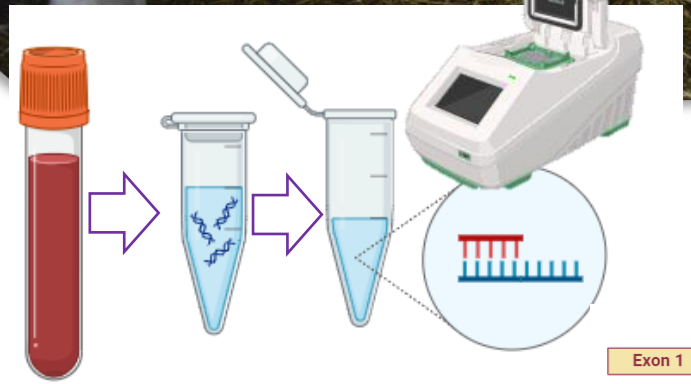
1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAMS?

Rasa Aragonesa rams

1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAMS?

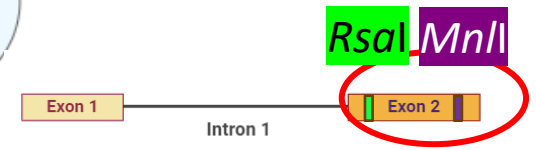
24 Rasa Aragonesa ram lambs born in autumn

Time of their first mating in spring (artificial vagina)

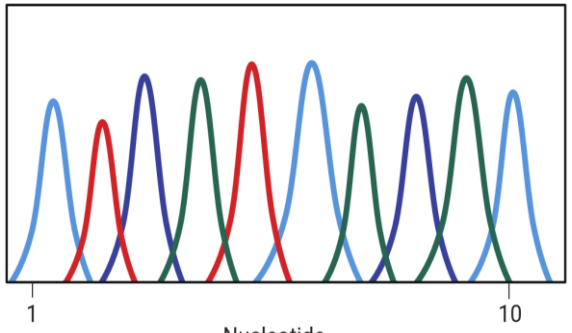


DNA extraction

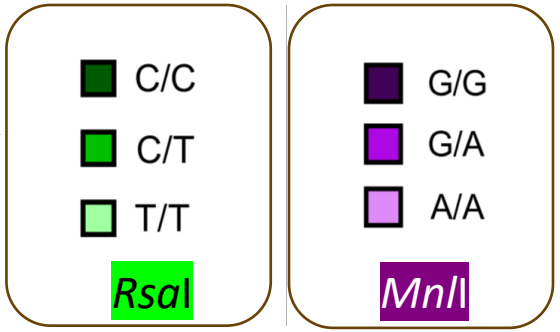
DNA amplification



Primers based on Calvo *et al*. (2018)



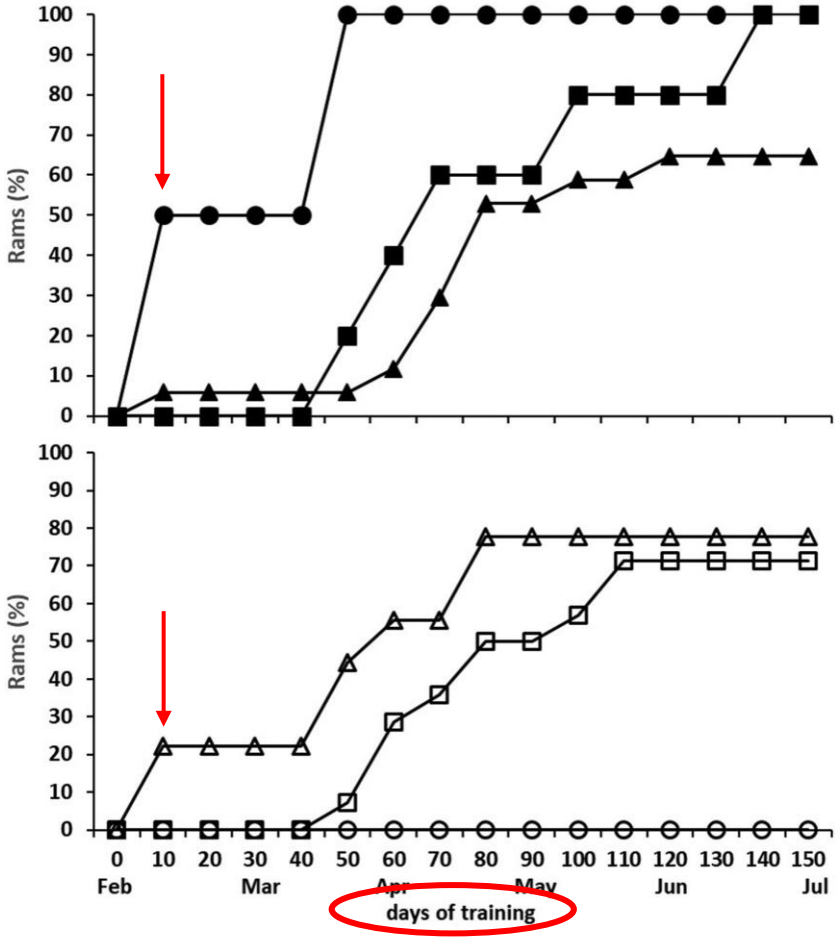
Genotyping
(STAB VIDA (Portugal))



Created in BioRender.com

1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAMS?

INFLUENCE OF *MTNR1A* POLYMORPHISMS ON REPRODUCTIVE BEHAVIOUR IN LAMB RAMS



▲ C/C
■ C/T
● T/T

*Rsa*I polymorphism

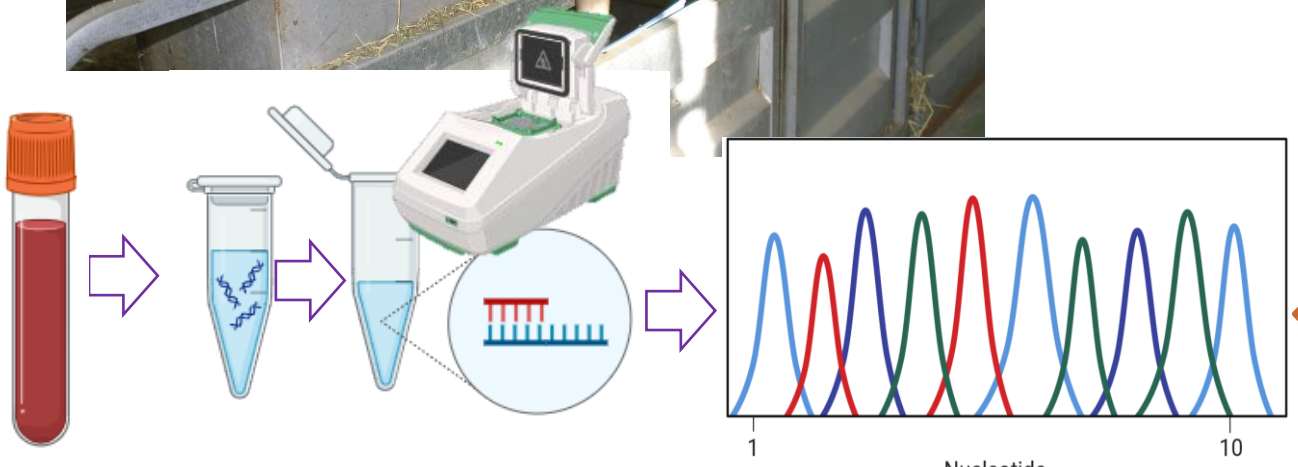
Δ G/G
□ A/G
○ A/A

*Mnl*I polymorphism

Distribution (%) of the first mating by rams with an estrus-synchronized ewe, ejaculating into an artificial vagina

1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAMS?

18 adult Rasa Aragonesa rams (>2.5 year-old)



DNA extraction

DNA amplification

Genotyping

(STAB VIDA (Portugal))

sexual activity

testicular volume

scrotal circumference

plasma testosterone concentrations

IN SPRING

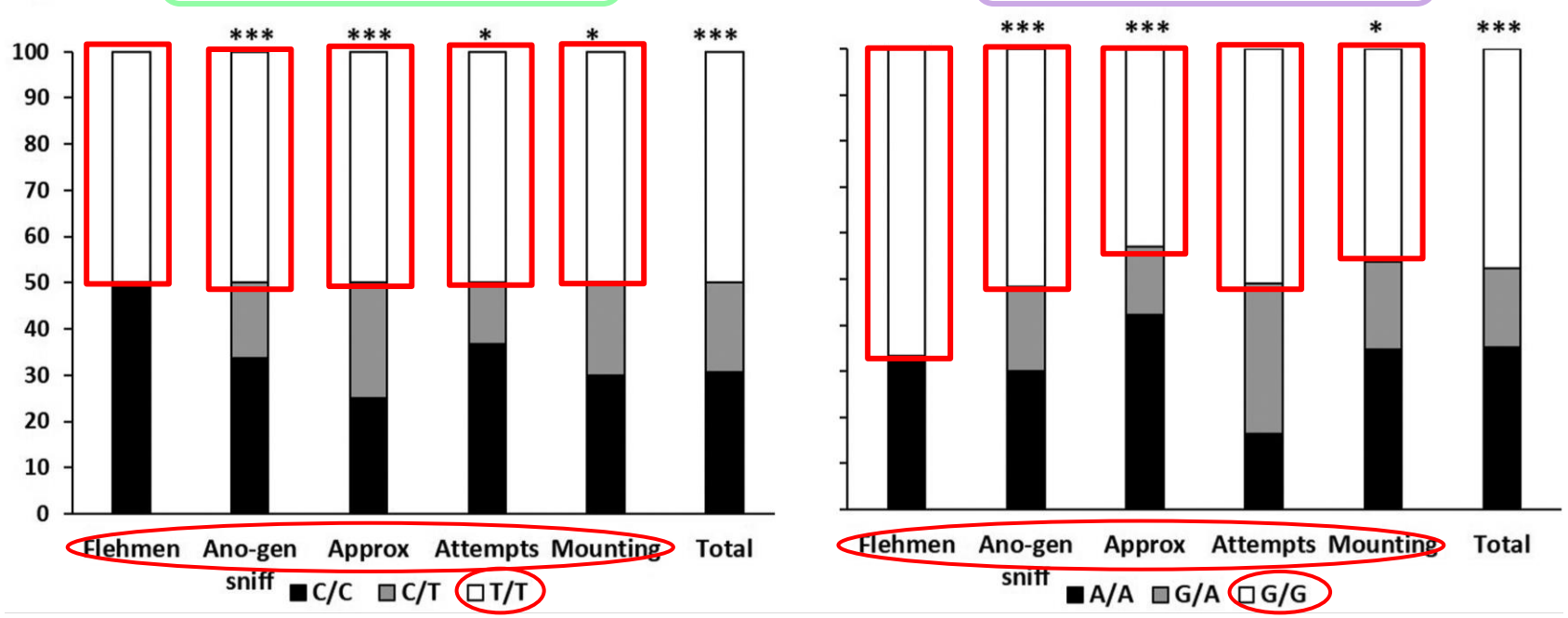
Created in BioRender.com

1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAM?

INFLUENCE OF *MTNR1A* POLYMORPHISMS ON REPRODUCTIVE BEHAVIOUR IN ADULT RAMS

*Rsa*I polymorphism

*Mnl*I polymorphism



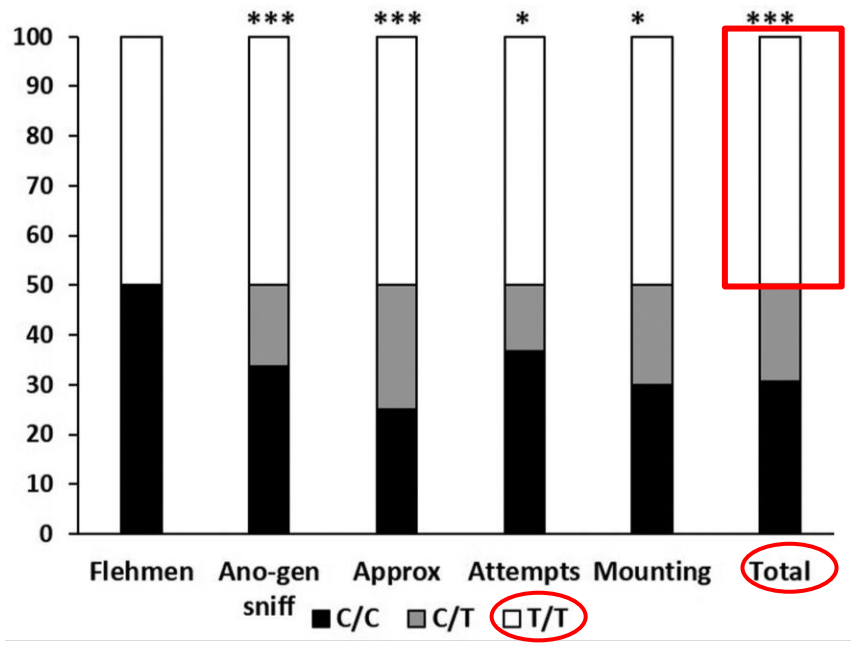
IN SPRING

Proportion (%) of signs of sexual activity in a 20-min individual serving capacity test (18 adult rams)

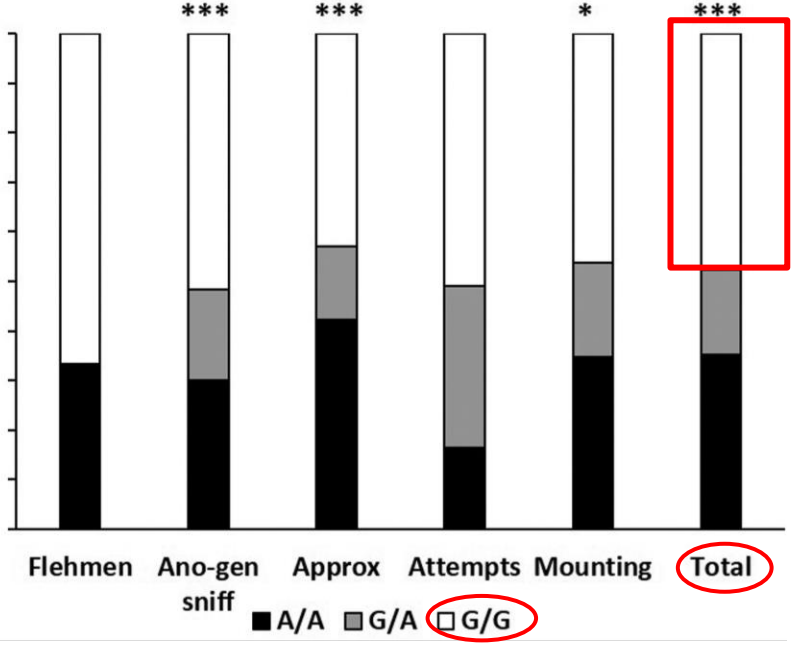
1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAM?

INFLUENCE OF *MTNR1A* POLYMORPHISMS ON REPRODUCTIVE BEHAVIOUR IN ADULT RAMS

*Rsa*I polymorphism



*Mnl*I polymorphism



sexual activity ✓

testicular volume ✗

scrotal circumference ✗

plasma testosterone concentrations ✗

IN SPRING

Proportion (%) of signs of sexual activity in a 20-min individual serving capacity test (18 adult rams)

1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAM?

Rasa Aragonesa ram lambs born in autumn that carry

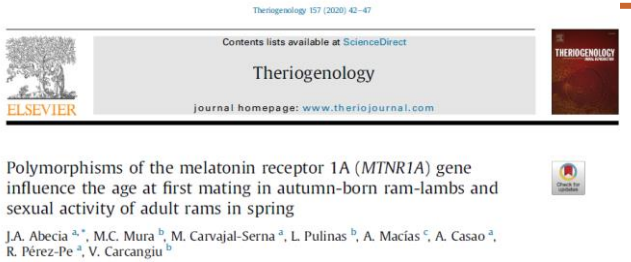
- TT genotype (*RsaI* polimorphism)
- GG genotype (*MnlI* polimorphism)

→ have a greater ability to reproduce in their first spring

Rasa Aragonesa adult rams that carry

- TT genotype (*RsaI* polimorphism)
- GG genotype (*MnlI* polimorphism)

→ exhibit a more intense reproductive behaviour (serving capacity test in spring)



Theriogenology 157 (2020) 42–47

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

ELSEVIER

Polymorphisms of the melatonin receptor 1A (*MTNR1A*) gene influence the age at first mating in autumn-born ram-lambs and sexual activity of adult rams in spring

J.A. Abecia [a,*], M.C. Mura [b], M. Carvajal-Serna [a], L. Pulinas [b], A. Macías [c], A. Casao [a], R. Pérez-Pe [a], V. Carcangiu [b]

RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTION IN OVINE

IN EWES

IN RAMS

TT and GG: % ovarian cyclicity throughout the year

TT and GG genotypes seems to be related to a less marked reproductive seasonality in both genders

RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTION IN OVINE

IN EWES

TT and GG: % ovarian cyclicity throughout the year

IN RAMS

Are these genotypes of rams influencing the fertility results?

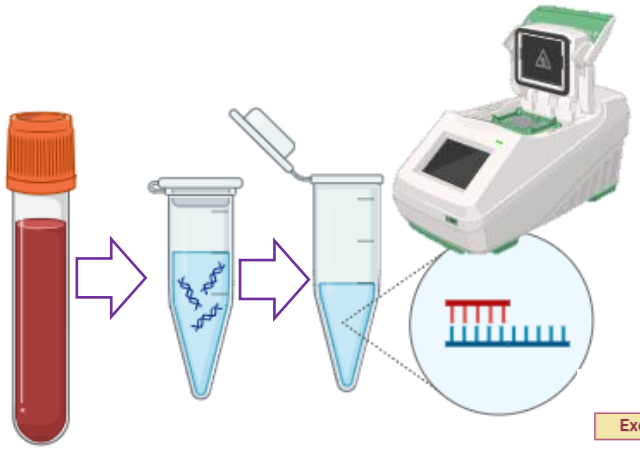
AIM:

To evaluate the effect of the polymorphisms of the *MNTR 1A* gene of rams on the fertility of the ewes after artificial insemination

2. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND FERTILITY AFTER AI?

Rams used in the artificial insemination (AI) program of the Rasa Aragonesa breed

26 rams were genotyped



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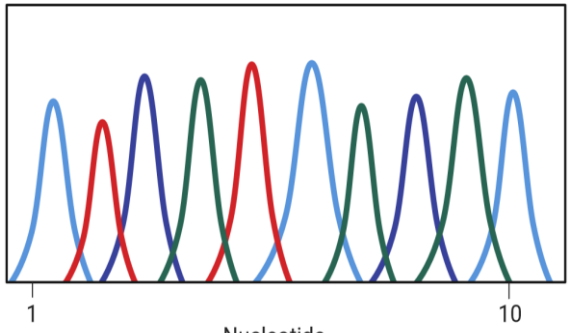
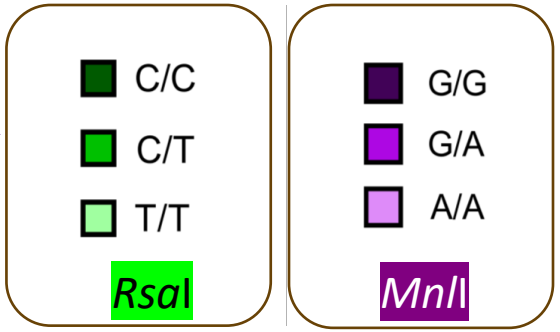


Genotyping
(STAB VIDA (Portugal))



Created in BioRender.com

2. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND FERTILITY AFTER AI?

Rams used in the artificial insemination (AI) program of the Rasa Aragonesa breed

26 rams were genotyped

for the RsaI polymorphism
- CC (n=19; 2,127 AI)
- CT (n=6; 832 AI)
- TT (n=7; 1,373 AI)

for the MnlI polymorphism
- GG (n=13; 2,200 AI)
- GA (n=10; 1,606 AI)
- AA (n=3; 526 AI)

AI of 4,332 ewes (45 farms)

¿?

¿?

Fertility rate (pregnant or not)

2. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND FERTILITY AFTER AI?

FERTILITY RESULTS OBTAINED FROM RAMS WITH DIFFERENT *MTNR1A* POLYMORPHISMS

For the *Rsa*I polymorphism:

CC rams: 63%
CT rams: 64%
⟺ (P<0.05)
TT rams: 60%

TT rams led to a lower fertility rate than CC and CT

For the *Mnl*I polymorphism:

GA rams: 67% ⟺ (P<0.001) GG rams: 61% ⟺ (P<0.001) AA rams: 55%

AA rams led to a lower fertility rate than GA and GG

Considering season:

For the *Rsa*I polymorphism:

Reproductive season

CC rams: 66%
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⟺ (P<0.05)
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For the *Mnl*I polymorphism:

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Non reproductive season: NO significant differences

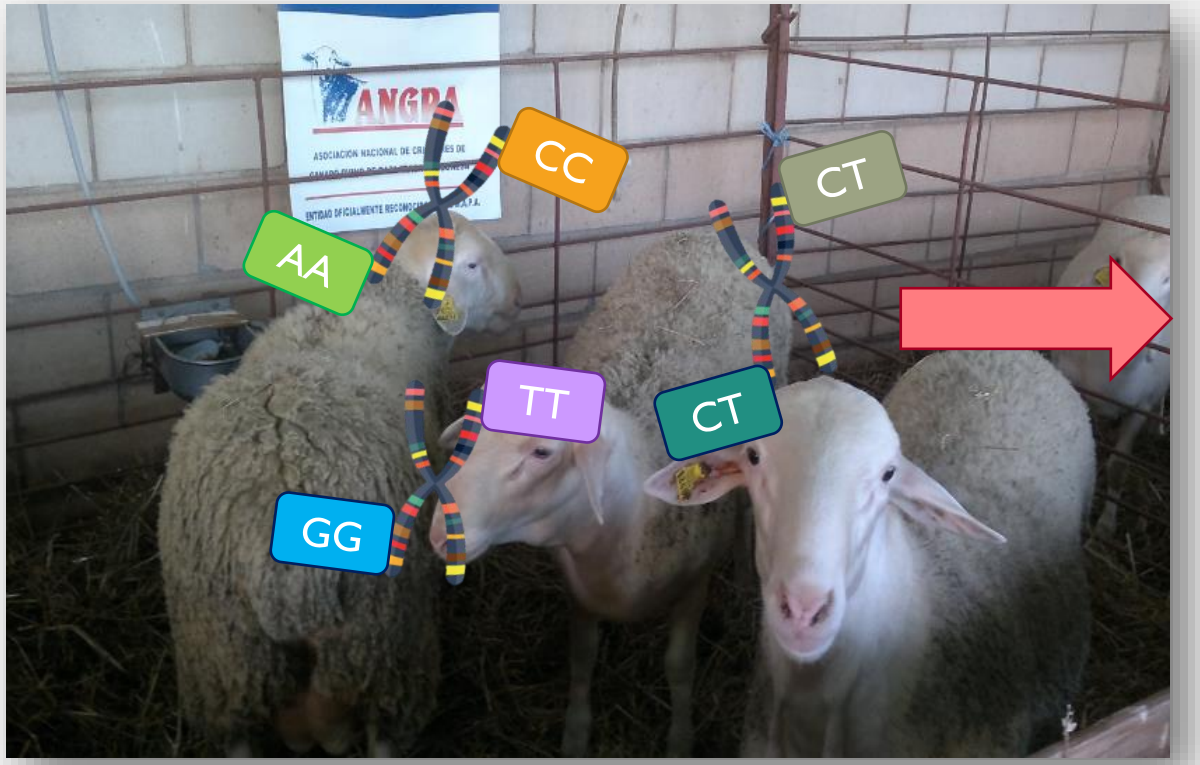
IN CONCLUSION

Carrying one or another genotype of the *MTNR 1A* gene by rams seems to influence the fertility rate of ewes after AI, specifically during the reproductive season

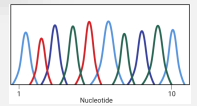
Were these observed differences due to differences in seminal quality?

OBJECTIVE:

Study of the influence of *MNTR 1A* polymorphisms on sperm quality



Eighteen Rasa Aragonesa rams of proven fertility



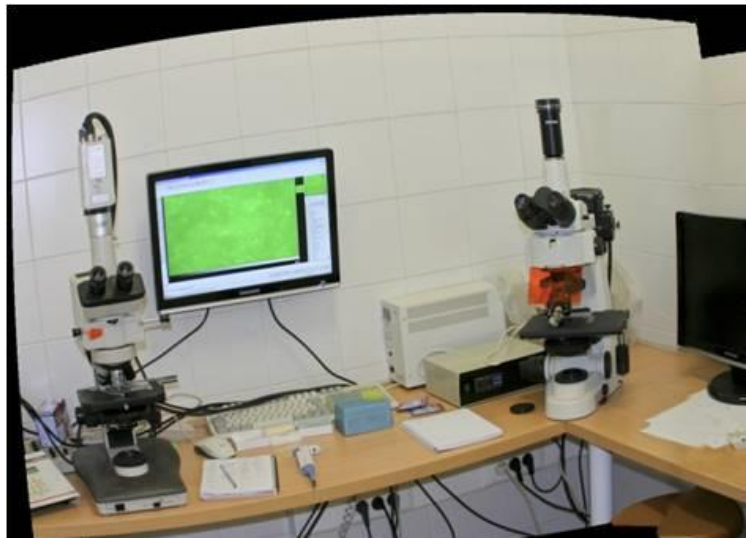
3. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND SPERM QUALITY?

EXPERIMENTAL DESIGN

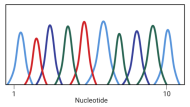
Weekly semen collection

once a month

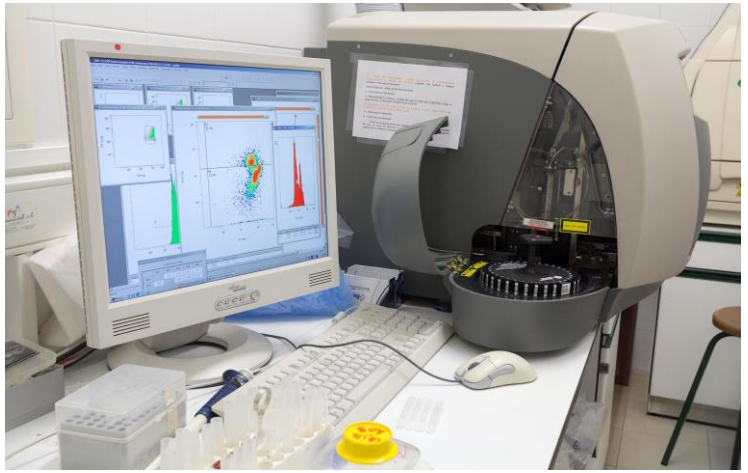
Refrigerated samples



18 Rasa Aragonesa rams of proven fertility
(AI program in farms)



Semen analysis



Once a month throughout a whole year
(from September to August)

3. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND SPERM QUALITY?

EXPERIMENTAL DESIGN

Weekly semen collection

once a month

Refrigerated samples

18 Rasa Aragonesa rams of proven fertility
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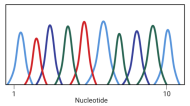
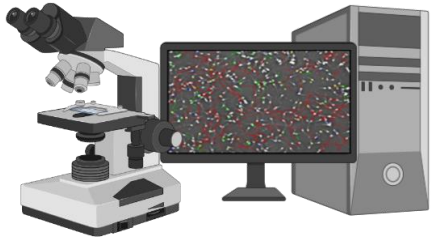
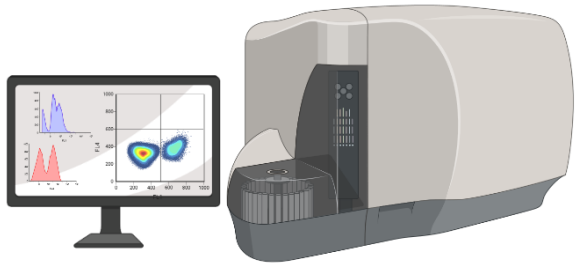


Semen analysis



Motility (CASA system)



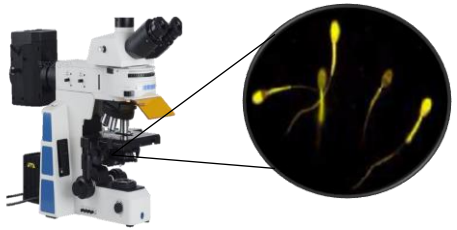
Viability (membrane integrity)

Oxidative damage (ROS levels)

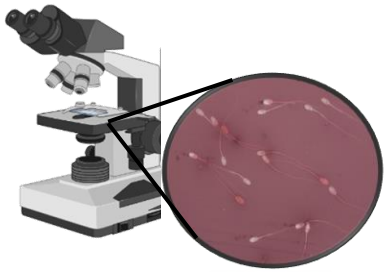
Phosphatidylserine (PS) translocation (annexin binding)

DNA fragmentation (TUNEL assay)

Flow cytometry



Capacitation status
(chlortetracycline staining)



Morphology
(eosin/nigrosine staining)

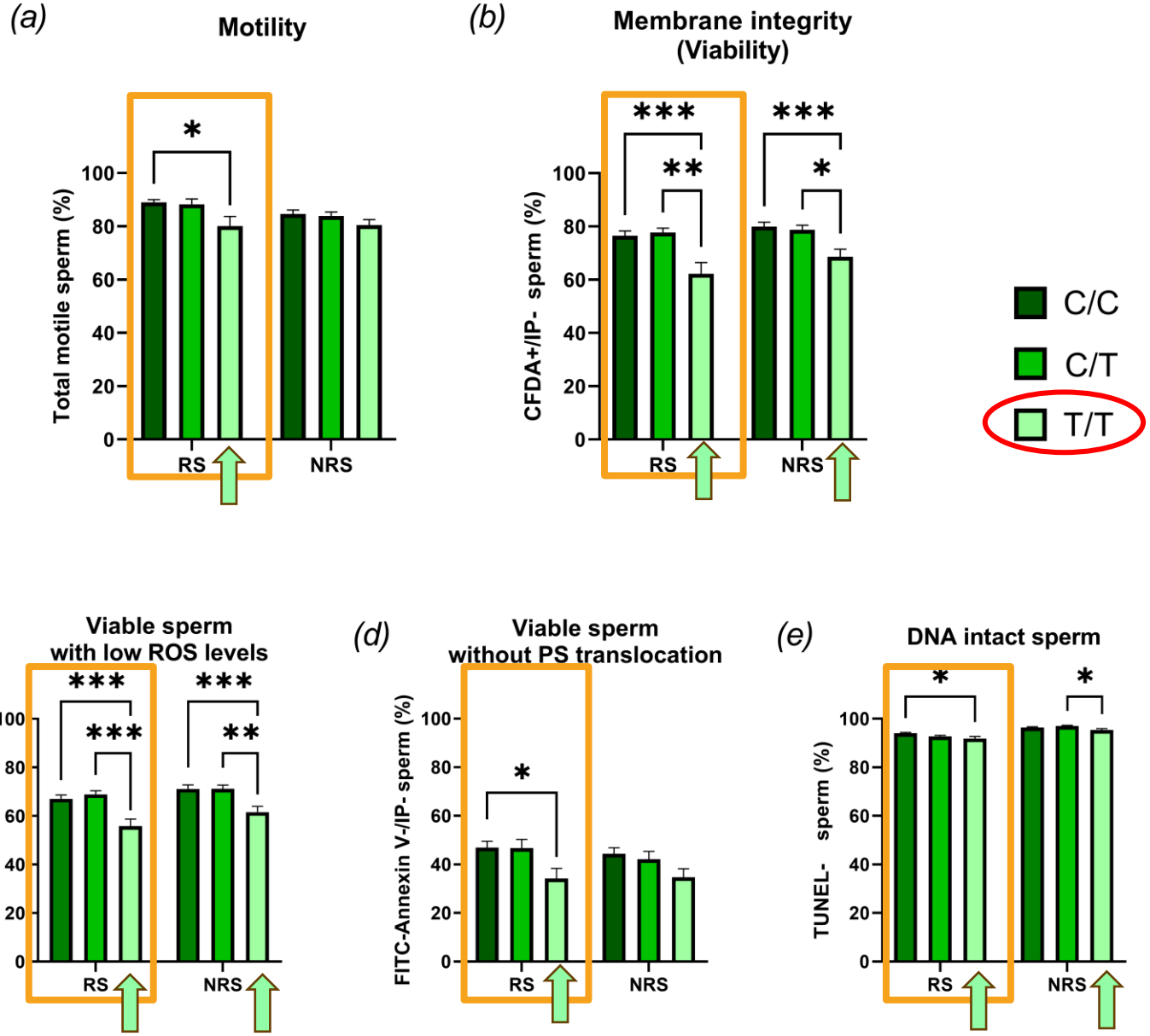
Once a month throughout a whole year
(from September to August)

RS: Reproductive season

NRS: Non Reproductive season

3. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND SPERM QUALITY?

EFFECT OF *RsaI* POLYMORPHISM ON SPERM QUALITY



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⟺ (P<0.05)
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TT rams led to a lower fertility rate than CC and CT

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GA rams: 67% ⟺ (P<0.001) GG rams: 61% ⟺ (P<0.001) AA rams: 55%

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Considering season:

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Non reproductive season: NO significant differences

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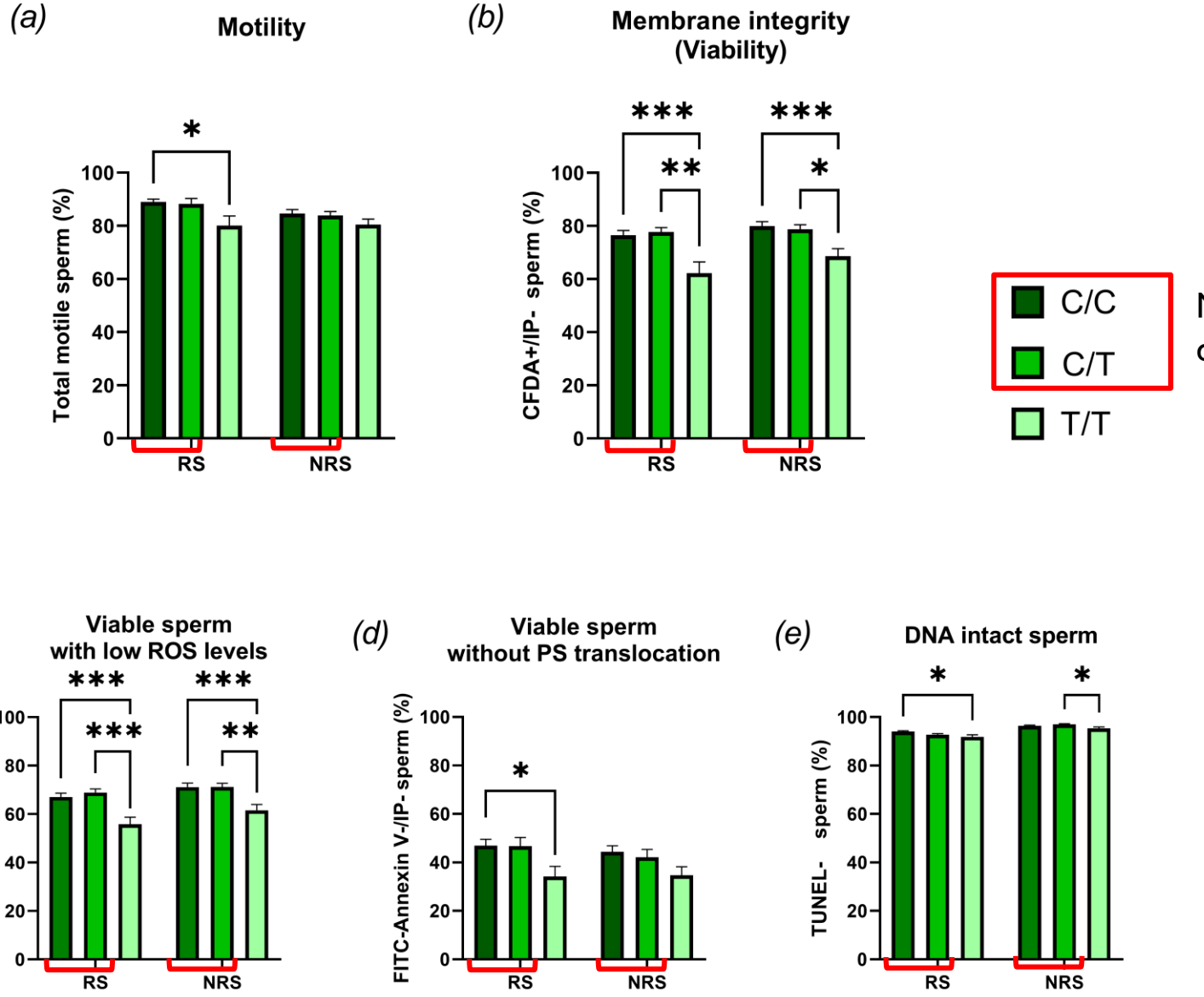

Non significant differences

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EFFECT OF *MnlI* POLYMORPHISM ON SPERM QUALITY

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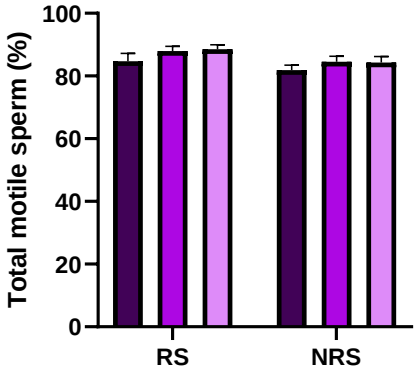
(a)



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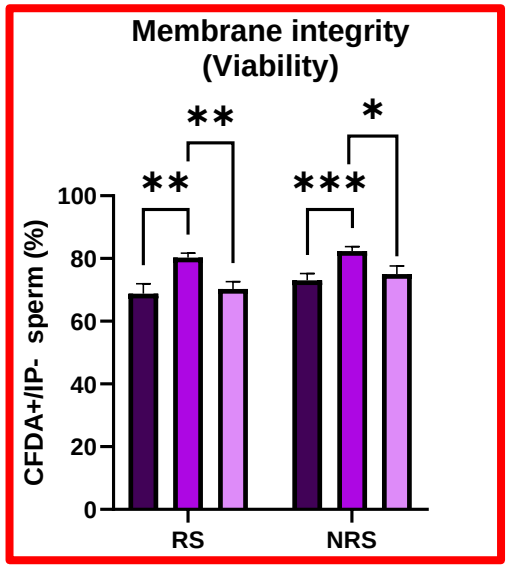

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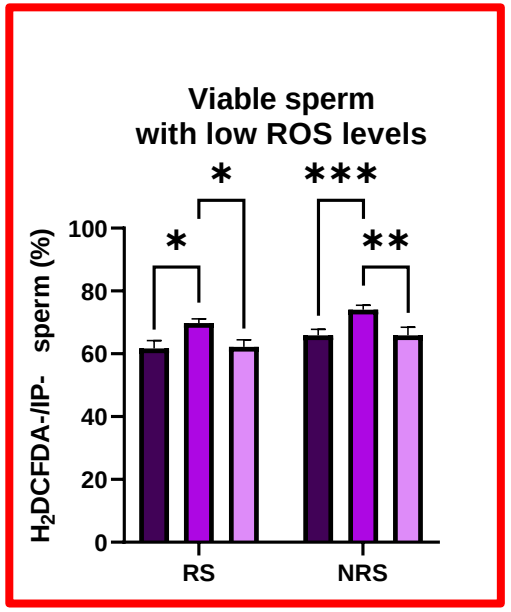

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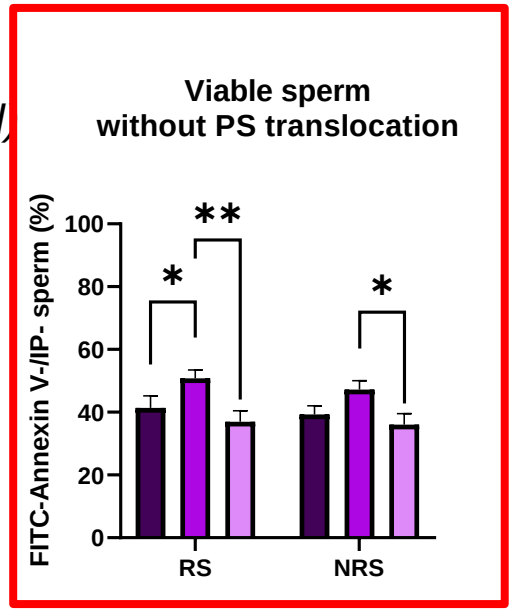

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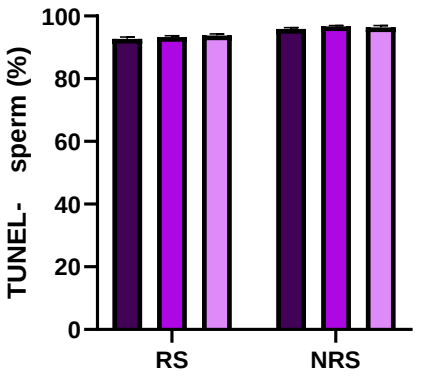

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Non reproductive season: NO significant differences

3. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND SPERM QUALITY?

Parameter	Mixed ANOVA results						
	RsaI	MnlI	Season	RsaI*season	MnlI*season	RsaI*MnlI	RsaI*MnlI*season
Total motility	P = 0.002	ns	P = 0.041	ns	ns	P = 0.018	ns
Progressive motility	ns	ns	P = 0.036	ns	ns	ns	ns
Membrane integrity (viability)	P < 0.001	P < 0.001	P = 0.030	ns	ns	P = 0.008	ns
Viable sperm with low ROS levels	P < 0.001	P < 0.001	P = 0.019	ns	ns	P = 0.010	ns
Viable sperm without phosphatidylserine (PS) translocation	P < 0.001	P < 0.001	ns	ns	ns	ns (P=0.062)	ns
Non-capacitated sperm	ns	ns	P = 0.001	ns	ns	ns	ns
Capacitated sperm	ns	ns	P = 0.001	ns	ns	ns	ns
Acrosome reacted sperm	ns	ns	ns	ns	ns	ns	ns
DNA intact sperm	P = 0.048	ns	P < 0.001	ns	ns	ns	ns
Normal morphology	ns	ns	ns	ns	ns	ns	ns

3. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND SPERM QUALITY?

Genotypes of the 18 rams used according to *Rsa*I and *Mnl*I polymorphisms

		***Rsa*I polymorphism**			
		C/C	C/T	T/T	Total
***Mnl*I polymorphism**	G/G	1 (5.6 %)	2 (11.1 %)	4 (22.2 %)	7 (38.9 %)
	G/A	4 (22.2 %)	3 (16.7 %)	0 (0.0 %)	7 (38.9 %)
	A/A	4 (22.2 %)	0 (0.0 %)	0 (0.0 %)	4 (22.2 %)
	Total	9 (50 %)	5 (27.8 %)	4 (22.2 %)	18

Allele frequency

G = 0.58 A = 0.41

Allele frequency

C = 0.63 T = 0.36

1. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND REPRODUCTIVE SEASONALITY IN RAMS?

Genotypes of the 24 Rasa Aragonesa lambs used according to *Rsa*I and *Mnl*I polymorphisms

		***Rsa*I polymorphism**			
***Mnl*I polymorphism**		C/C	C/T	T/T	Total
	G/G	6 (25.0 %)	1 (4.1 %)	2 (8.3 %)	9 (37.5 %)
	G/A	10 (41.67 %)	4 (16.6 %)	0 (0.0 %)	14 (58.3 %)
	A/A	1 (4.1 %)	0 (0.0 %)	0 (0.0 %)	1 (4.1 %)
	Total	17 (50 %)	5 (27.8 %)	2 (22.2 %)	24

COMPARISON OF THE PERCENTAGES OF GENOTYPES WITH OTHER STUDIES IN RASA ARAGONESA RAMS

Percentages of genotypes according to *Rsa*I and *Mnl*I polymorphisms in a screening with 158 lambs

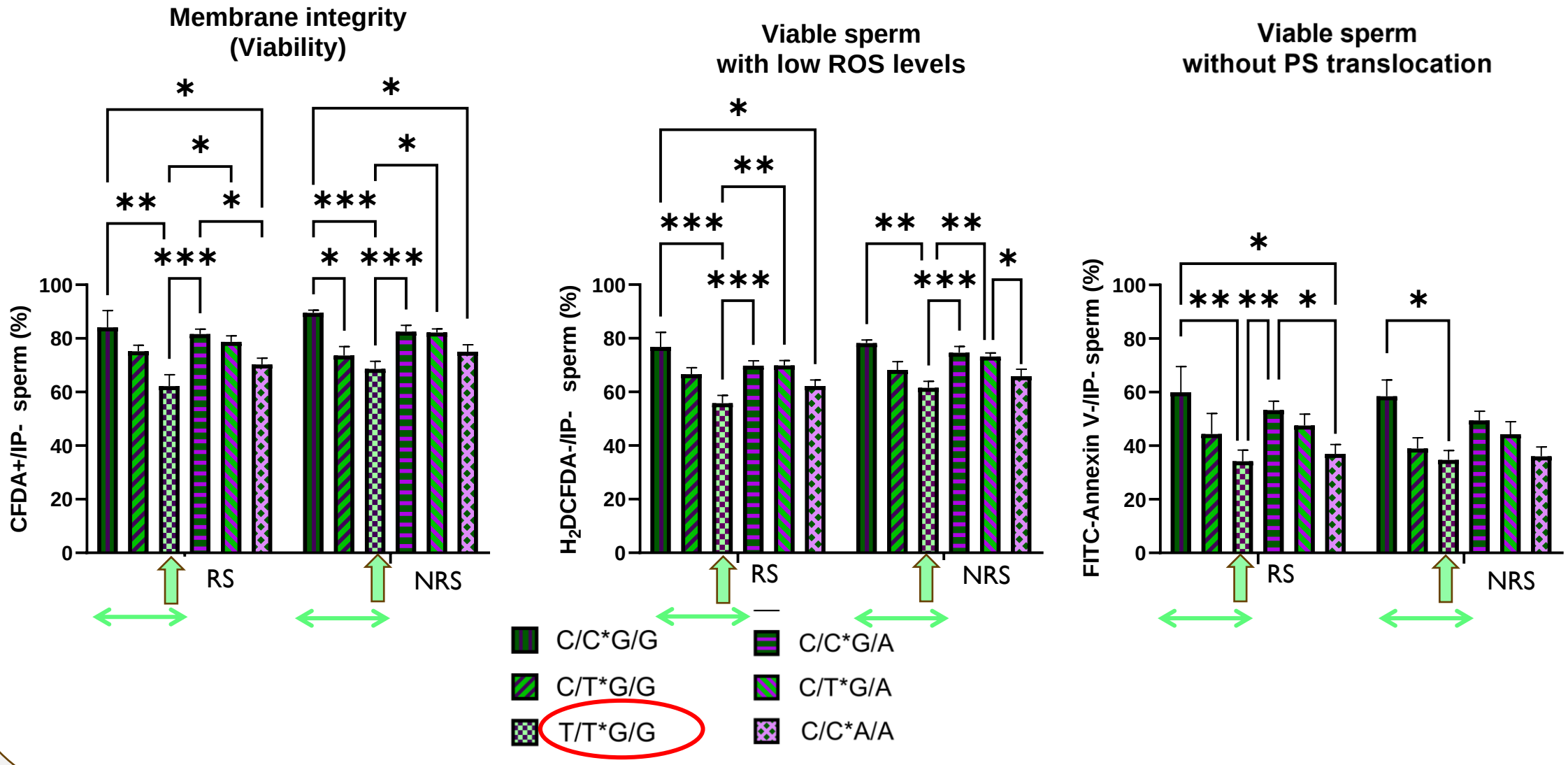
		***Rsa*I polymorphism (%)**			
		C/C	C/T	T/T	Total
***Mnl*I polymorphism (%)**	G/G	15.18	25.31	10.75	**51.26**
	G/A	18.98	18.98	0	**37.97**
	A/A	10.75	0	0	**10.75**
	Total	**44.93**	**44.30**	**10.75**	100

Poor reproductive performance?

Effect on embryo or neonatal survival?

3. IS THERE ANY RELATIONSHIP BETWEEN *MTNR1A* POLYMORPHISMS AND SPERM QUALITY?

EFFECT OF COMBINATION OF BOTH POLYMORPHISMS ON SPERM QUALITY



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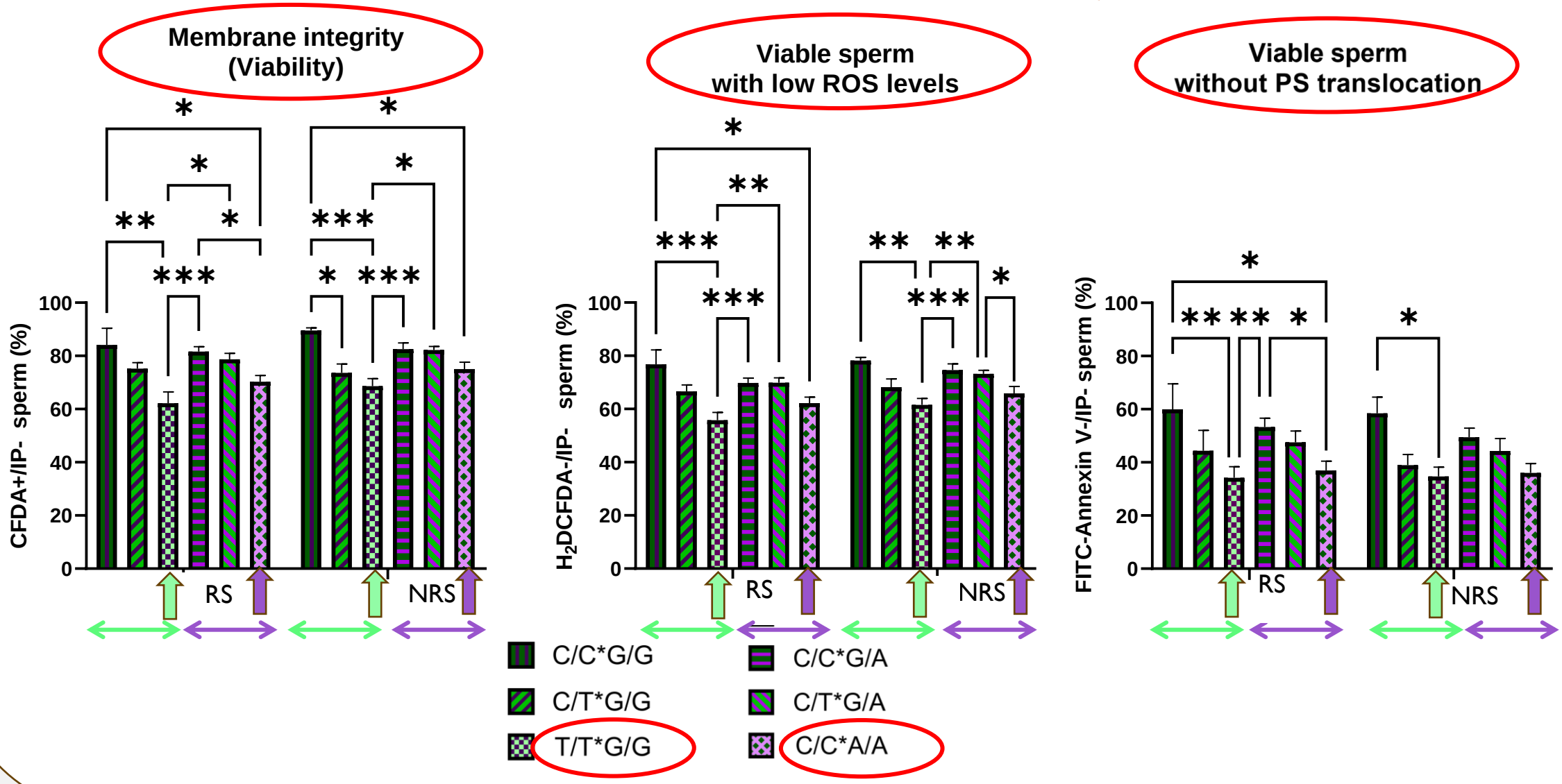


IN CONCLUSION

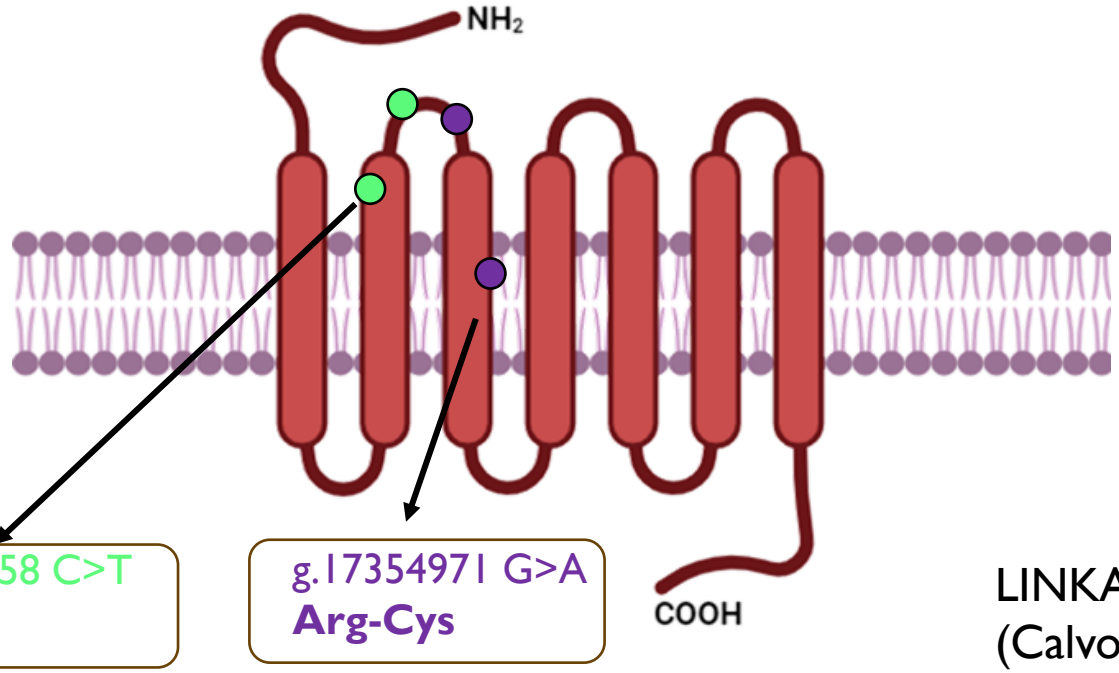
TT rams for *Rsa*I and AA for *Mnl*I polymorphisms presented lower seminal quality, particularly during the reproductive season

Mutations in the MT1 receptor gene in rams could reduce their seminal quality

IT IS IMPORTANT TO HIGHLIGHT THAT…

RsaI (g.17355458 C>T) | *MnlI* (g. 17355452 G>A)

SILENT MUTATIONS: no changes in the amino acid sequence of the protein (Carcangiu *et al.*, 2009)



LINKAGE DISEQUILIBRIUM (Calvo *et al.*, 2018)

Genotyping of rams based on Melatonin Receptor 1 (MT1) gene polymorphisms could be a powerful tool in male selection for sires in artificial insemination or natural mating programs

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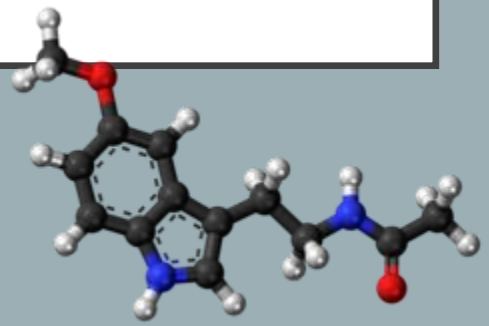
THE GENOTYPING OF RAMS BASED ON MELATONIN RECEPTOR 1 GENE POLYMORPHISMS COULD BE A USEFUL TOOL FOR A MORE CORRECT AND RATIONAL USE OF ANIMALS IN FARMING



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- Melissa Carvajal-Serna
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- Agustí Noya
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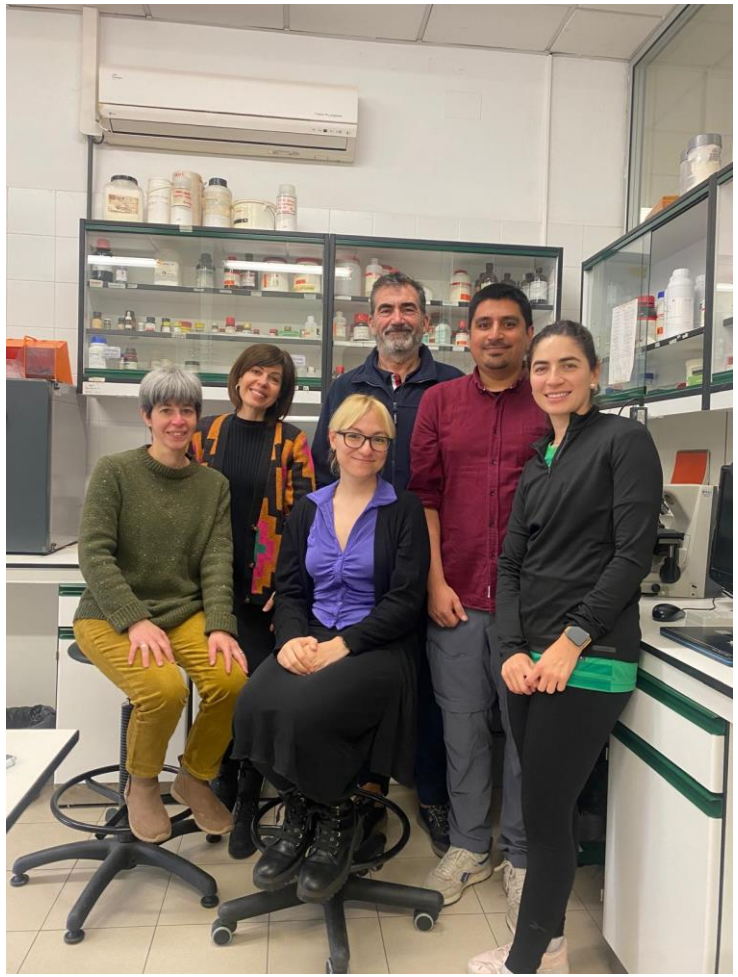

BIOFITER
BIOLOGÍA, FISIOLOGÍA Y TECNOLOGÍAS DE LA REPRODUCCIÓN

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- María Consuelo Mura
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ANGRA
Asociación Nacional de criadores de Ganado ovino selecto de raza Rasa Aragonesa

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Centro de Transferencia Agroalimentaria (CTA)

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THANK YOU!