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## ► To cite this version:

Marta Dordas-Perpinyà. Is AMH plasmatic level a biomarker to assess efficacy and reversibility of anti-GnRH immunization in stallions?. ESAR 2023, Sep 2023, Nantes, France. hal-04225747

**HAL Id: hal-04225747**

**<https://hal.inrae.fr/hal-04225747>**

Submitted on 3 Oct 2023

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# Is AMH plasmatic level a biomarker to assess efficacy and reversibility of anti-GnRH immunization in stallions?

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Anti-GnRH immunization = an alternative to surgical castration  
 ⇒ ↓ FSH & ↓ LH ⇒ ↓ spermatogenesis & ↓ steroidogenesis  
 ↓ size of testicles & scrotum, ↓ stallion behaviour ⇒ ↑ management  
 Theoretically reversible effects,  
 in stallions proved only after prime-immunizations  
 After several boosters ⇒ reversibility ?

AMH = Secretion of Sertoli cells  
 At puberty : spermatogenesis & steroidogenesis ⇒ ↓ [AMH]<sub>blood</sub>  
 Cryptorchids (ectopic intra-abdominal testis) : high [AMH]<sub>blood</sub>  
 ⇔ 0 spermatogenesis 0 steroidogenesis in ectopic testis

## Aim

Test 2 hypothesis :

- 1- ↓ FSH & ↓ LH ⇒ ↓ spermatogenesis & ↓ steroidogenesis induced by anti-GnRH immunization ⇒ ↑ [AMH]<sub>blood</sub> ?
- 2- Long time of repeated immunization boosters ⇒ long time without FSH and LH stimulation of Sertoli cells ⇒ definitive loss of secretory function of Sertoli cells ? ⇒ ↓ [AMH]<sub>blood</sub> ?

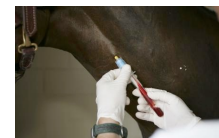
## Materials and Methods



Among 192 Lusitanian horses used for historical shows :  
 29 stallions (6 to 19 year-old) followed during 2 years blood sampled every month

- 5/29 controls : NEVER anti-GnRH immunized

Immunizations ⇔ 1ml Improvac® IM injections  
 Prime-immunization : 2 injections 28 days apart  
 11 months after previous injection, 2 booster-injections 28 days apart

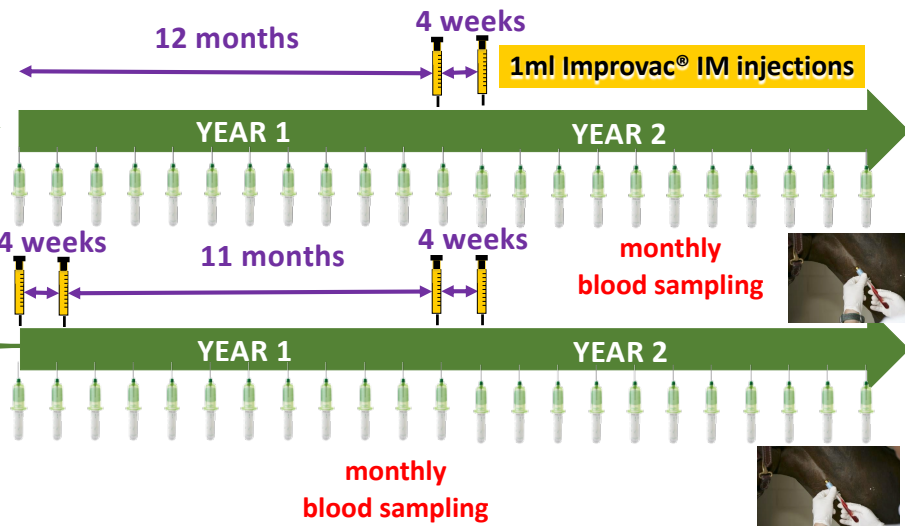


Immediately centrifugation  
 To freeze plasma until hormonal measurements

- 3/29 prime-immunized only Year 2

- 7/29 prime-immunized Year 1

- 12/29 already immunized before Year 1  
 for 4/12 : Year 1 = 1<sup>st</sup> booster injections  
 for 6/12 : Year 1 = 2<sup>nd</sup> booster injections  
 for 2/12 : Year 1 = 3<sup>rd</sup> booster injections

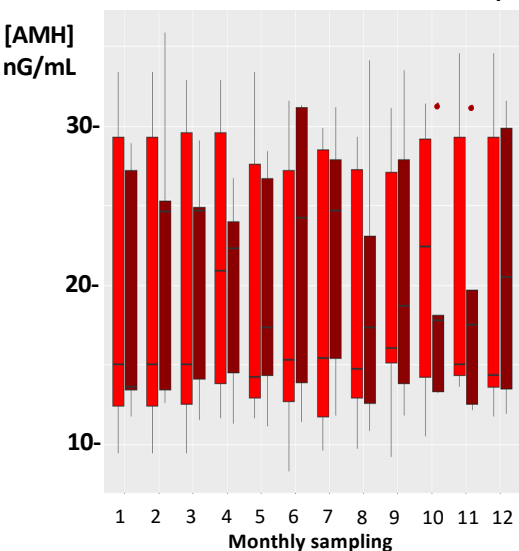


[E17β]<sub>plasma</sub> By ELISA kit (DE<sub>4399</sub>)<sup>®</sup>  
 [testosterone]<sub>plasma</sub>  
 [androstenedione]<sub>plasma</sub> } By RIA kits  
 Data for Year 1 presented in the XII<sup>th</sup> ISER  
 (J Equine Vet Sci 2018;66:26-28)

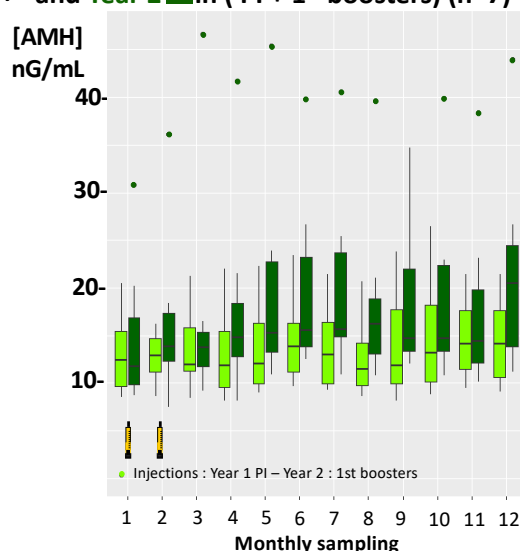
[AMH]<sub>plasma</sub> By AMH GENII ELISA kit<sup>®</sup>  
 Statistical analysis :  
 Linear mixed effects model,  
 ANOVA  
 Student's test on paired series  
 (same horse Year1 – Year 2)

## Results

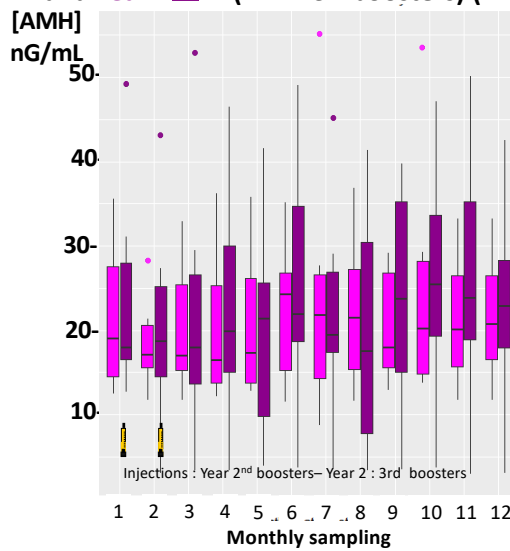
Comparison of [AMH]<sub>blood</sub> along Year 1 and Year 2 in controls not immunized (n=5)



Comparison of [AMH]<sub>blood</sub> along Year 1 and Year 2 in (PI + 1<sup>st</sup> boosters) (n=7)



Comparison of [AMH]<sub>blood</sub> along Year 1 and Year 2 in (2<sup>nd</sup> + 3<sup>rd</sup> boosters) (n=6)

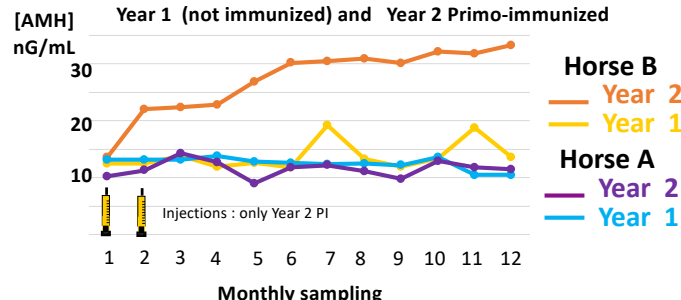


No significant difference  
 Between  
 Controls/immunized  
 groups  
 In each group  
 Between year 1 and year 2

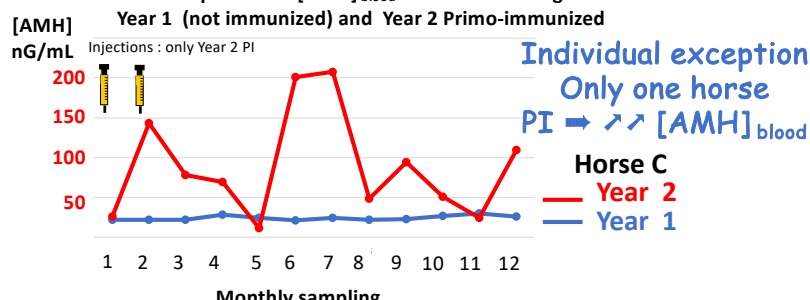
[AMH]<sub>blood</sub> ⇔ high individual variability

1<sup>st</sup> 3<sup>rd</sup> boosters ⇔ 0 effect on [AMH]<sub>blood</sub>

Comparison of [AMH]<sub>blood</sub> in 2/3 horses along Year 1 (not immunized) and Year 2 Primo-immunized



Comparison of [AMH]<sub>blood</sub> in 3<sup>rd</sup> horse along Year 1 (not immunized) and Year 2 Primo-immunized



Individual exceptions  
 - 1/6 : Year 1 = 2<sup>nd</sup> booster in year 2 after 1<sup>st</sup> injection  
 ↓ [AMH]<sub>blood</sub> < 4nG/ML (-80%)  
 - 1/2 year 1 = 3<sup>rd</sup> booster in all 24 samples  
 [AMH]<sub>blood</sub> < 4nG/ML  
 Low [AMH]<sub>blood</sub> ⇔ no more reversible ??

### Aknowledgments

For grants: ifce, Institut français du cheval et de l'équitation

For access to many males horses: PUYDUFOU

## Conclusion

Initial 2 hypothesis : not confirmed !

Few individual exceptions : hypothesis confirmed ? Why ?