

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

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Introduction:

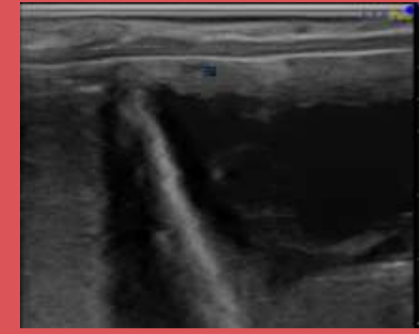
Placentitis

- ⇒ Ascending bacterial colonization of the placenta
- ⇒ Most important cause of abortion in mares

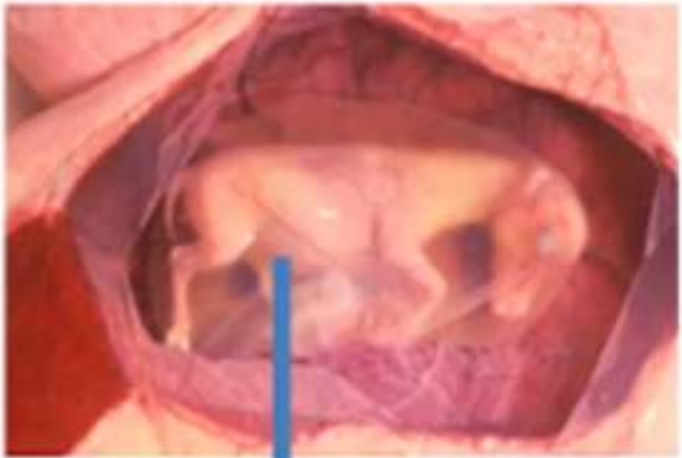
Actual diagnosis:

Ultrasonography but low sensitivity

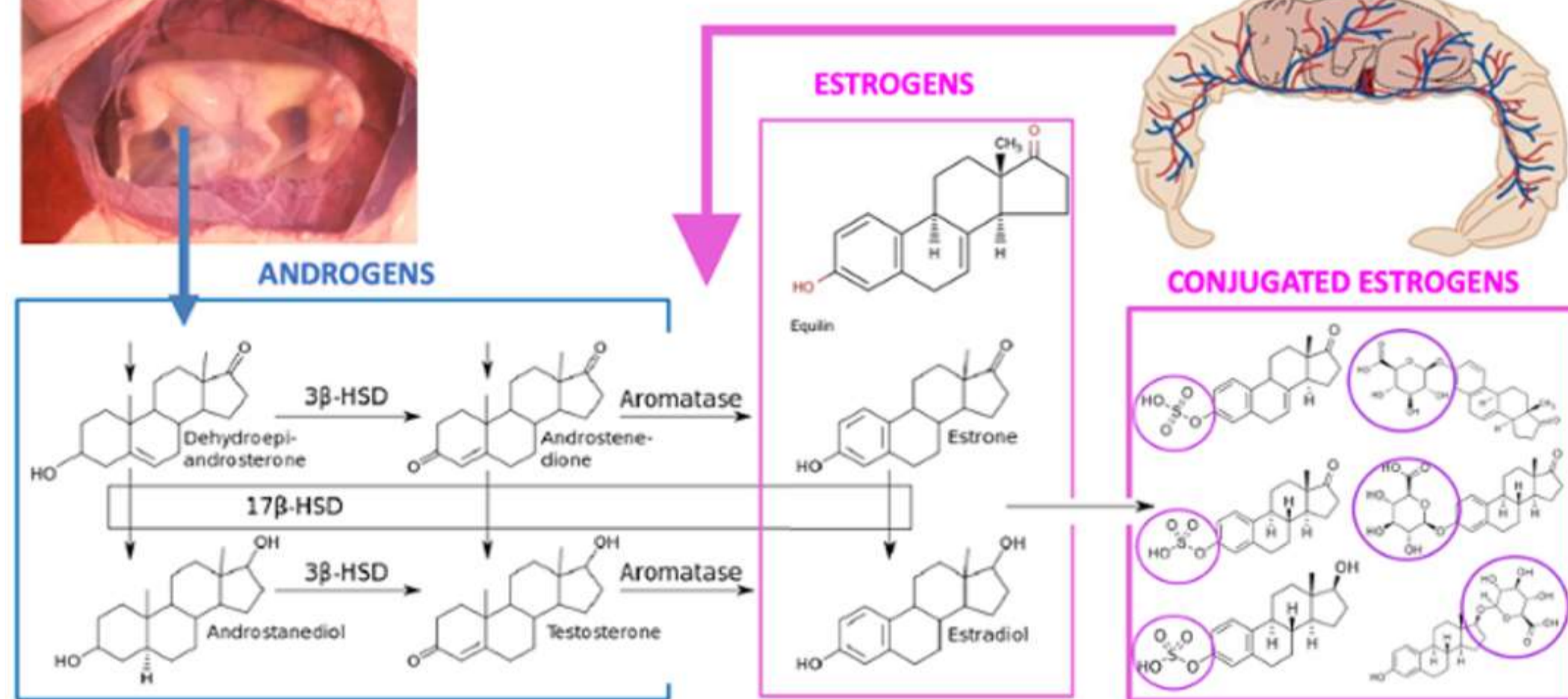
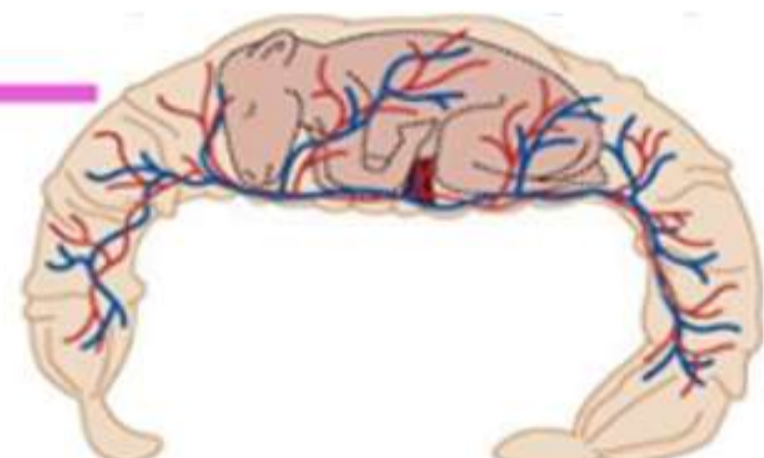
Placentitis ⇒ impairment of sexual steroids production



Equine conceptus



Allantochorion (Equine placenta)



Analytical methods currently used to measure estrogens :

⇒ IMMUNOASSAYS

- Lack of specificity and accuracy
- Cross reaction
- Not specific for the target species

Aims of this study:

- To determine [estrogens] from 4 to 11 months of pregnancy using Liquid Chromatography coupled to Mass Spectrometer (LC-MS/MS)
 - + Sensitivity, precision and accuracy
 - + Double identification of the compound (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:

Animals and sampling:

- 1x/month, in 17 Spanish Purebred (SPB) and 13 ShowJumping (SJ) mares



- Transrectal ultrasonography (Renaudin *et al.*, 1997) and blood sampled

- Exclusion criteria: signs of placentitis during pregnancy or after foaling



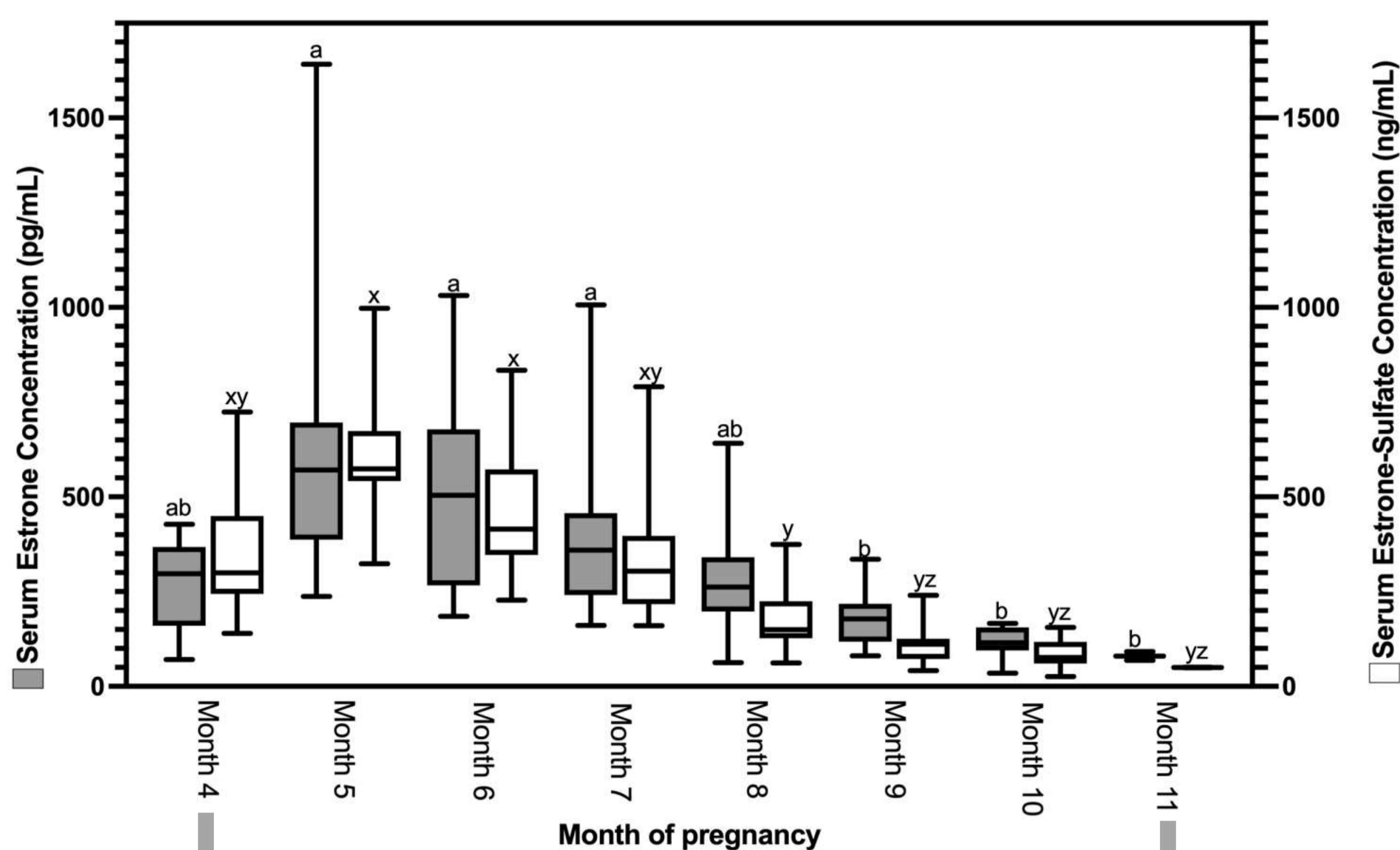
Determination of [estrogens] with LC-MS/MS (Dufour *et al.*, 2021):

- ① Extraction with acetonitrile
 - ② Evaporation
 - ③ Derivatization with dansyl chloride
 - ④ Injection for analysis
- LLOQ for Estradiol (E2): 2.0 pg/mL, Estrone (E1): 2.0 pg/mL and Estrone-sulfate (E1S): 0.5 ng/mL

Statistics (Graphpad Prism®): Double blind prospective study:

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

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



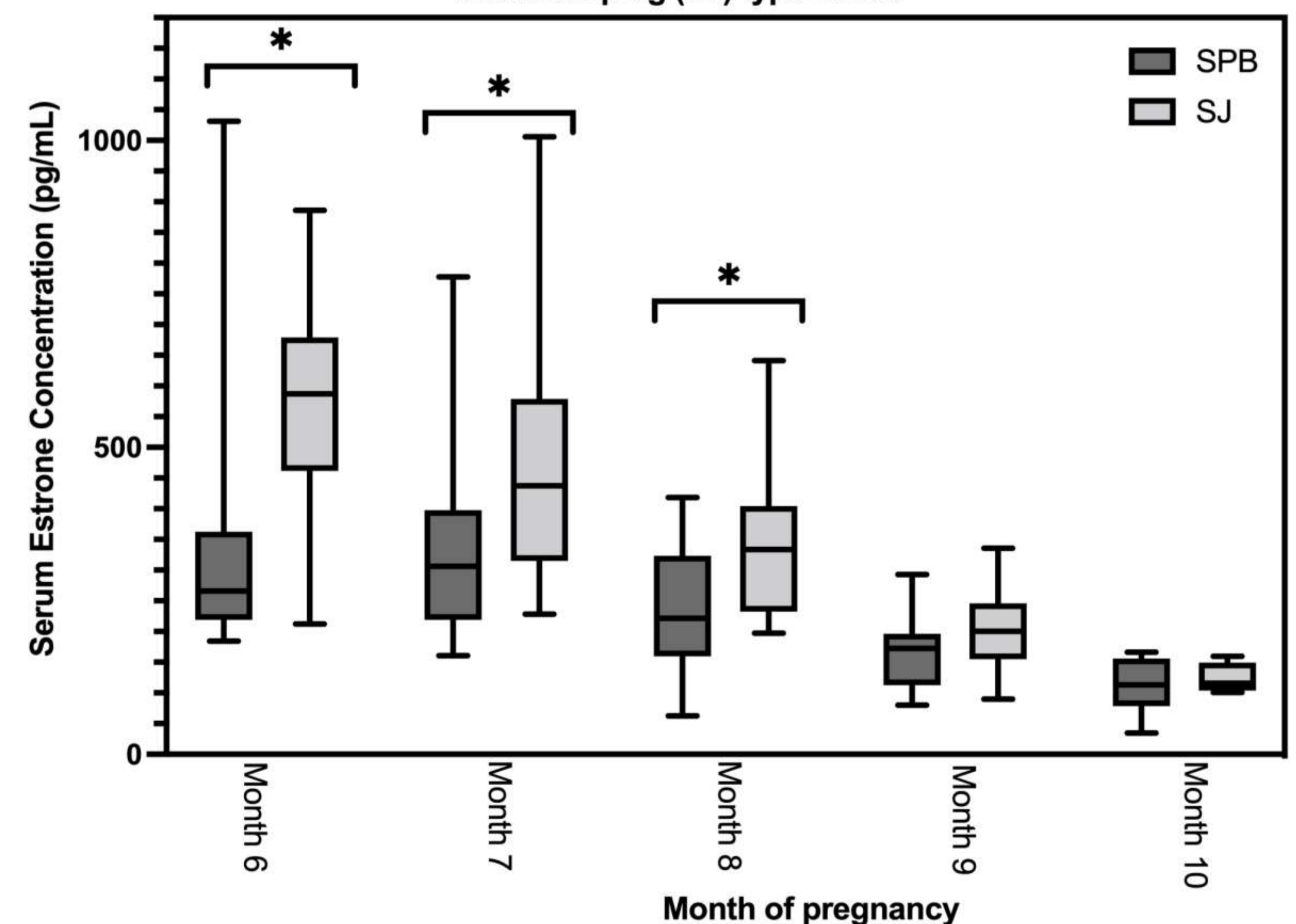
Results:

Peak values of E1 and E1S observed at 5 month
After 6 months, E1S was decreasing quicker than E1

Conclusion:

↓ sulfonation activity quicker than aromatization of the allantochorion

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



Results:

No effect of age and parity on [estrogens] but breed effect for non-sulfonated estrogens (higher in SJ than in SPB)

Conclusion:

Higher production of non-sulfonated estrogens in SJ > SPB = sulfonation activity of the allantochorion between breed

Perspectives:

No breed effect on E1S concentration, its measurement by LC-MS/MS assays would remain a potential diagnosis of placentitis.