



LIÈGE université
**Center for Interdisciplinary
Research on Medicines**

Liquid Chromatography and Mass Spectrometry assay for estrogens in mares' pregnancy

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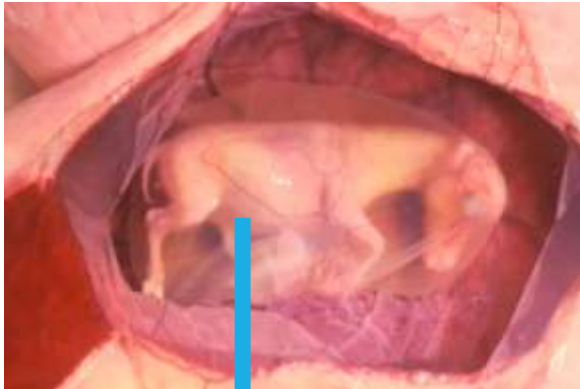
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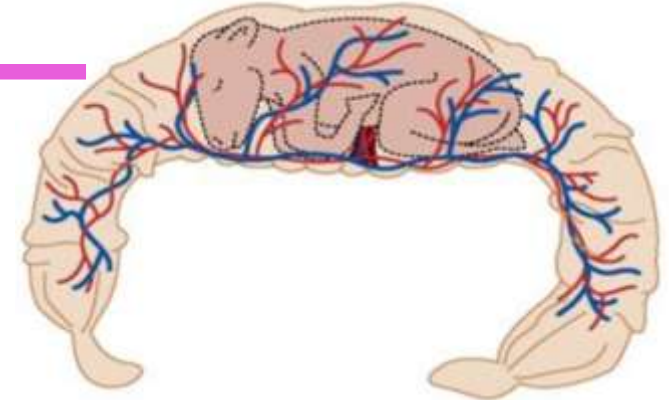
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Sexual steroids metabolism in mares

Equine conceptus



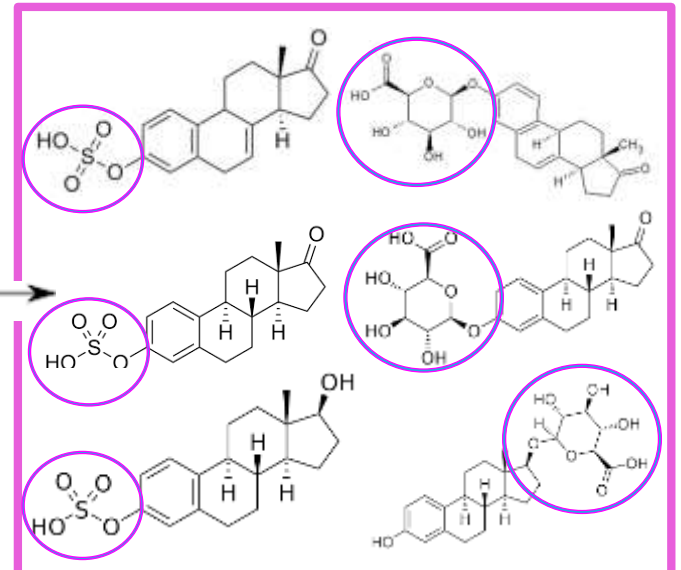
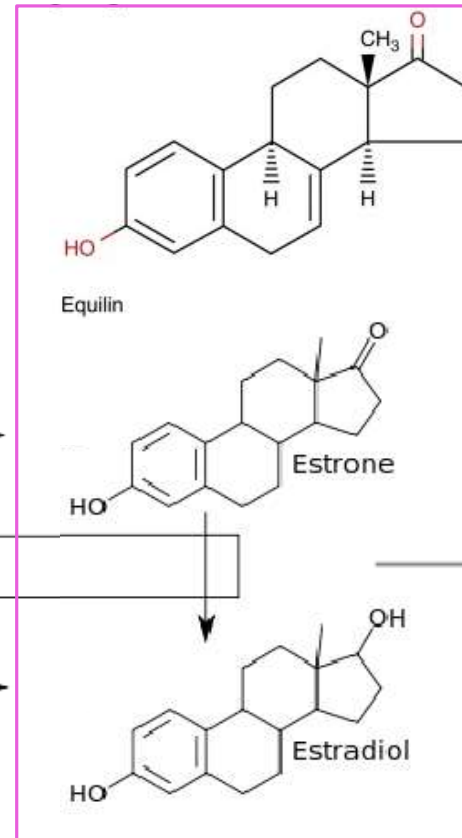
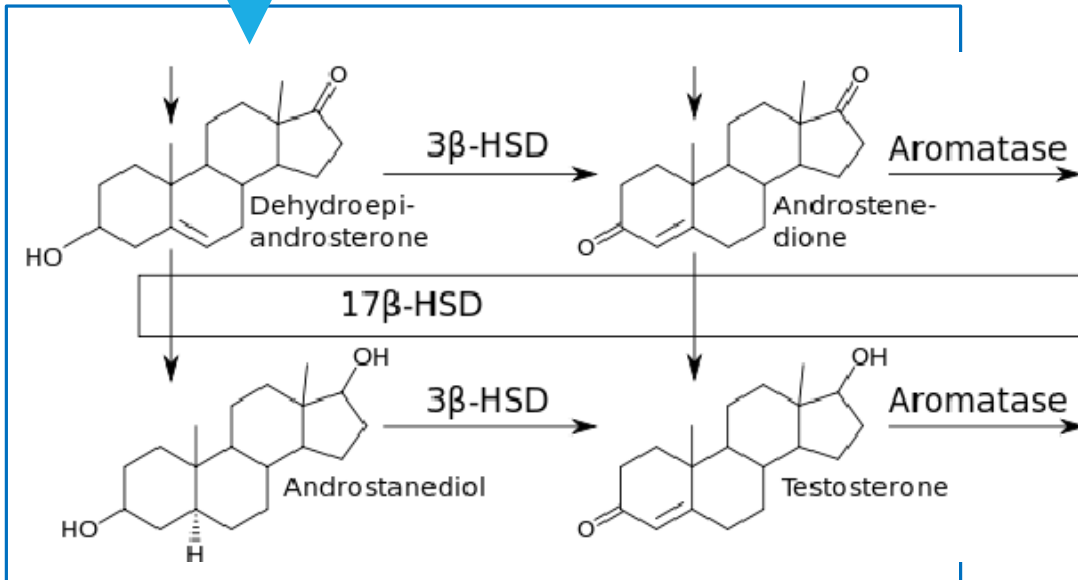
Allantochorion (Placenta)

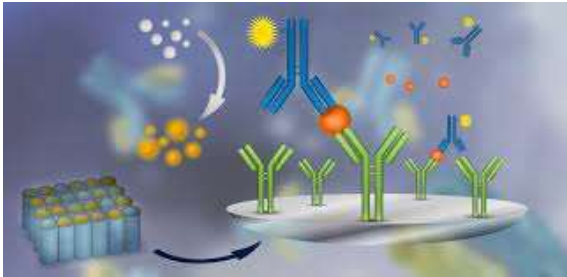


ANDROGENS

ESTROGENS

CONJUGATED ESTROGENS





Analytical method
currently used to
measure estrogens:
immunoassays

↓ Specificity and
Accuracy

Cross reaction

Not specific for the target
species

Aims of this study

- To determine [estrogens] from 4 to 11 months in healthy pregnancies using LC-MS/MS

↑ Sensitivity, precision
and accuracy

Double identification
(m/Z and RT)

Validated for the target
species





- To investigate potential relationship between [estrogens] in maternal sera and mare's age, parity and breed

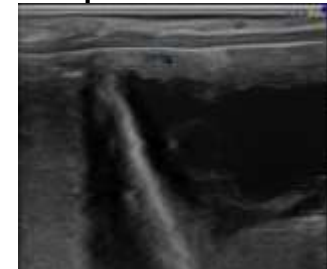
Methods

Statistics (Graphpad Prism®): Double blind prospective study

- *Kruskall-Wallis test*: [estrogens] between months with a Dunn post-test
- *Mann-Whitney test*: breeds at the same month of pregnancy for [estrogens]

Animals and sampling :

- 1x / month in 17 Spanish Purebred (SPB)  and 13 Show Jumping (SJ) mares 
- Transrectal ultrasonography (Renaudin *et al.*, 1997) and blood sampled
- Exclusion criteria : placentitis signs 



Ultrasonography
of the placenta



Placentitis



Weak foal

Determination of [estrogens] with LC-MS/MS (Dufour *et al.*, 2021):

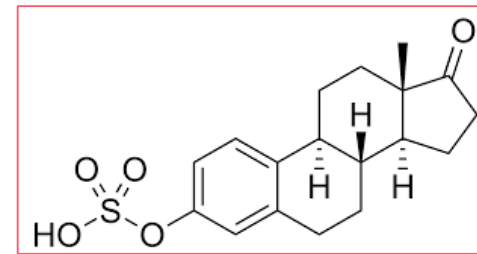
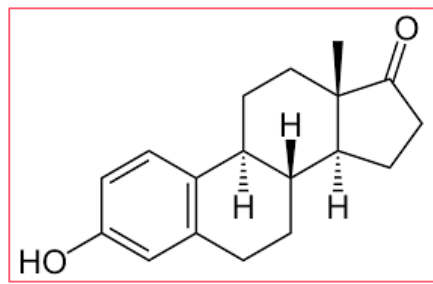
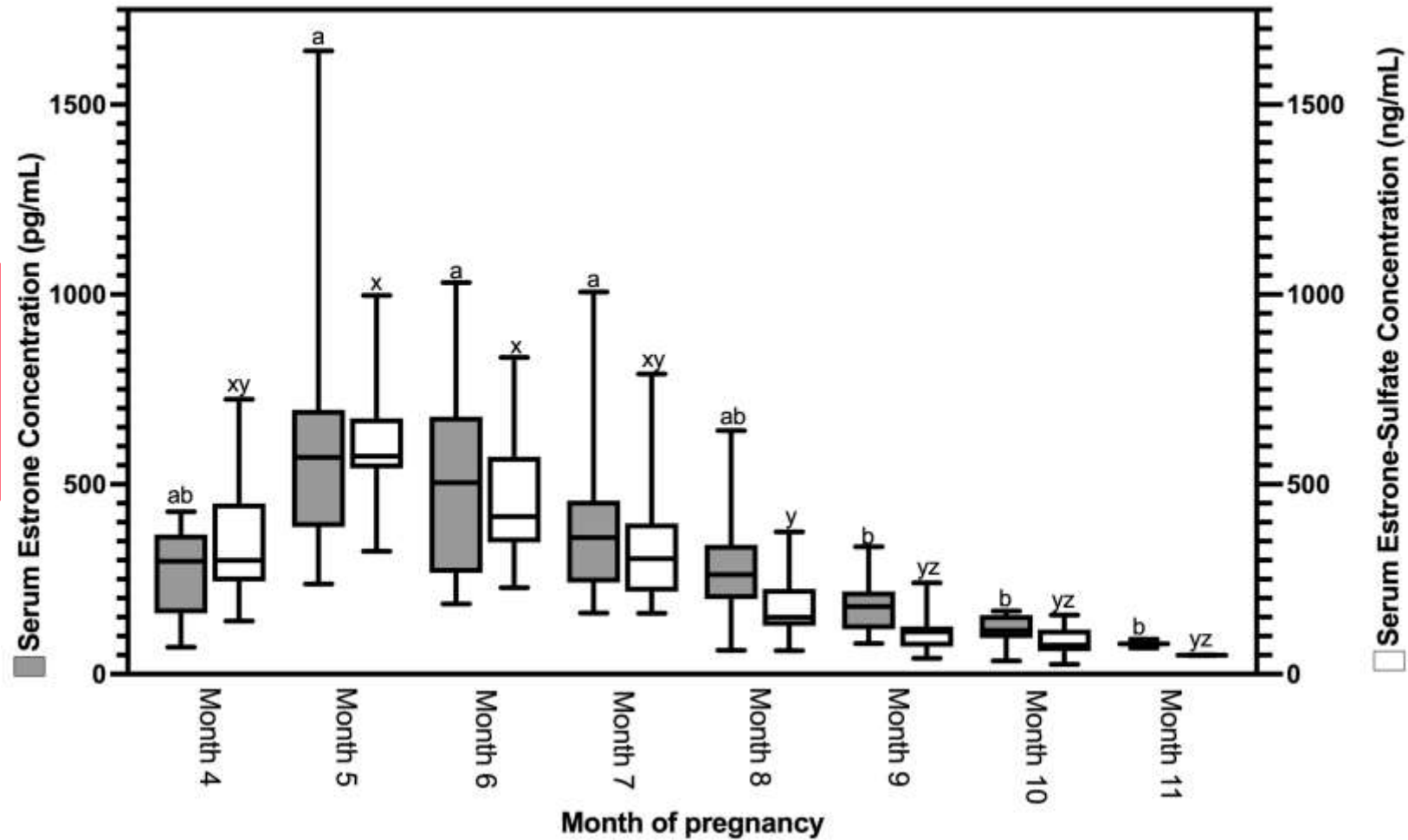
- ① Extraction with acetonitrile
- ② Evaporation
- ③ Derivatization with dansyl chloride
- ④ Injection for analysis

LLOQ for Estradiol: 2.0 pg/mL, Estrone: 2.0 pg/mL and Estrone-sulfate: 0.5 ng/mL



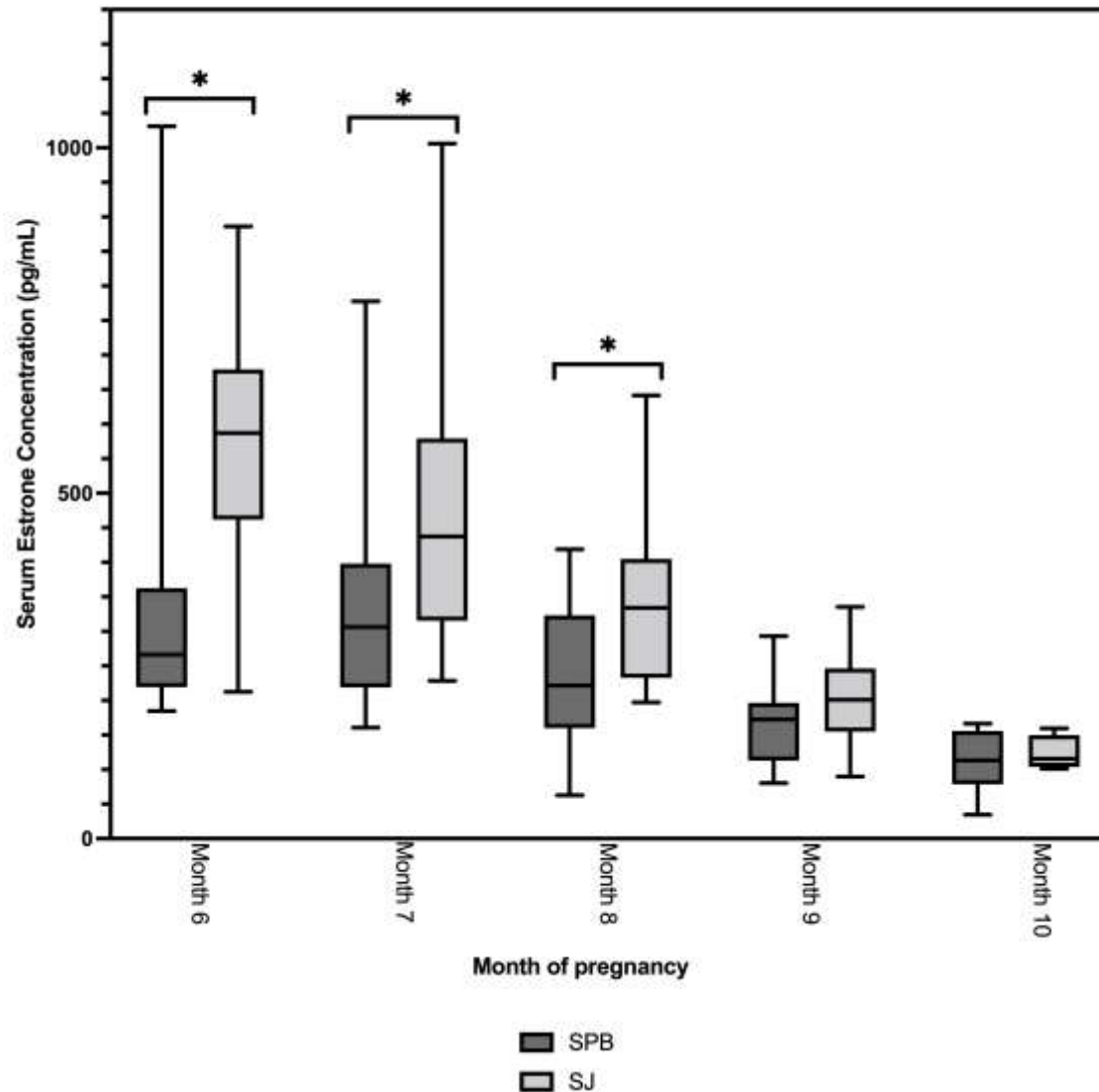
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Evolution of estrone (E1) and estrone-sulfate (E1S) serum concentrations from 4 to 11 months of pregnancy



Ledeck et al., Evolution of 17- β -estradiol, estrone and estrone-sulfate concentrations in late pregnancy of different breeds of mares using Liquid Chromatography and Mass Spectrometry. Theriogenology. 2022 Sep 1;189:86-91.

Comparison of Estrone (E1) concentration between Spanish PureBreed (SPB) and ShowJumping (SJ) type mares



Results

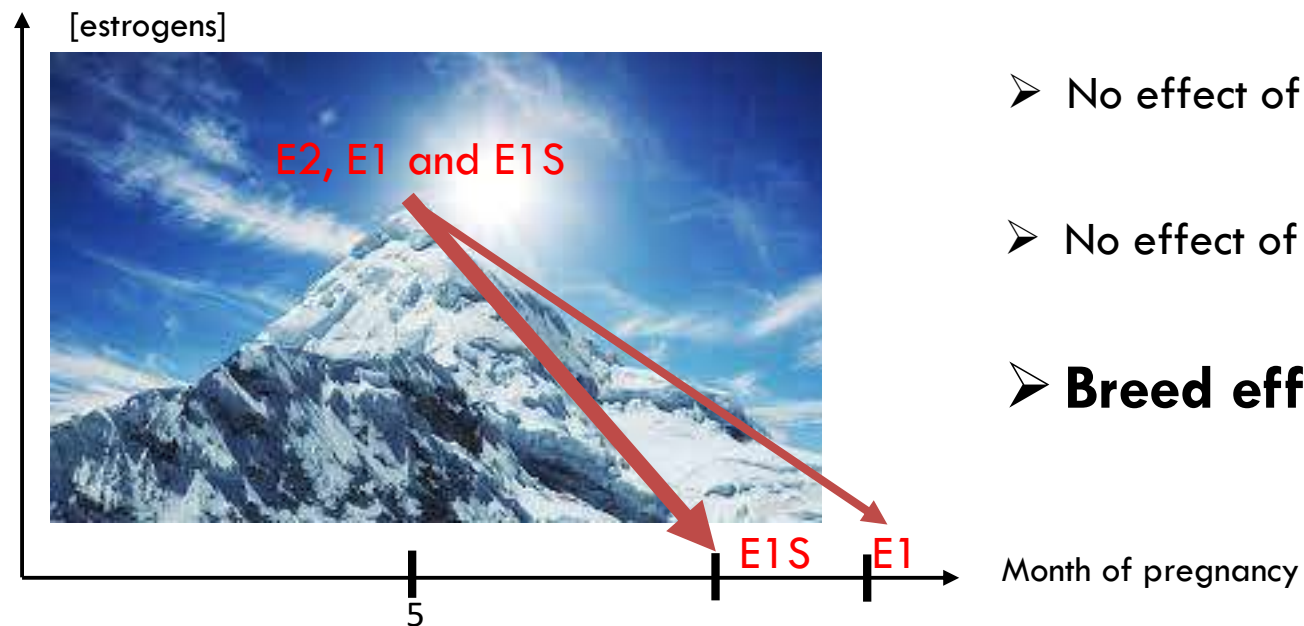
Spanish Purebred (SPB)



ShowJumping (SJ)



Ledek et al., Evolution of 17-β-estradiol, estrone and estrone-sulfate concentrations in late pregnancy of different breeds of mares using Liquid Chromatography and Mass Spectrometry. Theriogenology. 2022 Sep 1;189:86-91.



- No effect of age
- No effect of parity
- **Breed effect**

↓ sulfonation activity quicker than aromatization activity of the allantochorion

Spanish Purebred (SPB)



E1 and E2

<
E1S

=



Show Jumping (SJ)

= sulfonation activity of the allantochorion between breed

Perspectives: E1S remain a potential diagnosis of placentitis → Next step



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THANK YOU FOR YOUR ATTENTION!

