Enrofloxacin administration to late pregnant mares does not cause articular lesions or decrease tendon strength in the resulting foals

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Enrofloxacin would be an excellent antimicrobial to treat bacterial infections in pregnant mares if proven safe. Previous work demonstrated that enrofloxacin crosses the equine placenta and reaches therapeutic concentrations in the fetal fluids without apparent affects on the fetus, and that ciprofloxacin, an active metabolite of enrofloxacin, accumulates in the allantoic fluid over time (i.e., up to 1,000 ng/mL). However, it is unknown if *in utero* exposure to enrofloxacin and ciprofloxacin affect cartilage development in the weight-bearing foal. We hypothesized that enrofloxacin administration to late-term pregnant mares would not produce cartilaginous lesions or tendonopathies in the resulting foal. The objectives of this study were to determine: (i) if *in utero* exposure to enrofloxacin and ciprofloxacin resulted in clinical lameness or lesions in articular cartilage or tendons of the foal, and (ii) if *in utero* exposure resulted in decreased tensile strength or cartilage thickness in the resulting foals. Clinically healthy mares (7-23 years old) were enrolled in the study at 280 days gestation, and were assigned to: 1) control (n=5), 2) therapeutic dose of enrofloxacin (n=6, 7.5mg/kg PO), or 3) double therapeutic dose enrofloxacin (n=6, 15 mg/kg PO). Enrofloxacin was administrated once a day for 14 days, starting 280 days gestation. Maternal plasma samples were collected daily and plasma preserved at -80ºC. Transabdominal ultrasound guided fetal fluid sampling was performed in a subset of mares (n=4) at 330-335d gestation, and fetal fluids were collected for enrofloxacin and ciprofloxacin concentrations. Mares were allowed to carry the foals to term. After foaling, mares and foals were maintained on pasture for 30d, until foal euthanasia. Proximal articular surfaces of foal long bones and fetal membranes were examined macroscopically and stained with HE for histopathological evaluations. Tissues were graded for structural, cellular, and extracellular changes. Tensile testing was conducted on superficial flexor tendons by loading until failure (Instron 8511, Norwood, MA). Histomorphometric and extracellular matrix analyses were evaluated using a non-parametric Kruskal-Wallis one-way analysis of variance. Enrofloxacin and ciprofloxacin concentrations were measured by LC-MS/MS. Tensile data were analyzed using ANOVA with repeated measures and Tukey post-hoc test in R Version 3.2.2 (https://www.r-project.org/). Significance was set at $p<0.05$. Peak plasma concentrations were 1040 ± 228 ng/ml (enrofloxacin) and 210 ± 25 ng/ml (ciprofloxacin) for the 7.5 mg/kg group and 1638 ± 246 ng/ml (enrofloxacin) and 327 ± 21 ng/ml (ciprofloxacin) for the 15 mg/kg group on day 1. Both enrofloxacin and ciprofloxacin were still detectable in the amniotic (7.4 ±4.7, 15.3 ±12.4 ng/ml) and allantoic (11.2 ±1.0, 148.3 ± 61.1 ng/ml) fluids thirty to forty days after administration of the last dose. No differences were seen in macroscopic, cytological, or extracellular matrix lesions in the cartilage of foals from control or treated groups. No differences in tendon yield stress were detected between the control (7.6± 1.0 MPa), 7.5mg/kg (8.6 ± 1.5 MPa) and 15 mg/kg (9.2 ± 1.9 MPa) groups. These findings suggest that enrofloxacin administration to the late pregnant mare reaches therapeutic drug concentrations in the fetus and fetal fluids without detrimental effects on the cartilage and tendons of the resulting foal. Additionally, ciprofloxacin concentrations in the allantoic fluid may remain elevated for many days after administration of last enrofloxacin dose. This suggests that enrofloxacin may be a useful antibiotic option for late pregnant mares suffering severe bacterial infections.

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