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Hormonal Strategy for Advance of Mating Season in Mares Scored on Follicular Diameter

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Abstract

The equine productive in Brazil has been distinguished by the horses athletes. In this scenario, we highlight the use of reproduction biotecnology, which look to reproduce the best animals and increase the pregnancy rate through the induction of the first ovulation of the year. The objective of this study was to use intravaginal progesterone devices in mares with follicular diameters greater and smaller than 20 mm, aiming to advance the first ovulation of the mating season. Twenty-two crossbred mares, classified as: G1 - animals with a follicle ≤ 20 mm, and G2 - animals with follicles ≥ 20 mm, were given intravaginal progesterone device. After seven days, the follicular growth in the two groups was checked by daily ultrasonography. Device were removed when the animals got a follicle ≥ 35 mm and, one day after implant withdrawal, animals have reached 35 mm in both groups have had their ovulation induced. The data were tabulated and the statistical analyses of the variables were done using the procedure PROC GENMOD of the statistical program SAS, version 8.01. The percentage of animals that responded to the treatment, that is, had a dominant follicle greater than 35 mm, was significantly higher (P<0.05) in the animals of the G2 group (62.5%), compared to G1, which presented 28,5mm. The average time, in days, for the appearance of the dominant follicle was also higher (P<0.05) for the G2 group. The use of progesterone devices anticipated the first ovulation of the mating season, showing the efficiency and viability of technique.

Keywords: Equine Reproduction; Progesterone Implant; Reproductive Season

INTRODUCTION

In Brazil, the productive horse chain has shown significant growth in recent years. Data showed that the annual gross revenues in this chain increased from R$ 7.5 billion in 2006 to R$ 16 billion in 2015 (Lima and Cintra, 2015), as well as being an activity that generates many direct and indirect jobs (MAPA, 2017).

This growth happens mainly due to the creation of horses destined to the sports segment. Unlike the animals directed to the labor segment, usually associated with cattle breeding, the sport or recreational horse requires more exceptional care and investments (Lima and Cintra, 2015). In this scenario, the use of the reproductive biotechniques in the equine species becomes essential to reproduce only the best animals (Bortot and Zappa, 2013).

Considering the reproductive aspects, it should be regarded as that mares have particular characteristics, such as seasonality, which makes them be considered as not very productive. An alternative to increase the reproductive period of mares is to use artificial cycles, administering exogenous hormones (Jardim et al., 2015). Several techniques have been studied to anticipate the first ovulation of the mating season; among these techniques, the use of progesterone is highlighted (P4) (Rodrigues et al., 2012).

The Intravaginal devices of P4 may be efficient in inducing the first ovulation of the year in some mares since, in the equine species, the P4 does not inhibit the folliculogenesis and the ovulation (McKinnon et al., 2011). The use of P4 is based on the hypothesis that the abrupt increase and decrease of P4 levels in anestrus mares can stimulate the production and release of GnRH by the hypothalamus, with consequent increase in the release of LH and FSH by the pituitary, fundamental for the stimulation of follicular growth and ovulation (Ginther, 2017).

Using the P4 device, the dominant follicle present at the time of insertion regresses, but in response to the decrease of the P4 level, a new follicle develops after ten days of treatment, reaching 35 mm. Therefore, when the device is removed, mares rapidly enter estrus and ovulate within 4 to 8 days (Staempfli et al., 2011).

The success of treatment with P4 depends on the period and the follicular diameter, and the more developed the transitional period, the higher the probability of the mare ovulates after the treatment (Polasek et al., 2017). According to McKinnon et al. (2011), the mares that best respond to this technique are those with multiple follicles with a diameter between 20 and 30 mm, regardless of whether they are presenting estrus or not.

Currently, it is a consensus among researchers to seek for sustainability in several segments, including in the agricultural sector, which is notoriously related to the evolution of the production systems (Balbino et al., 2012).

Considering the scarcity of work related to sustainable production in the equine chain, mainly about the use of breeding biotechniques, the objective of this study was to use intravaginal progesterone devices in mares with follicular diameters greater and smaller than 20mm, aiming to advance the first ovulation of the mating season.

MATERIALS AND METHODS

The experiment was carried out at Farming School/ BIOTEC, at the University Center of Maringá / UNICESUMAR, Maringá, state of Parana (23 ° 25'S, 51º57'W and altitude of 550 meters), from 08/20/2017 to 10/20/2017.

Were used Twenty-two crossbred mares, with the score of the body condition and ages compatible with reproductive activity, between 5 and 12 years old. The animals were kept in paddocks, with Tifton 85 grazing, supplemented with concentrate, water and mineral salt *ad libidum*. All the mares were with the vaccination and the verification updated.

The mares were evaluated by ultrasound examination (Aloka SSD-500 ™) twice, with an interval of 10 days, to verify the conditions of the uterus and ovaries, as well as to observe the presence or absence of corpus luteum, characterizing or not the anestrous. The diameter of the follicles was also measured through the mean of their horizontal and vertical measurements, which allowed classifying the animals in the two studied treatments: group 1 (G1), composed of 14 animals, with the presence of follicles less than or equal to 20mm, and group 2 (G2), with 8 animals with follicles greater than or equal to 20mm in diameter.

After the ultrasonographic examination, the perianal and vulvar region of the animals were washed and the bovine intravaginal device (DIB® - Zoetis, São Paulo - SP, Brazil), containing 1g of progesterone, was introduced in all of them without the plastic rope located in the lower end. A solution of terramycin and hydrocortisone (Terra-Cortril Spray®, Pfizer, New York-NY, USA) was sprayed on the implants before placing them in the animals to avoid possible vaginitis. Also, if the animals stayed for 14 days with implants, also avoiding vaginitis, the devices would be removed and the animals classified as non-responsive to the treatment.

After seven days, the two groups were undergone to daily sonographic examinations to verify follicular growth and the presence or absence of uterine edema.

The implants were removed when the animals had a follicle greater than or equal to 35mm (preovulatory) and on the same day 2.5mg of estradiol benzoate (BE) was applied. One day after the withdrawing of the implant, all the animals had their ovulation induced by the use of a combination of hCG (Vetecor ®, Hertape, Juatuba - MG, Brazil) with deslorelin (GnRH - Sincrorrelin ®, Ourofino, Cravinhos - SP, Brazil), in the recommended doses by the manufacturer, being 1500 IU and 750 µg, respectively. The ovulation of all the animals was verified by sonographic examination, approximately 40 hours after the induction.

Analyses statistical: After the experiment was carried out, the data were tabulated and the statistical analyzes of the variables were done using the procedure PROC GENMOD of the statistical program SAS (2000), version 8.01.

The protocols of the biotechniques used to collect the data of this research were approved by the Committee of Ethics in the Use of Animals of the University Center of Maringá (008/2017 CEUA) / UNICESUMAR.

RESULTS AND DISCUSSION

The results of the animals percentage that responded to the treatment, in other words, presented a follicle larger than 35mm in diameter, the time for the appearance of the dominant follicle, time for ovulation in days and the rate of ovulation in crossbred mares during the transition phase from anestrous to reproductive season, treated with an intravaginal P4 device, as a function of the diameter of the follicle, are described in Table 1.

The percentage of animals that responded to the treatment, that is, had a dominant follicle greater than 35 mm, was significantly higher ($P<0.05$) in the animals of the G2 group (62.5%), compared to G1, which presented 28,5mm. The average time, in days, for the appearance of the dominant follicle was also higher ($P<0.05$) for the G2 group.

Data close to those obtained in this research were reported by De Oliveira Filho et al. (2012), who observed that in an experiment with Quarter Horse mares, after removal of the P4 implant, 80% (8/10) of the mares had a dominant follicle. Only 20% (2/10) did not present.

A higher number of females with dominant follicle in the spring transition, using P4, was observed by Botelho (2012). In the experiment performed with Mangalarga Marchador mares treated with P4, the author found that 77.27% (34/44) had the dominant follicle, whereas in the control group only 22.7% (10/44) were detected.

Table 1 - Percentage of animals that responded to treatment, the time for the appearance of the dominant follicle, size of the dominant follicle at the moment of the implant withdrawal, time for the ovulation and ovulation rate in crossbred mares treated with intravaginal progesterone device, in the function of the follicle diameter.

Variable	G1	G2
Animals that responded to the treatment (%)	28,5 ± 0,46[a]	62,5 ± 0,51[b]
Time (days) for the appearance of the dominant follicle (35 mm)	8,0 ± 2,23[a]	9,50 ± 0,57[b]
Time for ovulation (days)	3,0 ± 0,66[a]	3,0 ± 0,50[a]
Ovulation rate (%)	80,00[a]	100,00[a]

1[a,b] equal letters in the same line do not differ to each other statistically (P>0.05).
2 G1 = animals with the presence of a follicle less than or equal to 20mm at the time of implantation of the device containing progesterone; G2 = animals with the presence of a follicle greater than or equal to 20 mm at the time of progesterone implantation.

The findings of this research showed that the device did not cause suppression of follicular growth, contrary to that reported by Schutzer (2012), who observed inhibition of follicular growth during treatment with P4.

Most of the P4 experiments mention in the literature are based on the hypothesis that the increase and subsequent abrupt drop in P4 levels in anestrus mares may stimulate the production and release of GnRH by the hypothalamus, with increased release of LH hormones and FSH by the pituitary gland, which is essential for the stimulation of follicular growth and ovulation (McKinnon et al., 2011). Using the P4 device, the dominant follicle present at the time of insertion retreats, although in response to the falling of the P4 level, a new follicle develops after ten days of treatment reaching 35mm. Therefore, when the device is removed, the mares rapidly enter in estrus and ovulate after a few days, on average 4 to 8 (Staempfli et al., 2011), reported that agrees with the findings of this research.

It was possible to verify in this study that the follicles presented a normal growth, however, at the moment of implant removal, no differences were observed (P<0.05) in the diameter of the largest follicle (24.14 mm vs. 31.75 mm)

Handler et al. (2006) did not find differences in the diameter of the dominant follicle in mares treated with P4 device as well. Polasek et al. (2017), working with crossbred mares, reported that the duration of P4 insertion had no effect on the diameter of the largest follicle, however, researchers reported that the animals in the control group presented smaller diameters (30.0mm) when compared to animals from the P4 implant group (33.80mm).

The treatment with P4 does not inhibit the release of the follicle-stimulating hormone (FSH). Thus, follicular development continues and follicle diameter increases, even during the treatment period (Guinter, 2017). Reway (2017) experimented with devices containing 1.44 g of P4 in crossbred mares aged between 6 to 14 years and reported that in 57.1% of the animals (12/21), was observed the follicular growth and dominant ovulation follicle.

The ovulation rate of the females in this study was calculated about the number of mares that presented a follicle of 35mm in diameter. This rate was not altered (P>0.05) by the use of the P4 implant (Table 1). Polasek et al. (2017) also found no statistical difference in ovulation rates among the treated (P4) (64.3%) and control (without P4) (43.7%) groups, using anestrous crossbred mares and initiating the transition to reproductive season. On the other hand, Schutzer (2012) reported that 46% (7/15) of the mares ovulated within 60 days after starting treatment with P4 implants, and only 13.46% (2/15) ovulated in the control group. Results that showed the superiority of ovulation rate in mares submitted to treatment with P4 implant were also demonstrated by De Oliveira Filho et al. (2012), who reported that 80% (8/10) of the mares ovulated after treatment against 20% (2/10) of the mares group without P4.

The use of P4 implants may be an option to increase the amount of mares to be ovulated during the transition period from anestrus to the reproductive season, since P4 inhibits the preovulatory peak of luteinizing hormone (LH), which accumulates in the pituitary as reserves, facilitating the ovulation of these follicles, a fact that was also observed in the present study (Staempfli et al., 2011).

Mares with small and inactive ovaries should not be chosen for this treatment, because if there is a dominant follicle at the time of P4 withdrawal, it may progress rapidly to ovulation (McKinnon et al., 2011). However, if only small follicles are present at the time of device withdrawal, they may take 7 to 10 days for a new follicle to grow, become dominant and ovulate (Larsen and Norman, 2010).

Some authors note that the success of treatment with P4 depends on the period and the follicular diameter that the animal presents on the treatment day, since, in general, the more developed the transitional period; the higher the probability of the mare ovulates after the treatment (Polasek et al., 2017).

According to Allen and Wilsher (2017) over the years, the rate of seasonal pregnancy in pure mares has increased from 70 to 90%, mainly due to the use of techniques that include the application of artificial lighting to induce cyclicity in the period from anestrous to early spring associated with exogenous hormones, including P4.

In fact, this protocol contributes to the sustainability of the equine chain, since it results in a better productive rate, However, other works (Polasek et al., 2017; Schutzer, 2012; Newcombe et al., 2001) that applied only P4 were shown more sustainable, reveling lower investment, lower generation of waste, more significant savings in terms of management and use of electric energy to maintain artificial lighting, as well as useful productive indexes.

These statements are corroborated by the present research, which demonstrated, in addition to the benefits mentioned above, that the use of progesterone devices induced the cyclicity of the recipients of equine embryos and anticipated the first ovulation of the mating season. It is also worth noting that, when the mating season is started earlier by anticipating the first ovulation, a larger number of embryos can be collected from the animals during the reproductive period, also increasing the profitability of the producer.

CONCLUSIONS

According to the results obtained in this research, we conclude that the use of progesterone devices induced the cyclicity of the recipients of equine embryos, showing that this technique can make the management of equine females more efficient, productive and sustainable from the economic and environmental point of view.

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