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# Effect of anti-GnRH vaccine on Leydig cells in stallions

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**Anti-GnRH immunization = an alternative to surgical castration (↘ spermatogenesis & ↘ steroidogenesis)**  
↘ size of testicles & scrotum, ↘ stallion behaviour ⇒ ↗ management)  
**Theoretically reversible effects, and in stallions proved only after prime-immunizations**  
**After several boosters → reversibility ?**

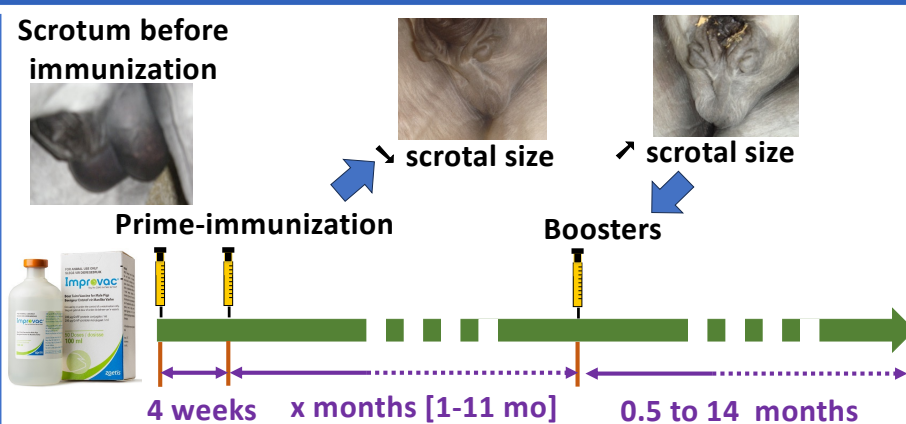
## Aim

Quantification of Leydig cell modifications induced by different numbers of anti-GnRH immunization injections

## Materials and Methods

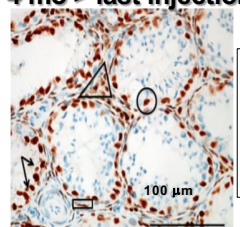


- Among 192 Lusitanian horses used for historical shows :
- 12 control stallions (unvaccinated) (4 to 9 year-old)
  - 28 anti-GnRH immunized stallions : (4 to 17 year-old)
  - 14/28 only prime-immunized (PI) (= 2 injections)
  - 4/28 PI + 1 booster (= 3 injections)
  - 2/28 PI + 2 boosters (= 4 injections)
  - 1/28 PI + 3 boosters (= 5 injections)
  - 4/28 PI + 4 boosters (= 6 injections)
  - 1/28 PI + 6 boosters (= 8 injections)
  - 2/28 PI + 8 boosters (= 10 injections)

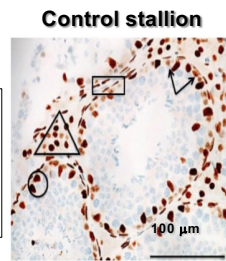


Immunizations ⇔ 1ml Improvac® IM injections

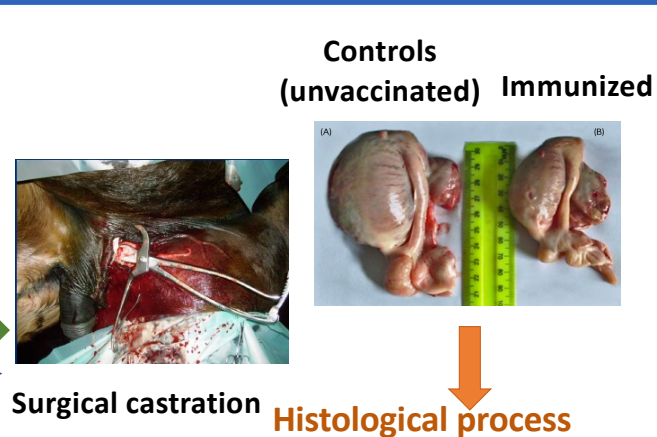
Immunized horse  
Castrated  
4 mo > last injection



△ Leydig cells  
□ myoid cells  
○ Sertoli cells  
→ spermatogonia



Statistical analysis by ANOVA

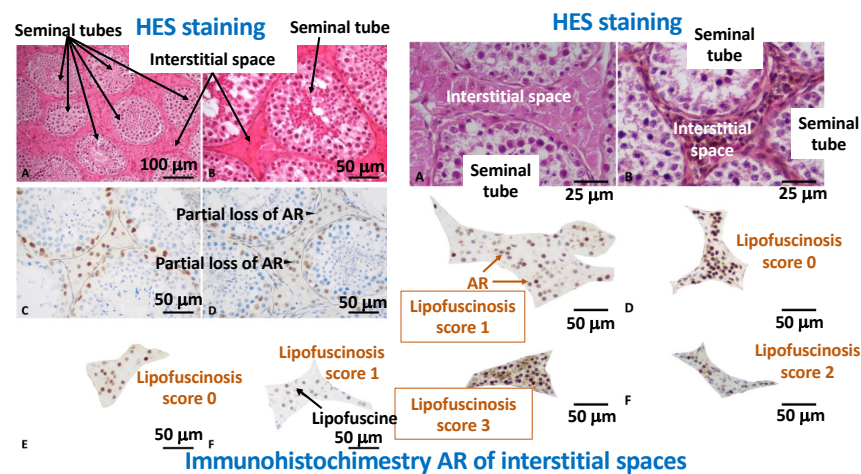
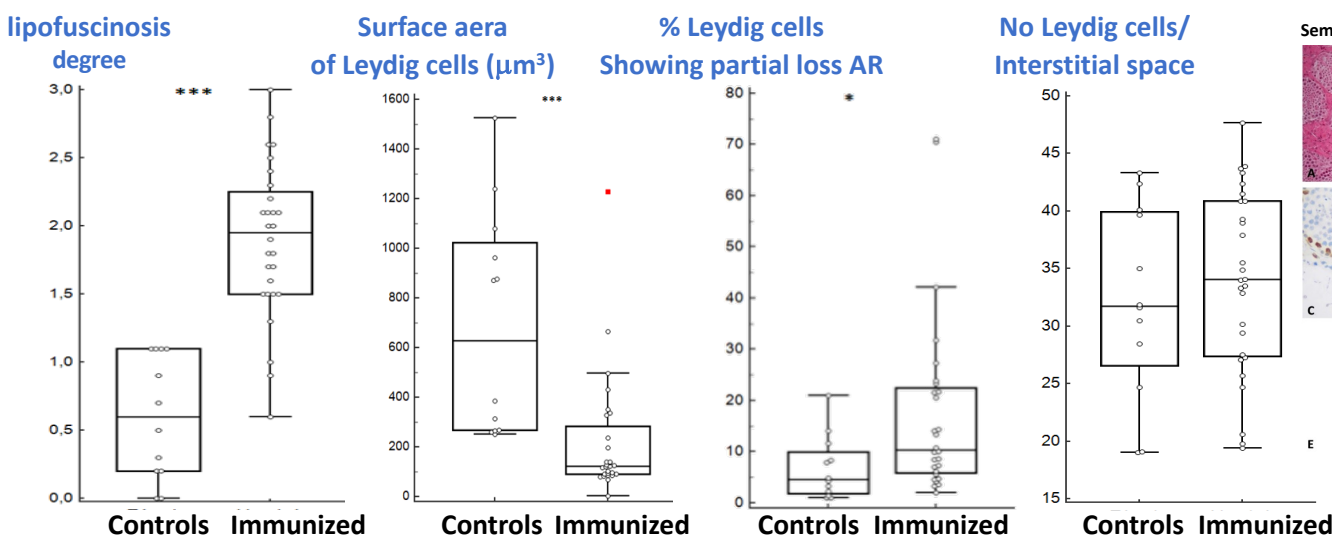


- Fixation in formaldehyde 10%
- Hematoxyline-Eosine-Saffron (HES) staining
- Androgen receptor (AR) immunohistochemistry

- Leydig cells evaluations on 15 interstitial spaces/horse**
- Lipofuscinosis degree (0 to 3)
  - Surface area
  - No Leydig cells/interstitial space
  - % Leydig cells showing partial loss of AR

## Results

Data about testicular size and histological evaluation of spermatogenesis were presented in the XIII<sup>th</sup> ISER (J Equine Vet Sci 2023;125:27)



Examples of histological views of testes from controls and immunized stallions

Leydig cells	CONTROLS Mean ± SD	IMMUNIZED Mean ± SD	P
Lipofuscinosis degree	0.6 ± 0.45	1.9 ± 0.6	0.001
Surface area (µm³)	221 ± 247	723 ± 489	0.001
% leydig cells showing partial loss of AR	7 ± 6	18 ± 18	0.05
No Leydig cells/ interstitial space	32 ± 8	34 ± 8	N.S

Anti-GnRH immunization  
→ No effect on number Leydig Cells  
→ ↘ size of Leydig cells  
→ ↘ expression of their androgène-receptors  
→ ↗ storage of lupofuscine  
Large individual variability of effects among the 28 immunized stallions

Effects significantly correlated with effects on both ↘ testicular size & ↘ spermatogenesis  
NONE correlation with both age of stallion, number of injections, time from the 1<sup>st</sup> immunization, time from the last immunization

## Conclusion

Anti-GnRH immunization → ↘ activity & functions of Leydig cells // ↘ steroidogenesis  
But none irreversible effect on Leydig cells (0 cellular degeneration 0 loss of hormonal reactivity) even after many boosters and long time of immunization ( many years)  
Large individual variability of time of effects after both prime-immunization and boosters.

## Aknowledgments

For grants



For access to many males horses

