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Impact of a progesterone-releasing intravaginal device and inflammatory reaction on ovarian activity in embryo-recipient anestrus mares

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ABSTRACT

This study aimed to correlate the inflammatory reaction (IR) caused by a progesterone-releasing intravaginal device (P4) with ovarian activity and pregnancy rate (PR) in embryo-recipient anestrus mares (to decrease the spring transitional period). 50 animals were assigned to three groups: GP4 (P4 group; n = 16), GP4OH (P4 + oxytetracycline hydrochloride and hydrocortisone sprayed onto the device; n = 14), and GNP4 (no intravaginal P4; n = 20). The administration protocol for GP4 was: Day 0, 750 mg P4 + ovarian examination by ultrasonography (US) + vaginal sample collection; Day 8, US; Day 11, P4 removal + 7.5 mg PGF2α + US + second vaginal sample collection; Days 13 to 16, US; Days 17 to 21, US + 750 IU hCG to mares with follicles 35 mm or more in diameter; Days 19 to 23 US (ovulation check); Days 24 to 28, embryo transfer + intravenous flunixin meglumine; and Days 30, US pregnancy diagnosis. The GP4OH and GNP4 mares received the same administration protocol as GP4, except that no P4 device was administered to the GNP4 group on Day 0. Although neutrophil-mediated IR occurred in the GP4 and GP4OH groups, the IR was significantly reduced in GP4OH as compared with that in GP4 ($P < 0.0001$). From Day 0 to Day 17, the GP4 and GP4OH mares developed a greater number of follicles per animal than did the GNP4 mares ($P < 0.05$), and the average diameter of the follicles was larger in the GP4 and GP4OH mares. The ovulation rates in GP4, GP4OH, and GNP4 mares were, respectively, 43.7%, 64.3%, and 30.0%, and ovulation occurred at 6.8, 6.5, and 23 days after P4 removal ($P < 0.05$). On Day 17, endometrial edema was verified in 50%, 64.2%, and 35.0% of the GP4, GP4OH, and GNP4 mares, and the PRs after embryo transfer were 80%, 100%, and 66.6%, respectively. Although intravaginal devices caused IR in both the device-recipient groups ($P = 0.0001$), IR and vaginitis had no negative impact on follicle diameter, ovulation rate, period to ovulation after the removal of P4, endometrial edema, or PR. In addition, P4 reactivated the ovarian function and the IR eliminated a large percentage of bacteria (*Bacillus* spp., *Enterobacter* spp., *Proteus* spp., *Pseudomonas* spp., and *Staphylococcus* spp.), especially in GP4; the application of oxytetracycline hydrochloride and hydrocortisone on the devices reduced the severity of vaginitis.

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1. Introduction

Brazil has the largest population of horses in Latin America and the third largest in the world. The combined population of horses, mules, and donkeys is close to 8 million, and horse production alone generates millions of dollars annually [1].

Mares are physiologically polyestrus and exhibit seasonal cycling in the spring and summer in response to the increase in the duration of daylight [2]. An annual transitional phase before the regular estrus cycles occurs in horses (i.e., exit from anestrus progressing to the first ovulation) [3,4]. This transitional phase is characterized by irregular cycles and a high incidence of large anovulatory follicles [5] that become atretic [6].

Brightness management (increasing hours of light/day) and feed or hormone management protocols have been used to reduce the duration of the transitional phase. Among others, artificial lighting for 60 days [7]; administration of GnRH or its analogs [8], FSH [9], or dopamine antagonists [10]; and devices with long-acting progestogens (DP4) have been employed [11,12]. The use of a progesterone-releasing intravaginal device (P4) helps mares in the transitional phase [12], stimulates ovarian activity, and synchronizes estrus [13], follicular wave, and ovulation [14].

From a strategic perspective, the use of P4 artificially increases the luteal phase, blocking the hypothalamic-pituitary axis [15]. However, it also results in early diestrus surges of FSH that initiate the development of follicles [16]. The secretion of FSH is not affected by P4 but is regulated by estradiol and inhibin produced by the follicles during the diestrus phase [17].

The efficiency of P4 is closely related to the season of the year. They are most effective at the end of the transitional phase than during the anestrus or early transitional phases because of the inadequate response of the hypothalamic-pituitary axis [12,13,18–21].

The use of DP4 is limited in routine practice because they cause vaginitis [12], which is characterized by mild-to-moderately cloudy vaginal discharge [13,22], mainly containing *Streptococcus zooepidemicus* [20]. However, vaginal discharge ceases 48 hours after the removal of the devices, causing no endometritis or pregnancy-related issues [6].

Whereas the uterine environment is free of pathogens, active vaginal flora is rich in nonpathogenic microorganisms [23,24]. The composition of the vaginal flora undergoes changes in response to exogenous and endogenous factors [25]. Bacteria such as *S. zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* have been isolated from the clitoral cavity, vestibulum [24], and deep vaginal walls of healthy mares [26].

Any changes in the vaginal flora, like those resulting, for example, from the use of antibiotics, can result in an imbalance of the microflora. This leads to the selective multiplication of microorganisms that were previously inhibited, which now start to colonize not only the vagina but also the uterus [27]. Therefore, we hypothesize that a combination of antibiotics and corticosteroids may prevent or at least reduce the severity of vaginitis caused by the use of the P4 intravaginal device without compromising the ovarian activity and PR.

The present study aimed to examine the relationship between the inflammatory reaction (IR) caused by the P4 11 days after insertion and the reproductive parameters, including ovarian follicular recruitment, follicular dynamics, ovulation, and pregnancy, in crossbred embryo-recipient anestrus mares.

2. Materials and methods

2.1. Embryo-recipient mares

This study included 50 embryo-recipient mares (Mangalarga × Quarter horse) produced for commercial purposes. The average age of the mares was 5.7 years (range, 4–12 years) and the average body condition score was 3.0 (1 = very thin; 5 = fat [28]). The animals were grazed in the paddocks of *Cynodon dactylon* (15.0%–35.0% crude protein; 63.0% total digestible nutrients), with free access to water and mineral salt. The paddock was located at latitude 25°32′05″S and longitude 49°12′23″W, and the average temperature was 12 °C in July 2014.

The embryo-recipient mares were randomly subjected to transrectal palpation and ultrasound examinations based on their ovarian (follicles) findings and absence of uterine edema as the criteria; animals with follicles less than 15 mm in diameter [2] and no CL [29] were considered to be in the anestrus phase and those with follicle diameters of 20 to 25 mm [12], in the late transitional phase. The exclusion criteria were as follows: the presence of uterine edema and follicles 35 mm or more in diameter [30] on ultrasonography (US), history of treatment with antibiotics and/or anti-inflammatory drugs for less than 15 days, presence of an exudate in the vaginal vault, and body condition score less than 2 [28]. The presence of endometrial edema and endometrial folds, along with the estrous behavioral manifestation, was considered a function of circulating estrogen [31].

The recipient mares were divided into three application groups (Experimental protocol, Fig. 1): (1) GP4 (group with a progesterone-releasing intravaginal device [P4]; n = 16); (2) GP4OH (group with a P4 with oxytetracycline and hydrocortisone sprayed onto the device; n = 14); and (3) GNP4 (control group with no P4 device; n = 20). The administration protocol for all three groups was the same (except for GNP4, which did not receive the P4 on Day 0): Day 0, P4 + ovarian US + sample collection (SC) from the vagina; Day 8, US; Day 11, P4 removal + PGF2α + SC + US; Days 13 to 16, US; Days 17 to 21, US + detection of follicles 35 mm or more in diameter and/or grade two or three uterine edema (scale according to Ley, 2006 [32]) + 750 IU hCG; Days 19 to 23, US for ovulation check; Days 24 to 28, embryo transfer; and Days 30 to 34, US pregnancy diagnosis.

After establishing the application groups, the animals were examined by transrectal US (5.0-MHz linear transducer; Mindray 2200; China) on Day 0 (start of the administration protocols), Day 8, Day 11, Days 13 to 16, Days 17 to 21, Days 19 to 23, Days 24 to 28, and Days 30 to 34 to track the ovarian follicular dynamics, ovulation, embryo