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Ovarian dynamics and estrous cycle length in the donkey (Equus asinus)

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ABSTRACT

Nine jennies were monitored daily by ultrasonography during three complete ovarian cycles in order to evaluate if the timing of luteolysis and the growth pattern of the ovulatory follicle (OVF) before, during and after luteolysis are related to the length of the interovulatory interval (IOI). Blood samples for progesterone determination were obtained daily during one of the cycles of each jenny. The cycles were classified according to the length of the IOI into three groups: Short IOI (21.2 ± 0.3 d, n = 10), medium IOI (23.9 ± 0.4 d, n = 7), and long IOI (26.2 ± 0.3 d, n = 10). Neither the time of luteolysis onset nor the time of luteolysis completion were significantly different between groups. The length of the IOI was mainly determined by the duration of the follicular phase, as the intervals from luteolysis onset to ovulation and from luteolysis completion to ovulation were directly correlated with the length of the IOI ($p < 0.001$ and $p < 0.01$ respectively). Multiple regression analysis revealed that the length of the IOI was negatively correlated with the size of the OVF at day 13 ($p < 0.01$), with its growth rate from day 13 to day 15 ($p < 0.05$) and with its growth rate from day 15 to day 18 ($p < 0.01$), and positively correlated with the final diameter of the OVF ($p < 0.01$). The correlation between the observed IOIs and those predicted by the multiple regression equation was highly significant ($r = 0.91$, $p < 0.001$), but the predictive ability of a simplified equation using only the diameter of the OVF at day 18 was almost as good ($r = 0.89$, $p < 0.001$). Estrus signs lasted longer and were more intense as the length of the IOI increased, and this was associated with a longer period of low progesterone concentrations during the follicular phase of jennies with longer cycles. It is concluded that the length of the luteal phase in jennies is relatively constant, and that most of the variation in the length of the IOI is associated with differences in the size of the OVF at the time of luteolysis onset and with its growth rate during the following five days.

1. Introduction

The donkey is a domestic animal of fundamental economic importance for inhabitants of rural areas around the world [1–3]. Even in developed regions, efforts are being carried out to protect endangered local breeds and to improve their reproduction [4–6]. However, very few studies concerning the reproductive physiology of the jenny have been conducted. This lack of information hampers efforts to improve the generally low reproductive efficiency of the species [7], or to preserve the growing number of endangered breeds [4,8].

Several studies have been conducted in the last decade to characterize the ovarian follicular dynamics and some patterns of reproductive hormones in different donkey breeds [2,4–11]. Some variation in the mean interovulatory interval (IOI) has been found in those studies, and the factors that have been reported to affect the cycle length in jennies include breed [11], season [4,10,11], age [6], body condition score [6] and year of study [4].

Considerable individual variation has also been found within each study, and although some of the factors mentioned above, such as age and body condition score, may partially explain individual differences in cycle length, they do not provide information about the ovarian dynamics behind such individual differences. Knowledge of the relation between cycle length and basic ovarian events can be of great help to improve reproductive efficiency and to fine-tune the use of assisted reproductive technologies [12].

The objective of this study was to evaluate if the timing of luteolysis and the growth pattern of the ovulatory follicle (OVF)

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immediately before, during and after luteolysis are related to the length of the IOI in jennies. The relation of these factors with the timing, duration and intensity of estrus signs was also evaluated.

2. Material and methods

2.1. Animals and experimental design

The study was carried out from the beginning of March to the end of May at an experimental farm located in the Central Plateau of Mexico. The experimental protocol was approved by an Institutional Committee for the Care and Use of Experimental Animals according to the Mexican Federal Law on Animal Health (Title III, Chapter I – Animal Welfare) and the Mexican Official Norm NOM-062-ZOO-1999.

Nine healthy "Mexican Burro" female donkeys were used for this experiment; their ages ranged from 3 to 9 years, their weights ranged from 120 to 150 kg, and their average body condition score was 3 on a scale of 1–5 [1]. The jennies were kept in a large pen with *ad libitum* access to water, and they were fed alfalfa hay, oat hay and commercial concentrate. Estrus detection was carried out individually by a single observer every morning using a 7-year old Jack. Once estrus was detected for the first time the jennies were monitored daily by ultrasonography using an Aloka 500 equipment with a 5 MHz linear transducer until ovulation was detected and recorded. Estrus detection and ultrasonographical observations of the ovaries were carried out daily for three consecutive estrous cycles after the first recorded ovulation.

Every day the diameter and location of every follicle ≥10 mm in diameter was recorded. The recorded diameter was the average value of the measurements taken at the largest diameter and at a line perpendicular to the largest diameter. Ovulation was confirmed by an abrupt decrease in the size of a preovulatory follicle [9].

2.2. Progesterone determinations

Blood samples for progesterone determination were collected daily by jugular puncture for an entire estrous cycle of each jenny. The samples were collected into vacuum tubes with no anticoagulant and allowed to clot at room temperature. Serum was separated and stored at −20 °C until assayed. Progesterone concentrations of all the samples were measured in a single assay run by solid-phase radioimmunoassay (COAT-A-COUNT®, Siemmens Diagnostics, Los Angeles). The sensitivity of the assay was 0.27 ng/ml and the intra-assay variation coefficient was 4.96%.

2.3. Definitions

Luteolysis was considered to have started when progesterone concentrations first declined by 50% or more between two consecutive days, and to have finished when progesterone concentrations declined to less than 2 ng/ml [15].

The day of ovulation was considered as day 0 of each cycle [13]. The interovulatory interval (IOI) was defined as the number of days between successive ovulations as detected by ultrasonography in two successive estrous cycles [4,11]. The ovulatory follicle (OVF) was defined as the largest follicle on the day prior to ovulation. Its diameter at earlier days was obtained retrospectively from ultrasonographical records as far back as that follicle could be identified as the largest follicle on the side of ovulation [13]. A secondary follicular wave was defined as an anovulatory wave in which the largest follicle attained a diameter of at least 22 mm before starting to regress.

Sexual behavior was classified on a scale from 0 to 2, where 0 = no estrus signs, 1 = the jenny allows the male to approach and shows mouth-clapping as the only estrus sign or in combination with kicking, moving, or switching of the tail, indicating that the animal is in transition into estrus or going out of estrus, and 2 = mouth clapping plus at least one of the following: clitoral winking or raising the tail at any moment during the teasing period [14]. The duration of estrus in each cycle was considered as the number of days when behavior was rated as either 1 or 2, and the period with full estrus signs was the number of days when behavior was rated as 2.

2.4. Statistical analysis

In an initial analysis, Student's t tests were used to compare the IOI and other variables between cycles in which a secondary follicular wave was identified and those with no secondary follicular wave. As no significant effect of this factor was found for any variable, data were re-grouped according to the length of the IOI, using as reference the mean value for all cycles (23.7 ± 0.2 days). Thus, the 27 estrous cycles that were monitored were classified as short (IOI ≤ 22 d), medium (IOI 23–24 d, i.e. within one day of the mean value) or long (IOI ≥ 25 d), and values of different variables were compared between these groups by analysis of variance. The effect of day post-ovulation was included as an additional factor when analyzing variables that were recorded repeatedly. When needed, Tukey's multiple comparisons tests were carried out. Linear regression analysis was used to further explore the relation between IOI and other variables.

The repeatability of IOIs and of the diameter of the OVF at day −1 were calculated as the intra-class correlation from the variance components between and within jennies [16].

3. Results

3.1. Secondary follicular waves

A secondary follicular wave was identified in 48% (13/27) of the cycles that were monitored in this study, while in the rest of the cycles the only follicular wave was the one culminating in ovulation. The mean IOI was no different between cycles with or without secondary waves ($p > 0.05$), and short, medium-length and long IOIs were represented in both types of cycle. No significant differences were found between cycles with or without secondary waves in the variables shown on Table 1, which mainly refers to events that occur after the end of secondary waves and after the onset of the major ovulatory follicular wave. Thus, data from both types of cycle were pooled for further analysis, and the rest of the paper does not differentiate between cycles with or without secondary waves. .

3.2. Classification according to length of the IOI

A single ovulation occurred in all the estrous cycles monitored in this study. Fig. 1 shows the distribution of IOIs and their classification according to length. Average IOIs were 21.2 ± 0.3, 23.9 ± 0.4 and 26.2 ± 0.3 days for short ($n = 10$), medium ($n = 7$) and long ($n = 10$) cycles, respectively. Individual intervals ranged from 20 to 30 days. The within-jenny repeatability of IOIs was 0.49 ($p < 0.05$).

3.3. Progesterone concentrations and the timing of luteolysis

Data from the 9 cycles (one from each jenny) in which progesterone concentrations were determined shows that there was little variation in luteal lifespan or function. Progesterone concentrations were not different ($p > 0.05$) between short, middle or long cycles