Ovulation induction in Caribbean jennies in the tropics

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Abstract

The population of some endangered donkey breeds in tropical regions has declined recently. Therefore, there is an increasing interest to improve the reproductive management of these animals. Ovulation induction agents are often used in equine practice to optimize reproduction techniques. However, limited information exists to support its use in donkeys. Therefore, we compared 2 ovulation-inducing agents (commonly used in equine practice) to hasten ovulation in Caribbean jennies in the tropics. Five cycles of 8 Caribbean jennies were used. Jennies were monitored via transrectal ultrasonography and in 4 estrous cycles they had ovulation induced with either 2,500 IU of hCG or 1.8 mg of deslorelin acetate when a follicle measuring between 27 - 30 mm or > 30 mm in diameter was diagnosed. In the Control-assigned cycle, jennies were not treated with hormones and had spontaneous ovulation. Follicle diameters affected ($p < 0.05$) the interval to ovulation. When given with a follicle > 30 mm, either treatment reduced ($p < 0.05$) the interval to ovulation. A higher percentage of jennies ovulated within 48 hours after hCG or deslorelin acetate with > 30 mm follicle ($p < 0.05$), and with deslorelin acetate with 27 - 30 mm follicle ($p < 0.05$) than in the Control-assigned cycle. In conclusion, Caribbean jennies in tropics with > 30 mm follicle responded to ovulation induction with hCG or deslorelin acetate, with ovulation occurring within 48 hours.

Keywords: Donkey, breeding, mule, hCG, GnRH analog

Introduction

Interest in donkey production has increased worldwide. In the Americas, donkeys have been used for agricultural work and for producing top mules whereas in the orient, donkeys are used to produce milk for cosmetic manufacturing and for meat.[1,2] In addition, many donkey breeds have been threatened with extinction.[3-5] Therefore, there is an increasing interest to improve the reproductive management in donkey species.

Guidelines developed for horses have been used in donkeys over the years for reproductive management. One of the most common techniques applied in horse breeding programs is ovulation induction to manipulate the reproductive cycle and optimize breeding management. This technique improves artificial insemination efficiency, particularly in advanced assisted reproduction techniques, as it predicts ovulation timing. Although this procedure is routinely used in horses, limited information exists to support its use in donkeys. Recently, some studies have reported the efficacy of GnRH analogs (e.g., deslorelin acetate, buserelin, and histrelin acetate) and human chorionic gonadotropin (hCG) to hasten ovulation in jennies.[6-9] Although GnRH analogs and hCG have similar efficacy to hasten ovulation in jennies,[6,9] these hormones act differently on the ovaries. Human chorionic gonadotropin binds to LH receptors in granulosa cells directly to induce ovulation[10] whereas GnRH analogs induce endogenous release of LH by the adenohypophysis.[11,12] In mares, hCG has been reported to have a shorter interval to induce ovulation than GnRH analogs;[13] however, it has not been observed in Brazilian Northwest[9] or Martina Franca[6] jennies in the subtropical and temperate environment, respectively. To the best of the authors' knowledge, there has been no study evaluating ovulation-inducing agents in jennies in tropical regions. Although there are only a few studies on follicular dynamics of jennies, they differ in results that may be attributed to the differences in breeds and location of the donkeys.[14-16] These differences conceivably could affect response to ovulation inducing agents. Additionally, the population of donkeys in some countries in the tropical zone (e.g., Brazil, Ecuador, and Colombia) has declined that may lead these animals to be threatened with extinction.[3] Therefore, we compared deslorelin acetate, a GnRH analog, and hCG to hasten ovulation in Caribbean jennies in the tropical zone.

Materials and methods

Study was approved by the institutional animal care and use committee (protocol 15.12.032). It was conducted from Sep-

tember 2018 to April 2019 at Ross University School of Veterinary Medicine, Basseterre, St. Kitts, West Indies (17°18′N 62°44′W), close to the Equator (tropical zone). Five estrous cycles of 8 reproductively sound, nonpregnant Caribbean Jennies, ages 3 - 12 (± 5) years were enrolled. Jennies were housed in outdoor grass paddocks under natural light, fed freshly-cut New Guinea grass (*Megathyrsus maximus*) with free access to water.

Jennies were examined via transrectal ultrasonography (5 MHz linear transducer, Sonosite, Bothell, WA) to monitor ovarian activity and follicular growth. Five days after detection of a corpus luteum in 1 ovary, jennies received intramuscular prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 0.25 mg cloprostenol sodium, Estrumate®, Merck Animal Health, Madison, NJ) for induction of estrus. Four days after $PGF_{2\alpha}$ treatment, jennies started to be monitored daily. Five successive estrous cycles of each jenny was studied and randomly assigned to each of 5 treatment groups in a crossover design. In the Control-assigned cycle, jennies were not treated with hormones and had spontaneous ovulation, whereas the other 4 estrous cycles were used to evaluate the ovulation-inducing treatment at various follicular sizes. Jennies had ovulation induced with either intravenous 2,500 IU of hCG (Chorulon, MSD Animal Health, Summit, NJ) or 1.8 mg of intramuscular deslorelin acetate (Sucromate®, Thorn BioScience LLC, Louisville, KY) as follows: hCG was given when a follicle measuring between 27 and 30 mm (G1) or > 30 mm (G2) in diameter was diagnosed; deslorelin acetate was given when a follicle measuring between 27 and 30 mm (G3) or > 30 mm (G4) in diameter was diagnosed. Starting 24 hours posttreatment, jennies were monitored every 6 hours via transrectal ultrasonography until ovulation. Number of jennies ovulating within 48 hours, the interval between ovulation-inducing agent treatment and ovulation, preovulatory follicular size, length of estrous cycle, and endometrial edema (0: no edema, 1: mild edema, 2: moderate edema, 3: evident edema, 4: exacerbated edema)[17] were recorded. Jennies did not receive an injection of $PGF_{2\alpha}$ to bring them back to estrus between treatments. All jennies were submitted to every treatment.

Data analyses

GraphPad Prism 8.0.1. (GraphPad Software, San Diego, CA) was used. Data are summarized as mean ± SD. The Gaussian distribution of the data was evaluated using the Kolmogorov-Smirnov normality test. Mixed model and Tukey's posthoc test were used to compare the interval between treatment of an ovulation-inducing agent, follicular diameters, number of follicles > 27 mm, and days of the cycle. Edema scores were considered categorical variables and analyzed with nonparametric tests (Kruskal-Wallis test followed by Dunn's test). Chi-square test was used to compare the success of the ovulation-inducing treatments (the percentage of jennies ovulating within 48 hours after treatment or > 48 hours after treatment, indicating unsuccessful induction) and between intervals until ovulation in control and treated cycles. Multiple linear regression model was used to evaluate the relationship between ovulation and endometrial edema score. Ovulation was evaluated by a logistic regression model, where ovulation was the dependent variable and edema score the explanatory variable. Correlations between ovulation and edema score were categorized as high ($r \geq 0.7$), moderate ($0.5 \leq r < 0.7$), and poor ($r < 0.5$). Significance was set at $p \leq 0.05$ for all tests and $p < 0.10$ and > 0.05 as a statistical trend.

Results

Follicular diameter at ovulation induction and the length of the estrous cycle, were similar ($p > 0.05$) among groups. However, jennies in G3 tended to have smaller ($p = 0.068$) follicular diameter 12 hours before ovulation was detected compared to Control cycle whereas this parameter was similar ($p > 0.1$) to Control cycle in other groups. More jennies in the G3 group (GnRH, 27 - 30 mm) ovulated ($p < 0.05$) within 48 hours compared to Control-matched group, but not different ($p > 0.05$) than G1 (hCG, 27 - 30 mm; Table). Jennies in G1 group tended to have more ovulations ($p = 0.056$) within 48 hours after treatment than control group. A higher percentage of jennies had ovulation diagnosed ($p = 0.015$) within 48 hours in both G2 and G4 groups (hCG and GnRH, > 30 mm, respectively) compared to control-assigned cycle. However, there was no difference ($p > 0.05$) among treated groups in the percentage of jennies ovulating within 48 hours (Table). The mean interval between the detection of a predefined follicular size and ovulation was longer ($p < 0.001$) in jennies with 27 - 30 mm follicles with the respective matched cycle (Table). Also, in G2 and G4 groups, this interval was shorter ($p < 0.05$) by treatments compared to Control-matched cycle (Table). Additionally, there were no differences in the number of follicles > 27 mm, the number of ovulations per cycle, or the endometrial edema score among assigned cycles (Table). All jennies had single ovulation throughout the experimental cycles, and no jennies failed to ovulate. Edema score during the predefined follicular sizes and ovulation were not associated events in these jennies ($r = 0.0186$).

Discussion

We evaluated hCG and deslorelin acetate use to induce ovulation in Caribbean jennies in the tropics. Although ovulation-inducing agents (e.g., hCG and GnRH analogs) have been described to induce ovulation in jennies,[6-9,18] there was no study assessing the efficacy of these hormones to hasten ovulation in jennies in a tropical environment. Recently, some countries in the tropical zone (e.g., Brazil, Ecuador, and Colombia) have reported a decrease in herd number of donkeys, and therefore, there has been an increase in interest to improve the breeding management of this species to prevent threatened extinction.[3]

It is noteworthy that none of the jennies failed to ovulate during the study that was conducted in the tropics at ~ 17° North during the winter period (September 2018 - April 2019). Although a case of hemorrhagic anovulatory follicle has already been reported in Caribbean jennies,[30] donkeys' ovulation seem not to be affected by seasons since this species display less seasonality than horses in subtropical and temperate zones.[1,15] However, the follicular dynamics and reproductive management of this species are still not well described.[14,15,19] In a study by our group,[20] the follicular dynamics of Caribbean jennies in tropics were described which contradicted the existing information regarding the estrous cycle in this species in temperate and subtropical zones.[14,15,19] One follicular wave

per cycle was described in Egyptian jennies,[14] 2 or 3 waves in jennies in Ethiopia,[19] and we characterized 2 waves in Caribbean jennies.[20] We speculated that these differences are associated with the breed, origin, and location of the animals in each study.

In the first study of our group, the maximum follicular diameter before ovulation in Caribbean jennies was 34.64 ± 2.91 mm,[20] prompting us to test both intervals (27 - 30 and > 30 mm follicle) for ovulation induction. Although the diameter of preovulatory follicles have a large variation based on donkey's breed (30 - 45 mm),[6,7,9,18,21-23] in a Brazilian study assessing the efficacy of histrelin acetate to hasten ovulation in Northwest jennies with various follicle diameters, follicles 29 - 32 mm had the most satisfactory response.[7] In another study, the same authors reported that hCG and histrelin acetate had similar efficacy to induce ovulation in jennies with 29 - 32 mm follicles, with 90 and 100% of ovulation occurring between 36 - 48 hours after treatment, respectively.[9] Similar to other authors description, in the present study, the interval between detection of > 30 mm follicle and ovulation was reduced by treatments (hCG or deslorelin acetate). However, when jennies had ovulation induction treatment with 27 - 30 mm follicle, there was no change in this interval compared to the Control-matched cycle. Some jennies with 27 - 30 mm follicles had a potential to ovulate, but others were not able to respond to exogenous LH stimulus. It may be associated with the follicular maturation at 27 - 30 mm, as fewer jennies responded to the treatment (hCG, 37.5%; GnRH, 50%) with ovulation within 48 hours. Immature follicles fail to respond to exogenous gonadotropins due to lack of granulosa cells' LH receptors.[24] In both hCG and deslorelin acetate groups, a higher proportion of jennies ovulated within 48 hours after ovulation induction, implying that some jennies with follicles under 30 mm may have ovulation potential.[7] However, ovulation induction using hCG or deslorelin acetate was most efficient in Caribbean jennies with > 30 mm follicles. Deslorelin acetate induced ovulation within 48 hours more consistently than hCG at a follicular diameter between 27 and 30 mm in jennies in the present study. This lack in response to hCG is interesting in Caribbean jennies. The hCG is a glycoprotein with LH-like biological activity that binds directly to LH receptors in granulosa cells to induce ovulation,[10] whereas GnRH analogs act at the anterior pituitary to induce the secretion of endogenous LH.[11,12] Therefore, hCG might have a faster response and might be more efficient than GnRH analog, as hCG does

Table. Variables measured at several predefined follicular sizes in jennies with spontaneous ovulation (Control) and in jennies that had the ovulation induced with hCG or deslorelin acetate (GnRH)

Variables	Estrous cycle assigned	Follicle diameter	
		27 - 30 mm	> 30 mm
Mean number of follicles > 27 mm	Control	1.0 ± 0.0	1.0 ± 0.0
	hCG	1.0 ± 0.0	1.3 ± 0.4
	GnRH	1.0 ± 0.0	1.3 ± 0.4
Mean number of ovulations per cycle	Control	1.0 ± 0.0	1.0 ± 0.0
	hCG	1.0 ± 0.0	1.0 ± 0.0
	GnRH	1.0 ± 0.0	1.0 ± 0.0
Mean diameter of follicles with predefined follicular size (mm)	Control	28.9 ± 0.9	32.1 ± 1.6
	hCG	28.4 ± 1.1	32.2 ± 1.3
	GnRH	28.1 ± 0.9	31.7 ± 1.5
Mean diameter of preovulatory follicle 12 hours before ovulation (mm)	Control	37.2 ± 2.3*	37.2 ± 2.3
	hCG	34.9 ± 2.4	37.0 ± 3.3
	GnRH	32.7 ± 1.9*	34.4 ± 1.9
Mean interval from detection of predefined follicular size to ovulation (hours)	Control	121 ± 31.1.0^{X}	86.8 ± 28.8^{Ya}
	hCG	92.3 ± 40.4^{X}	50.3 ± 22.4^{Yb}
	GnRH	79.5 ± 37.8^{X}	42.7 ± 8.7^{Yb}
Mean length of estrous cycle	Control	23.8 ± 2.8	23.8 ± 2.8
	hCG	23.9 ± 3.9	22.5 ± 2.1
	GnRH	22.9 ± 2.2	21.6 ± 2.1
Median/mode of edema score at each specific time lap	Control	0.5/0	1.5/1
	hCG	0.5/0	1.0/1
	GnRH	0.5/0	1.0/1
Percentage of jennies responding with ovulation within 48 hours	Control	0%^{b*}	12.5%^{b}
	hCG	37.5%^{ab*}	62.5%^{a}
	GnRH	50.0%^{a}	75.0%^{a}
Percentage of jennies with unsuccessful induction of ovulation	Control	100%	87.5%
	hCG	62.5%	37.5%
	GnRH	50.0%	25.5%

Edema score: 0, no edema, and 4, exacerbated edema. Unsuccessful induction of ovulation: ovulation detected > 48 hours after administartion of induction agent. Lines with different superscript capital letters (^{X,Y}) and columns with different superscript lowercase letters (^{a,b}) represent differences ($p < 0.05$). Columns with superscript (*) trend to be different ($p < 0.10$ and > 0.05).

not need endogenous LH to induce ovulation.[13] However, this was not observed in this study.

It is also important to note that repeated doses of hCG may induce antibody development in mares and may reduce its effectiveness.[10,25,26] Although hCG antibody development in jennies is still unknown, it is an important information to take into account in advanced breeding programs, especially whenever a female jenny or mare will be bred many times in a season. Jennies used in the present study were feral and had not been manipulated previously. Although hCG antibody was not assessed in this study, the authors assumed that our results were not affected by hCG antibodies.

One jenny in each treated cycle (hCG and GnRH) ovulated before 36 hours after treatment. We speculated that it may be associated with an early endogenous hormonal stimulus in these jennies. Also, the number of follicles ≥ 27 mm was similar among groups and all jennies had single ovulation in all cycles. Of interest was the observation by other authors who reported that the percentage of jennies with multiple ovulations varied between 5 to 32% depending on the breed and location;[7,27-29] however, all Caribbean jennies in the present study had single ovulation in all estrous cycles. It may be a specific characteristic of this donkey breed; however, it must be evaluated in a larger number of animals. It was also noted in the present study that Caribbean jennies did not have an endometrial edema pattern associated with the phase of estrus that has been described by other authors.[7] In mares, a pronounced endometrial edema pattern was closely related to the estrous cycle and higher secretion of estradiol by the preovulatory follicle that decreased spontaneously before ovulation.[24,31] Therefore, endometrial edema cannot be used to predict the best time to induce ovulation in jennies or the time close to ovulation.

It is important to recognize that the present study was conducted with a limited number of jennies and estrous cycles. It is possible that if a larger number of jennies was used, different results could have been obtained. However, we feel that the results provided a good representation of the Caribbean donkeys' response in tropics to ovulation-inducing agents. We concluded that ovulation induction using hCG or deslorelin acetate in Caribbean jennies in tropics is most suitable with a follicle > 30 mm. GnRH induced ovulation within 48 hours more consistently than hCG; however, at a follicular diameter of 27 or 30 mm, the effect was not as predictable as it is in mares induced at a follicular diameter of 35 mm. Further studies are needed to improve the breeding management of this species in various environments.

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Conflict of interest

None to declare.

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