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Reproductive response in creole mares treated with deslorelin acetate

Respuesta reproductiva en yeguas criollas tratadas con acetato de deslorelina

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ABSTRACT

The deslorelin acetate effect on reproductive response was evaluated in creole mares in Michoacán State. Ten mares were treated intramuscularly and distributed in a Control group (CG; n = 5) 1 ml of distilled water as a placebo and the experimental group (DG; n = 5) 1.5 mg of deslorelin acetate. When the follicle reached 35 mm in diameter, the mares were treated according to the assigned group. The ovarian activity was monitored every 24 h. The incidence (%) of anovulatory hemorrhagic follicles (AHFs), ovulation rate (%), follicular growth per day (mm), follicular diameter at ovulation (mm), and time to ovulation (h) were determined. The incidence of AHFs was higher (X^2 = 3.83) in the CG than in DG (25.58% vs. 5.8%, respectively). The ovulation rate was higher (X^2 = 4.76) in DG than in CG (94.11% vs. 74.41%, respectively). The hormone administration did not affect (p> 0.05) the follicular growth per day or the follicular diameter, still, ovulation occurred (p <0.01) at 39.45 ± 2.95 h and 89.47 ± 3.62 h in the DG and CG, respectively. deslorelin acetate reduced the incidence of HAFs and increased the ovulation rate.

Keywords: creole mares, deslorelin acetate, anovulatory hemorrhagic follicles.

RESUMEN

El efecto del acetato de deslorelina sobre la respuesta reproductiva fue evaluado en yeguas criollas en el Estado de Michoacán. Diez yeguas tratadas intramuscularmente y distribuidas en un Grupo control (GC; n=5) 1 ml de agua destilada como placebo y el grupo experimental (GD; n= 5) 1.5 mg del acetato de deslorelina. Cuando el folículo alcanzó los 35 mm de diámetro, las yeguas se trataron en función del grupo asignado. La actividad ovárica fue monitoreada cada 24 h, y se determinó la incidencia (%) de folículos hemorrágicos anovulatorios (HAFs), tasa de ovulación (%), crecimiento folicular por día (mm), diámetro folicular a la ovulación (mm), y tiempo a la ovulación (h). La incidencia de HAFs fue superior (X^2=3.83) en el GC que en GD (25.58% *vs.* 5.8%, respectivamente). La tasa de ovulación fue superior (X^2= 4.76) en GD que en GC (94.11% *vs* 74.41%, respectivamente). La administración de la hormona, no afecto (p>0.05) el crecimiento folicular por día ni el diámetro folicular, pero la ovulación se presentó (p<0.01) a las 39.45±2.95 h y 89.47±3.62 h en el GD y GC, respectivamente. El acetato de deslorelina redujo la incidencia de HAFs e incrementó la tasa de ovulación.

Palabras clave: yeguas criollas, acetato de deslorelina, folículos hemorrágicos anovulatorios.

INTRODUCTION

Anovulation is one of the main causes of infertility in different domestic animal females, since it delays the opportunity for the female to become pregnant. One of the types of dysfunction observed in mares is the presence of persistent anovulatory follicles (PAF, McCue & Squires, 2002), hemorrhagic anovulatory follicles (AHFs; Cuervo-Arango & Newcombe, 2012), or also called luteinized follicles (LUF, Bashir *et al*., 2016). These structures occur when a preovulatory follicle that had a normal development, fails to rupture or ovulate and the follicular antrum fills with blood, coming from the rupture of venules and arterioles that irrigate granulosa cells. The presence of AHFs/LUF is the most common form of anovulation in the mare. Previous studies have reported an incidence of 22.2 % in mares in the United Kingdom (Lefranc & Allen, 2003), and 24% during the breeding season in quarter mares in the United States of America (Ginther *et al*., 2008); however, the presence of AHFs has also been reported during the transition season; in this sense, McCue & Squires (2002) determined an incidence of 8.2% of AHFs in 1845 estrous cycles recorded during a 5-year period at the University of Colorado, USA.

A mare with AHFs manifests typical signs of estrus repeatedly and prolonged; however, she fails to release the oocyte, so there will be no gestation. Such anovulatory follicles have been reported to negatively affect ovulation rate (Gerard & Robin, 2019).

Additionally, an AHF will usually undergo a process of luteinization, i.e. development of vascularized luteal tissue in the ovulation absence, the follicular antrum fills with blood, which is seen with echogenic foci and strands or clots that give the appearance of fibrin (Jacob *et al*., 2009).
The presence of AHFs has been associated with some risk factors, such as the timing of the reproductive season, age and the use of exogenous and hormonal substances (Ginther *et al*., 2008).

AHFs should generally be identified by ultrasound, and hormones have been used to increase the release or concentration of luteinizing hormone, including human chorionic gonadotropin (hCG), with results of 88.3% ovulation within the first 48 hours of application (McCue *et al*., 2007) and deslorelin acetate, a GnRH analogue, which has shown an effectiveness of 90.1% ovulation during the first 48 hours (McCue *et al*., 2007). In this same sense, Finan *et al*. (2016), determined that the use of deslorelin in follicles of size ≥30 mm showed a significant response with 93.75% ovulation. Few studies have been conducted in Mexico to determine the incidence of anovulatory failures in mares. It is possible that in some cases the technician administers exogenous hormones for their treatment, and in other cases they are left to pass until the mare presents an effective ovulation on her own.

The objective of the present study was to evaluate the reproductive response in Creole mares treated with deslorelin acetate.

MATERIAL AND METHODS

Study area: The present study was carried out during the months of April-August 2020, in Santa Clara de Valladares community in Tocumbo municipality, Michoacán, Mexico; located in the west of the state at 10231'19" West Longitude and 1942'10" North Latitude, at an altitude of 1604 m a.s.l, average rainfall of 800-1300 mm per year, with a sub-humid climate with summer rains and a temperature ranging between 16-26°C (INEGI, 2009).

Animals: 10 Creole mares were used, with an age between 3 and 11 years, live weight between 350 and 450 kilograms, height at withers between 1.55-1.65 meters and a body condition of 4 to 6 points on the Henneke (1984); none of them were lactating and none of them had a history of previous reproductive problems. The mares were fed the same diet, based on 8% of the live weight of the female, considering a concentration of 80:20 of forage and concentrate, respectively. Forage was oat hay and commercial feed as concentrate. Drinking water was available *ad libitum*.

Hormonal treatments: prior to the start of the experiment, the mares were subjected to a diagnostic ultrasound examination with a 7.5 MHz transrectal ultrasound equipment to determine their reproductive, ovarian and uterine status. All the mares were treated with a dose of 5 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Dinoprost, Lutalyse, Zoetis NZ®), to synchronize the estrous cycle by lysis of the corpus luteum, and to initiate the development of the follicular phase at the same time and obtain homogeneous ovarian conditions.

Subsequently, the mares were distributed in two groups: control group (CG; n=5), which received the intramuscular (im) application of 1 ml of distilled water as placebo, and the experimental group (GD; n=5), which received the application, im, of 1.5 mg of the hormone deslorelin hydrochloride. In both groups the application of placebo or hormone was performed when the dominant follicle reached a diameter ≥35 mm. A total of 77 estrous cycles were recorded, 43 in the CG and 34 in the GD.

Mares that exhibited ovulation and development of a functional corpus luteum around day 9 of the estrous cycle received a 5 mg (im) dose of $PGF_{2\alpha}$ (Dinoprost, Lutalyse, Zoetis NZ®) with the intention of restarting follicular dynamics.

Ovarian check-up: ovarian check-up of mares in both groups was performed with a 7.5 Mhz ultrasound (US) (Sonoescape S23, USA®), at 24 h intervals to determine the follicular growth rate per day and prior to the time of ovulation, ovarian check-up was performed at 6 h intervals.

The incidence (%) of anovulatory hemorrhagic follicles was established when upon observation with the US a follicle showed signs of internal hemorrhage forming the follicular stigma, but without ovulation taking place.

To evaluate reproductive efficiency, the following parameters were included: follicular growth per day, follicular diameter at ovulation, time to ovulation after hormone application and ovulation rate.

Follicular growth per day (mm), was evaluated by placing the US cursor crosswise from the top and bottom and right and left edges of each follicle 35 mm, to record the average diameter on each day of evaluation; follicular growth per day was calculated by subtracting the current diameter from that of the previous day.

Once the stigma was observed in the Graff follicle, the ultrasound review was performed at 6-h intervals, recording the diameter at ovulation (mm) and the ovulation time (h), which was considered as the time elapsed between the application of the hormone until follicular rupture, and was confirmed by observation of the hemorrhagic ovulatory fossa and with the appearance of one or more corpora lutea.

On the other hand, the ovulation rate (%) was considered as the total number of follicles that achieved ovulation in each of the estrous cycles evaluated in the mares of both groups, multiplied by 100 and divided by the number of dominant follicles that developed on the surface of the ovary.

Statistical analysis: the results obtained for the variables incidence (%) of anovulatory follicles and ovulation rate (%) were subjected to an X^2 test, in a 2x2 contingency table. Follicular growth per day (mm), follicular diameter at ovulation (mm) and time (h) to ovulation were evaluated by a repeated measures test in a mixed model, where the mare was the random effect, the treatment the fixed effect and the estrous cycle of each mare as a repeated measure. The comparison between treatments was performed using the least squares test. The significance value considered was $p<0.05$ and all analyses were performed in the SAS statistical package (SAS, 2013).

RESULTS

The incidence of anovulatory follicles was 25.58% (11/43 observed estrus) in CG mares; while in DG it was 5.8% (2/34 treated estrus). The differences observed between both groups were statistically significant ($X^2=3.83$).

Follicular growth per day in Creole mares from the state of Michoacán showed no differences ($p>0.05$) between the groups of mares, observing a daily growth of 2.15±0.19 mm for the CG and 2.42±0.16 mm in the GD.

Follicular diameter at ovulation showed no significant differences ($p>0.05$) between the groups studied, observing an average follicular diameter of 44.08±0.63 mm and 44.94±0.78 mm in mares of the GD and CG, respectively.

The time (h) to ovulation was 39.45±2.95 h for the GD, while for the CG it was 89.47±3.62 h. The differences observed between both groups were statistically significant ($p<0.01$) and represented a difference of 50 h in favor of the GD.

The ovulation rate was 74.41% (32/43 estrous cycles observed) and 94.11% (32/34 estrous cycles), in the CG and GD respectively; indicating a favorable effect of deslorelin acetate ($X^2=4.76$).

DISCUSSION

The incidence of AHFs observed in the present study was similar to that reported by Ginther *et al*. (2008), who observed 24% during the breeding season in quarter mares. In the same sense, Lefranc y Allen (2003), reported an incidence of AHFs 22.2 % in mares. On the contrary, other authors (McCue y Squires, 2002) observed an incidence of AHFs of less than 10% during the transition season.

Other studies, such as those conducted by Cuervo-Arango & Newcombe (2010), observed an effect of age on the incidence of AHFs of 13.1% in mares aged 6-10 years; while in mares older than 10 years, the incidence was 24%, indicating that the reproductive season and age of the mare may be predisposing factors to the formation of anovulatory hemorrhagic follicles (Ginther *et al*., 2008). Previous studies in Mexico (López *et al*., 2010), reported the presence of anovulatory follicles; however, the observed incidence is not indicated.

The mare is a species with seasonal reproduction and has an ovarian transition period in spring, between the winter anestrus and the renewal of its cyclicity during the reproductive season. During the spring transition, LH concentrations are low, possibly caused by the effect of melatonin blocking gonadotropin-releasing hormone (Satue & Gordon, 2020). This explains why mares can present anovulatory follicular waves, which can even reach the size of preovulatory follicles and become more predisposed AHFs (Watson & Al-Zi'abi, 2002).

With respect to age, in the mare as in other domestic animals, there are degenerative changes associated with AHFs, starting from the alteration in the synthesis of the glutathione peroxidase enzyme and the enzyme responsible for the elimination of reactive oxygen species; If this hormone is restricted, oocytes and follicle wall cells lose their ability to respond to collagenase, limiting the effect of LH hormone on the cells of the internal theca, allowing it to only thin the follicle walls (Ginther & Beg, 2011), a fact that even with the physical effect of ovulation is not able to break the follicular wall and release the genetic material, thus giving rise to the anovulatory follicle (Morel *et al*., 2005).

The use of exogenous and hormonal agents intended to induce ovulation, such as LH or GnRH analogues, have resulted in ovulatory alterations and/or AHF formation (Cuervo-Arango & Newcombe, 2010). Previous studies by Schauer *et al*. (2013), posited that LH hormone did not disrupt follicular growth patterns or ovulation; however, it did alter follicular fluid factors that could disrupt oocyte or follicle maturation (Burden *et al*., 2015). LH hormone allows through activation of matrix metalloproteinases and activation of plasminogens, tissue remodeling of the follicle to occur, since the higher the concentration of E_2 and LH, the more collagenase III increases in the ovarian stroma and collagenase I decreases in the periphery of the dominant follicle (Smok & Rojas, 2010). This hormone also has a close involvement with prostaglandins that are responsible for triggering ovulation with COX II (Cuervo-Arango & Martínez-Boví, 2016). LH production, prior to ovulation is responsible for luteinizing these follicles, allowing there to be an infiltration of fat cells through the follicle wall ,and in this way it luteinizes, a fact that blocks the exit of the oocyte.

In the present study, the application of deslorelin acetate was effective in reducing the incidence of AHFs in Creole mares in the state of Michoacán; it is possible that its effectiveness was due to its promoting effect on the amplitude and frequency of the hypophyseal LH release pulses, provoking the activation of collagenase and thus triggering ovulation, avoiding the formation of AHFs (Squires *et al*., 1994).

Regarding follicular growth per day, the results found in this study are similar to those reported by Donadeu & Pedersen (2008); however, McCue (2007a) and McCue (2007b), who observed daily follicular growth ranging from 2.1 to 4.3 mm per day. The alterations in this growth imply that the precise moment of ovulation cannot be estimated with certainty, since their behavior is unpredictable and they can ovulate spontaneously, which is why they require greater attention with respect to the moment of ovulation and should be monitored frequently by ultrasound (Dordas-Perpinyà *et al*., 2020).

One of the factors frequently used as a practical criterion for predicting ovulation in the mare is follicular diameter, since it is a simple and relatively easy method to monitor when experienced in the use of ultrasound (Cuervo-Arango & Newcombe, 2008). Studies have shown a range of 34 to 70 mm in the diameter of preovulatory follicles 24 h prior to ovulation, where the mare's breed, body condition and the time of year play an important role; therefore, hormonal treatments are used to predict the opportune moment to service the mare and increase reproductive efficiency.

In this regard, Cuervo-Arango & Newcombe (2008) found that the diameter of the preovulatory follicle decreases when a hormonal treatment is applied with respect to the follicular diameter of spontaneous or natural ovulation. These authors reported a follicular diameter of 38.82±0.83 and 44.18±1.22, treated with 1500 IU of hCG in spontaneously ovulating mares, respectively. They also observed a smaller follicular diameter in mares treated with $PGF_{2\alpha}$ (39.42±1.77 vs 47.77±2.00) with an implant of 2.6 mg deslorelin (34.25±0.65 vs 40.92±1.38) with respect to natural ovulation.

The use of different hormonal compounds resulted in a follicular diameter at ovulation similar to that observed in the present study. Dolezel *et al.* (2012), reported a follicular diameter between 44 and 48 mm. Dordas-Perpinyà *et al*. (2020), reported no significant differences in the follicular diameter of mares treated with buserelin acetate (6 mg) or eCG (1500 IU); they reached an average follicular diameter of 40.98±0.55 mm and 41.45±0.59 mm, respectively. In this same study, mares were treated with 3 or 6 mg of buserelin acetate and no significant differences were reported between both groups, finding a follicular diameter of 41.64±3.63 mm and 41.95±3.79 mm, respectively.

Similarly Dordas-Perpinyà *et al*. (2020), evaluated the efficacy of different hormonal treatments in mares and included 0.1 mg triptorelin, 1500 IU hCG, 3, 2 and 1 mg buserelin acetate and observed follicular diameter per treatment at ovulation of 42.1±2.8, 43. 1±4.2, 42.5±2.9, 42.6±2.8, 43.7±3.8 mm, respectively; being different ($p<0.01$) with respect to the follicular diameter at spontaneous ovulation, which was 45.6±5.3 mm, higher mean than the hormonal treatments and even slightly higher than that observed in our study.
Some authors (Cuervo-Arango & Newcombe, 2008) have pointed out that hormonal treatments to induce ovulation decrease the size of the preovulatory follicle, compared to natural ovulation; and although there is little information on this subject, it is possible that the increase in LH concentration, either by the administration of GnRH agonists such as deslorelin, buserelin or others, or by analogues such as hCG, cause a decrease in pituitary activity for the production of follicle stimulating hormone, limiting follicular growth, allowing earlier ovulation than when natural follicular growth is maintained.

The time elapsed since the application of deslorelin hormone and other GnRH analogues significantly reduces ovulation time. Previous studies (Miki *et al*., 2016), with mares with follicular diameter equal to or greater than 35 mm, have reported an increase in LH levels within the first 6 to 24 h of application in heavy draft mares, in contrast to LH peaks reported 1 to 2 days after ovulation, according to normal patterns reported for mares (Meinecke *et al*., 1987). The early release of LH decreases the possibility of luteinizing the preovulatory follicle and positively influences the decrease of anovulatory follicles.

The results obtained in the present study for ovulation rate are similar to those reported by Finan *et al*. (2016), who worked with Australian native mares, observing 93.7% ovulation, using a subcutaneous implant of 1.25 mg deslorelin 1.25 mg.

The results obtained in the present study with respect to ovulation, are similar to those obtained in different geographical position and breed of mares, a fact that could be predicted, since the hormone and its analogs showed positive results, proving its therapeutic response on gonadotrophs due to its LH analog effect (D'Oliveira *et al.,* 2019).

CONCLUSION

The administration of the hormone deslorelin acetate improved the reproductive response in Creole mares from the state of Michoacán, by reducing the incidence of AHFs, increasing the ovulation rate and reducing the time to ovulation.

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