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Comparison of different regimens of estradiol benzoate treatments followed by long-acting progesterone to prepare noncycling mares as embryo recipients

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ABSTRACT

The present study evaluated the influence of different regimens of estradiol benzoate (EB) treatments followed by a single dose of long-acting progesterone (LA P4) on plasma estrogen and P4 concentrations in noncyclic mares prepared as embryo recipients. Twenty-one anestrous mares were distributed into three groups (n = 7 mares per group), according to the EB dose received (single dose of 2.5 mg, total of 5 mg in decreasing doses, and total of 10 mg in decreasing doses), which was followed by a single administration of 1500 mg of LA P4 in all groups. Mares were reevaluated during the ovulatory phase and seven of them became part of the cyclic nontreated control group. Ultrasonography was performed to monitor endometrial edema, and blood samples were collected to measure estradiol (E2), estrogen conjugate (EC), and P4 by RIA. Maximum uterine edema was achieved 24 hours after administration of EB in all treated groups. Maximum E2 concentrations were observed 24 hours after the first EB injection in treated groups and there were no differences ($P > 0.05$) among treatments. Maximum EC concentration was observed 24 hours after the single EB injection in the 2.5-mg group, whereas in the 5- and 10-mg groups EC peaks were observed 48 hours after the first EB administration. Maximum P4 concentrations were detected 24 hours after LA P4 injection, although higher P4 concentrations were observed in the group treated with 2.5 mg of EB than in that treated with 10 mg of EB ($P < 0.05$). Because P4 concentrations were reduced after administration of high doses of EB, we also measured 17α-hydroxyprogesterone (17-OH-P) to test the hypothesis that high concentrations of EB would accelerate the conversion of P4 to 17-OH-P. However, 17-OH-P concentrations paralleled P4 profile in all groups, irrespective of EB doses. In summary, the three EB treatment regimens induced similar E2 peaks, although the observation of EC peaks 24 hours after E2 peaks in the 5- and 10-mg groups indicate that an excess of E2 was given, which was converted into EC to be inactivated. Administration of 10 mg of EB reduced P4 concentrations 24 hours after LA P4 was given. We demonstrated that the mechanism by which this reduction occurred was not by

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an increase in P4 metabolism to 17α-OH-P. In conclusion, the use of 2.5 mg of EB followed by 1500 mg of LA P4 appears to be a more appropriate regimen to treat noncyclic mares, although additional studies are needed to verify embryo survival with this treatment dose.

1. Introduction

Equine embryo transfer (ET) is a worldwide technique in which the highest number of transferred embryos per year is found in Brazil, Argentina, and the United States [1]. However, a major limiting factor in the ET programs is the reduced number and quality of recipient mares during the breeding season and especially during the early spring transitional period [2]. For some horse breed associations in the southern and northern hemisphere, the use of recipient mares early in the year outside the normal breeding season (spring through early fall) is very desirable [3] because there is economic pressure for foals to be born early in the foaling season to enhance their athletic performance.

To increase the availability of recipient mares in ET programs, exogenous estrogen and progesterone (P4) treatments are usually administered to noncyclic mares [2–6]. Currently, noncyclic recipient mares often receive estradiol benzoate (EB) [2,7,8], an ester of the natural estrogen 17β-estradiol (E2) with an approximate half-life of 3 days [9]. After the observation of endometrial edema, long-acting progesterone (LA P4; half-life of 7 days) is administered 3 to 8 days before ET [3,10], or altrenogest is given during 4 to 6 days before transfer [2,11], to achieve an appropriate progesterone concentration (≥2.5 ng/mL) [6] at the time of ET. These hormones (natural or synthetic) induce similar uterine changes to those which occur in cyclic mares [12], such as endometrial edema caused by estradiol followed by increased uterine tone and stimulus to histotrophic secretion as a result of exogenous progestin treatment [13]. The hormonal changes prepare the uterus for a possible pregnancy.

Although protocols for exogenous P4 have already been described regarding suitable P4 concentrations for pregnancy maintenance [14], EB administration is empirical with different reports of dose and administration frequencies for preparing noncyclic recipient mares such as a single dose of 2.5 mg [2,11] and decreasing doses of 5 [15] or 10 mg [7,8].

To our knowledge, there are no studies comparing EB regimens to select the treatment that causes endometrial edema and produce similar estrogen concentrations to those found in cyclic mares during estrus. The knowledge of an exogenous estrogen protocol that is more compatible with endogenous estrogen concentrations found in cyclic mares would optimize the hormone regimen in noncyclic embryo recipient mares. Therefore, the objective of this study was to evaluate different regimens of EB followed by a single dose of LA P4 on plasma estrogen and P4 concentrations in noncyclic mares and evaluate the degree of endometrial edema after treatments.

2. Material and methods

2.1. Animals and experimental groups

Twenty-one cross-bred mares from 5 to 15 years of age, weighing between 350 and 450 kg, were used in the study. Mares were maintained on coast-cross hay (*Cynodon dactylon*) with water and trace-mineralized salt *ad libitum*. The experiment was conducted from June to December in Botucatu, São Paulo, Brazil (latitude 22°53′09″ and longitude 48°26′42″). In the southern hemisphere, June through early September are winter months, and late September through early December is the spring season. Animal procedures were approved by the Ethics Committee on Animal Use at the School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (CEUA-200/2014).

Data were collected from anestrous mares that were used later in the season for data collection during the cyclic phase. Anestrous mares were those showing ovarian follicles less than 20 mm in diameter, absence of a CL, and P4 concentrations <1 ng/mL on three evaluations at 7-day intervals. Data from all anestrous mares were collected in July and August, under short-day photoperiod. Cyclic mares were those showing regular estrous cycles with ovulatory follicles and presence of a CL at least once in 21 days. Data from cyclic mares were collected between October and December, under increasing photoperiod.

The 21 anestrous mares selected to the experiment were randomly distributed into three groups (n = 7 mares per group), according to EB regimen followed by the same dose of LA P4: 2.5 mg EB + LA P4, 5 mg EB + LA P4, and 10 mg EB + LA P4. After treatments, the anestrous mares were monitored until cyclic activity was detected. Once in the cyclic reproductive phase, seven mares were randomly chosen out of the initial 21 to be part of the control group. Data collection in the control group started from the third estrous cycle of the breeding season. A cyclic nontreated control group was used to compare artificially induced reproductive phases to physiological reproductive phases.

2.2. Hormone treatments

Mares from the group 10 mg EB + LA P4 received 10 mg of EB (Estrogin, im) in decreasing doses (5, 3, and 2 mg on consecutive days), and 24 hours after the last EB administration, 1500 mg of LA P4 (Sincrogest Injetável, im) was given (Fig. 1A). The group 5 mg EB + LA P4 received 5 mg of EB in decreasing doses (3 and 2 mg on consecutive days), and 24 hours after the last EB administration, 1500 mg of LA P4 was given (Fig. 1B). The group 2.5 mg EB + LA P4 received a single 2.5-mg dose of EB, and 48 hours after its administration, mares were treated with 1500 mg of LA P4 (Fig. 1C). Control group consisted of cyclic mares that did not receive EB and LA P4 treatments. During the cyclic phase of the control group mares, ovulation induction was performed using 1500 IU of hCG (Vetecor, iv) after detection of a 35-mm follicle or more and uterine edema to synchronize evaluation days between natural and artificial cycles (Fig. 1D).