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Estradiol cypionate aided treatment for experimentally induced ascending placentitis in mares

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ABSTRACT

The overall goal of this study was to assess the efficacy of various therapeutic combinations of estradiol cypionate (ECP, a long-acting estrogen) and altrenogest (ALT, a long-acting progestin) in addition to basic treatment for placentitis with trimethoprim-sulfamethoxazole (TMS) and flunixin meglumine (FM). Specific outcomes measured in this experiment were (i) time from induction of bacterial placentitis to delivery, and foal parameters (high-risk, survival, and birth weight); and (ii) serum steroid concentrations (progesterone, 17α-hydroxyprogesterone, 17β-estradiol, and cortisol) in response to treatment. Pregnant mares (~300 days gestation, n = 46) were randomly assigned into healthy mares (control group, CONT, n = 8) and mares with experimentally induced ascending placentitis (n = 38). Placentitis was induced via intracervical inoculation of *Streptococcus equi subspecies zooepidemicus*. Thereafter, placentitis induced mares were randomly assigned into: (1) basic treatment, TMS+FM (n = 8); (2) basic treatment with ALT supplementation, TMS+FM+ALT (n = 8); (3) basic treatment with ECP supplementation, TMS+FM+ECP (n = 6); (4) basic treatment with ALT and ECP supplementation TMS+FM+ALT+ECP (n = 6); and (5) no treatment (INOC, n = 10). Treatments were started 48 h after bacterial inoculation and carried out for ten consecutive days. Blood samples were collected daily, and mares were assessed for signs of placentitis until the mare delivered, or for ten consecutive days after onset of treatment. Steroids were analyzed via RIA. Continuous data were analyzed by ANOVA, and categorical data analyzed by Fisher's exact test. Significance was set at $p < 0.05$. Foal survival at parturition and seven days post-delivery were similar across treated groups (66.7–100%), and to the CONT group. Similar to CONT group, mares in the TMS+FM+ECP group had no high-risk foals while mares in the other groups had higher incidences (50–75%) ($p < 0.05$). The inclusion of ECP in the treatments resulted in foals with body weight similar to CONT group ($p > 0.05$). There were no group effects or time by group interactions on concentrations of steroids assessed herein ($p > 0.05$). In conclusion, in addition to basic treatment TMS+FM, mares with experimentally induced ascending placentitis benefited from ECP supplementation. Conversely, ALT did not appear to make a difference in outcomes. The immunoassays used for measurements of steroid concentrations did not appear useful to assess treatment response.

1. Introduction

Ascending placentitis is an important cause of abortion, stillbirth and premature delivery of weak foals [1–3]. While there are regional variations in the bacterial and fungal agents associated with ascending placentitis in mares, β-hemolytic streptococci (*Streptococcus equi* subspecies *zooepidemicus* and *Streptococcus*

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equisimilis), and coliforms such as *Escherichia coli* are the predominant microbial isolates worldwide [1,4–6].

In ascending placentitis infection begins at the caudal placental pole (cervical star region), then bacteria spread cranial-ventrally towards the uterine body segments of the chorioallantois and gain access to the fetus either by migrating through umbilical vessels or through fetal fluids [6–8], consequently, reaching the fetus and becoming a potential cause of septicemia and fetal morbidity and mortality.

Placentitis is characterized by the production of pro-inflammatory cytokines such as IL-6 and IL-8, and prostaglandins (PGF2α and PGE2) [9,10]. Prostaglandin release increases uterine contractility and consequently increases the risk of premature delivery [11]. Inflammation and infection of the fetoplacental unit can induce premature activation of the fetal hypothalamic-pituitary-adrenal axis, thus accelerating fetal maturation before parturition [6,11,12]. Thus, early fetal maturation likely counterbalances premature delivery and may help improve the odds for foal survival [6,11]. It is worth noting that among farm animals, maturation of the equine fetus occurs latest in gestation [13]. This implies that any event that interferes with the normal function of the fetal-maternal unit such as placentitis or maternal disease could be devastating to the newborn foal.

Clinical diagnosis of ascending placentitis is based on the presence of clinical signs such premature udder development (with/without lactation), purulent/serosanguinous vulvar discharge, and ultrasonographic evidence of thickening and edema of the chorioallantois, and chorioallantois detachment from the endometrium at the caudal placental pole [14,15]. While overt clinical cases of ascending placentitis can be easily diagnosed, subtle and early cases can be missed using standard diagnostic means [6]. Recently, several molecular markers, including serum amyloid A, haptoglobin, 17β-estradiol, and alpha-fetoprotein have been identified as useful diagnostic tests for experimentally induced placentitis [16–21]. Some of these molecules are also suitable markers for spontaneous placentitis [20].

The equine fetoplacental unit is an intricate system involving the mare endometrium, the fetus, and the fetal membranes, where large quantities of steroids (estrogens, progestogens, and androgens) are produced and metabolized [18,22,23]. While the function of most equine fetoplacental steroids remains unknown, several studies have evaluated their concentrations to assess fetal well-being and placental health [12,18,24–26]. However, limited work has been carried out to determine the validity of using these steroid hormones as prognostic indicators in response to treatment of placentitis. Douglas [24] suggested that mares (from 100 d gestation to term) with low serum estrogen concentrations (<700 pg/mL), as determined by a commercial assay called "total-estrogens," were prone to abortion, whereas serum estrogen concentrations >1000 pg/mL resulted in the delivery of a live foal. In mares with experimentally induced ascending placentitis, progesterone concentrations were remarkably reduced in mares aborting in less than seven days compared to mares sustaining the pregnancy more than eight days post induction [25].

Treatment for bacterial placentitis is aimed at (i) eliminating or reducing the spread of microorganisms through the fetal membranes and fetus, (ii) keeping the uterus quiescent, and (iii) reducing the inflammatory response [6,27]. It has been suggested that these goals can be accomplished by treating mares with antimicrobials (i.e. a combination of penicillin and gentamicin, or trimethoprim-sulfamethoxazole), progestins (altrenogest or progesterone), and anti-inflammatories (flunixin meglumine, phenylbutazone, acetylsalicylic acid, pentoxifylline) [6,27–29]. If the treatment goals are accomplished, the gestation length of mares affected with placentitis should be similar to the expected normal duration of pregnancy (~330–340 days) and result in a live, well-developed foal with minimal health issues [6].

A number of controlled studies have shown the value of antimicrobial drugs, highlighting differences in drug selection (ability to cross membranes, the spectrum of activity, and potential toxicity to the fetus), duration of therapy, as well as immunomodulators in the treatment of experimentally induced ascending placentitis in mares [28–32]. However, the role of steroids hormone supplementation (estrogens and progestins) in the treatment of placentitis is poorly defined. Progestins have been included as a part of treatment in multiple placentitis studies [26,29–32], and one report failed to achieve an improvement in foal survival with the addition of altrenogest treatment [30]. However, it remains to be determined if progestins are beneficial for the treatment of placentitis. Estrogen therapy has been advocated as a necessary treatment for equine placentitis to reduce the risk of abortion [24]. Despite its anecdotal use in equine practice for years [24], to date, the treatment of placentitis with estrogens has not been critically evaluated under controlled experimental conditions.

The overall goal of this study was to assess the efficacy of various therapeutic combinations of a long-acting estrogen (estradiol cypionate; ECP) and a long-acting progestin (altrenogest; ALT) in addition to a basic treatment for placentitis with trimethoprim-sulfamethoxazole and flunixin meglumine (TMS+FM). Specific outcomes evaluated in this experiment were (i) time from induction of placentitis to delivery, gestational length, and foal parameters (high-risk, survival, and birth weight); and (ii) serum steroid concentrations (progesterone, 17α-hydroxyprogesterone, 17β-estradiol, and cortisol) in response to treatment. Our primary hypothesis was that the different treatment combinations (in particular ECP) would affect pregnancy outcomes and newborn foal parameters. Our secondary hypothesis was that measuring progestagens (progesterone and 17α-hydroxyprogesterone), 17β-estradiol, and cortisol could be used to assess response to treatment for experimentally induced ascending placentitis in mares. It was our expectation that the information obtained from the present study would enhance our understanding of the efficacy of various drugs and drug combinations in the treatment of equine placentitis.

2. Materials and methods

2.1. Mares and animal husbandry

All procedures carried out in the present study were approved by the Ethical Committee on Animal Experimentation of the Universidade Federal de Pelotas (UFPel) under protocol # 4750. Animal procedures carried out herein followed the guidelines of the European Union Directive (2010/63/EU) for animal experimentation. The mares were housed at Palma Farm of the UFPel, Capão do Leão, Rio Grande do Sul, Brazil. Forty-six pregnancies from 27 multiparous Criollo and Criollo-type mares (age 10 ± 2 years; parity 3 ± 0.5; body weight 437 ± 22 kg) were used in the experiment. None of the mares enrolled in this study had a history of subfertility or late-term pregnancy abnormality. Ovulation was determined by transrectal palpation and ultrasonography examinations performed every other day. All mares were bred via artificial insemination with fresh semen from a single fertile Criollo stallion (1.72 breeding/conception). Mares were maintained on pasture and supplemented with commercial concentrate pellets and water *ad libitum*. Before foaling, mares were kept in individual stalls at night and on pasture during the day. This study was carried out during the natural breeding season of the Southern Hemisphere from September–December for the years of 2012, 2013, and 2014.