Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 70 (2008) 1489–1497
Theriogenology
www.theriojournal.com

Oestrus cycle characteristics and prediction of ovulation in Catalonian jennies

E. Taberner *, A. Medrano, A. Peña, T. Rigau, J. Miró

Department of Animal Medicine and Surgery, School of Veterinary Medicine, Autonomous University of Barcelona, Campus UAB, Edifici V, E-08193 Bellaterra, Spain

Received 7 April 2008; received in revised form 10 June 2008; accepted 18 June 2008

Abstract

The Catalonian donkey breed is in danger of extinction, and much needs to be learned about the reproductive features of its females if breeding and conservation programmes are to be successful. This study reports the oestrous behaviour, oestrus cycle characteristics and dynamic ovarian events witnessed during 50 oestrous cycles (involving 106 ovulations) in 10 Catalonian jennies between March 2002 and January 2005. These jennies were teased, palpated transrectally and examined by ultrasound using a 5 MHz linear transducer—daily during oestrus and every other day during dioestrus. Predictors of ovulation were sought among the variables recorded.

The most evident signs of oestrus were mouth clapping (the frequent vertical opening and closing of the mouth with ears depressed against the extended neck) and occasional urinating and winking of the vulval lips (homotypical behaviour). Interactions between jennies in oestrus were also recorded, including mounting, herding/chasing, the Flehmen response, and vocalization (heterotypical behaviour).

Nine jennies ovulated regularly throughout the year; one had two anovulatory periods (54 and 35 days). The length of the oestrus cycle was 24.90 ± 0.26 days, with oestrus itself lasting 5.64 ± 0.20 days (mean ± S.E.M.) and dioestrus 19.83 ± 0.36 days. The incidence of single, double and triple ovulations was 55.66% ($n = 59$), 42.45% ($n = 45$) and 1.89% ($n = 2$), respectively. No significant difference was seen in the number of ovulations involving the left and right ovaries (52.63% [$n = 70$] compared to 47.37% [$n = 63$] respectively; $P > 0.05$). The mean interval between double ovulation was 1.44 ± 3.98 days. The mean diameter of the preovulatory follicle at day −1 was 44.9 ± 0.5 mm; the mean growth rate over the 5 days before ovulation was 3.7 mm/day.

Data on preovulatory changes in oestrous behaviour, follicle size, follicle texture, the echographic appearance of the follicle and uterus, and uterine tone were subjected to stepwise logistic regression analysis to detect predictors of ovulation. The logit function showed the best predictors to be follicle size, follicular texture and oestrous behaviour. Certain combinations of these three variables allow the prediction of ovulation within 24 h with a probability of >75%.

Keywords: Oestrus cycle; Prediction of ovulation time; Jennies

1. Introduction

The Catalonian donkey is an endangered local donkey breed that can be found in a number of Pyrenean and pre-Pyrenean areas of Catalonia (northeastern Spain) [1]. The Catalonian donkey has contributed to the development of other breeds, such as the American

* Corresponding author. Tel.: +34 935 811 959; fax: +34 935 812 006.
E-mail addresses: estertaberner@gmail.com (E. Taberner), jordi.miro@uab.cat (J. Miró).

0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.06.096

Mammoth Ass and the Italian Pantellaria, Ragusana and Martina Franca breeds.

Numerous publications describe the characteristics of the oestrus cycle and reproductive behaviour of mares (*Equus caballus*). However, studies on reproductive behaviour, the oestrus cycle and ovulation in donkeys are almost non-existent. Among the literature available, Nishikawa and Yamasaki [2] report the characteristics of the oestrus cycle in *Equus asinus*, Henry et al. [3] and Meira et al. [4] report the same in the Pega breed and in crossbreed jennies from Brazil, and Blanchard et al. [5] report the reproductive behaviour, the ovarian dynamics and the hormonal changes during the oestrous cycle of Mammoth Ass jennies in Texas. However, little is known about the differences between Catalonian and other jennies in these respects—a lack of basic knowledge that hinders the use of reproductive technologies [6].

The main aims of this study were to examine the oestrous cycle of the Catalonian donkey and to establish indicators that predict the exact moment of ovulation. Such knowledge should help maximize the production of live foals and help conserve the breed.

2. Materials and methods

2.1. Experimental animals and study location

This study was undertaken at the Experimental Farm and Countryside Service of the Veterinary Faculty of the Autonomous University of Barcelona (41°N) between March 2002 and January 2005. The animals involved were 10 clinically healthy, cycling Catalonian jennies aged 3–12 years. All were kept outdoors in a group and were fed grain forage, straw, hay, and water *ad libitum*. Body weight was monitored throughout the study; all the jennies showed a good body condition. Jacks were maintained in nearby paddocks and fed similar diets. Oestrous behaviour, oestrous cycle characteristics and the prediction of ovulation were studied during 50 oestrous cycles (106 ovulations).

2.2. Oestrus detection and ultrasonic examination

Jennies were teased, palpated transrectally and examined by ultrasound using a 5 MHz linear transducer (Aquila Vet, Esaote Pie Medical®, Pie Medical Equipment B.V., Maastricht, The Netherlands) daily during oestrus and every other day during dioestrus. The durations of 50 complete cycles were recorded, including the length of 36 oestrus periods and 23 dioestrus periods. The largest diameter of the preovulatory follicle and the changes in follicular shape were recorded. Ovulation was defined (day 0) as the day when the preovulatory follicle was irregular in outline or when the corpus rubrum was observed.

Six different variables were evaluated and categorized as below:

1. Oestrous behaviour

0 points	no oestrus, resistance
1 point	indifference
2 points	interested, mouth clapping
3 points	mouth clapping, urination, vulvar activity and immobilization response

2. Follicle size

0 points	<35 mm
1 point	35–39 mm
2 points	40–44 mm
3 points	>45 mm

3. Follicle texture

0 points	fibrous ovary
1 point	turgid
2 points	elastic
3 points	soft
4 points	oedematous and dolorous

4. Echographic appearance of the follicle

0 points	round
1 point	oval
2 points	triangular with ovulation conus
3 points	irregular

5. Echographic appearance of the uterus

0 points	grey and diffuse
1 point	irregular black zones
2 points	regular black zones

6. Uterine tone

0 points	poor
1 point	fair
2 points	good

2.3. Statistical analysis

All analyses were performed using the SAS® statistical package (Statistical Analysis System, SAS, Institute Inc., Release 8.2, Cary, NC, USA, 2001).

The durations of the oestrous cycle, oestrus and dioestrus were represented as means $\pm$ S.E.M. The PROC GLM procedure was used to compare the percentages of ovulation from the right and left ovaries. Differences between preovulatory follicle diameters in simple and double ovulations were determined using the PROC GLM procedure.

Preovulatory changes in oestrous behaviour, follicle texture, follicle size, echographic follicle appearance, uterine tone and echographic uterine appearance were analysed taking into account the number of days to ovulation. Data for most jennies were recorded on two or more occasions and analysed using the PROC MIXED (SAS software) routine including the effect of the individual jenny as a random factor.

The independent variables that influence ovulation were selected by stepwise logistic regression. The relationship between these variables and time to ovulation (up to 5 days) was studied using stepwise logistic regression for ordinal responses, employing the logit function as the link function. The logit function is defined as

$$P(x_i) = \frac{1}{1 + e^{-x_i'\beta}}$$

where, $P(x_i)$ is the probability of ovulation (or non-ovulation) within a given time, and $x_i'\beta$ the product of the vectors of the observed variables (x_i') in each individual and the partial regression coefficients (β). This analysis was undertaken using the PROC LOGISTIC routine. Variables with P-values of <0.05 were selected as predictive of ovulation.

3. Results

3.1. Sexual behaviour

Sexual behaviour was observed when the jennies were teased. On some occasions, the simple arrival of the jennies at the place where they were teased evoked oestrous signs such as mouth clapping (frequent vertical opening and closing of the mouth with ears depressed against the extended neck). During pre-copulatory interaction the attending jack vocalized, mounted without erection, nibbled the head, neck and extremities of the jenny, sniffed the perineal area and showed the Flehmen response. The jennies showed mouth clapping, sialorrhea, immobility and no aggression towards the jack—except for one jenny that maintained a permanently aggressive attitude towards the males. This jenny nonetheless showed a preovulatory follicle with a diameter of >35 mm, although ovulation was delayed. Occasionally, the older jennies showed tail raising, urinating and contraction and relaxation of the labia of the vulva with eversion of the clitoris.

Within their group the jennies also expressed heterotypical sexual behaviour, included chasing one another, mounting, the Flehmen response and sniffing.

3.2. Oestrus cycle and ovulation

Over the 30 months of the study, 9 of the 10 jennies cycled throughout the entire period, with no anovulatory season. Only one jenny showed anovulatory oestrus periods at the end of 2002 and 2003 (54 and 35 days, respectively), with follicles <30 mm in diameter.

The mean length of the oestrus cycle, oestrus and dioestrus was 24.9 ± 0.26 (range 22–29), 5.64 ± 0.2 (range 4–8) and 19.83 ± 0.36 (range 17–23) days, respectively (mean ± S.E.M.). Table 1 shows the mean data for individual jennies. The duration of oestrus was longer in December to February than in other months (6.75 ± 0.25 compared to 5.52 ± 0.22 days [mean ± S.E.M]).

A slightly (but not significantly) greater frequency of ovulation was recorded for the left ovary (52.63% [$n = 70$] compared to 47.37 [$n = 73$]; $P > 0.05$)

Table 1
Mean duration of the oestrus cycle, length of oestrus, and length of dioestrus in 10 Catalonian jennies

Jenny	Oestrus cycle (days)	*n*	Oestrus (days)	*n*	Dioestrus (days)	*n*
1	25.25 ± 0.84	8	5.88 ± 0.48	8	20.17 ± 0.83	6
2	24.71 ± 0.66	7	4.75 ± 0.75	4	20 ± 1.00	3
3	27 ± 0.41	4	6.25 ± 0.75	4	20.67 ± 0.88	3
4	23 ± 0	2	5 ± 0	3	18 ± 0	2
5	24 ± 0.55	5	5.33 ± 0.88	3	18.5 ± 1.50	2
6	26.4 ± 1.03	5	8 ± 0	1	–	–
7	25.5 ± 1.50	2	–	–	–	–
8	24.6 ± 0.60	5	5.33 ± 0.33	3	21 ± 0	2
9	23.57 ± 0.43	7	6 ± 0.41	4	18.33 ± 0.33	3
10	25 ± 0.45	5	5.5 ± 0.43	6	21.5 ± 0.50	2
Mean	24.90 ± 0.26	50	5.64 ± 0.20	36	19.83 ± 0.36	23

Results are expressed as means ± S.E.M.

Table 2
Distribution of ovulation (left and right ovaries)

Jenny	Left ovary (*n*)	Right ovary (*n*)
1	4	6
2	5	4
3	10	8
4	8	9
5	5	4
6	8	5
7	6	7
8	7	6
9	10	8
10	7	6
Total	70	63
Percentage (%)	52.63	47.37

(Table 2). The incidence of single, double and triple ovulations was 55.66% ($n = 59$), 42.45% ($n = 45$) and 1.89% ($n = 2$), respectively (Table 3). Some 45% of multiple ovulations occurred in just three jennies (jenny 3 produced more than one follicle in 7 out of 14 cycles studied, jenny 9 did so in 11 out of 12, and jenny 10 did so in 6 out of 10). Fourteen (41.18%) double ovulations occurred on the same day, 7 (20.59%) occurred with an interval of 1 day between ovulations, 9 (26.47%) occurred with an interval of 2 days, and 1 (2.94%) occurred with an interval of 4, 5, 6 or 9 days between the ovulation events. The mean of the interval between multiple ovulations was 1.44 ± 3.98 days (range 0–9 days). These multiple ovulations involved the left ovary alone on 11 occasions (23.4%), the right ovary alone on 9 occasions (19.15%), and both ovaries on 27 occasions (57.45%).

3.3. Follicular dynamics

The mean diameter of the preovulatory follicles at 1 day before ovulation was 44.9 ± 0.5 mm (mean ± S.E.M.) (range 35–60 mm). In multiple ovulations the average size of the preovulatory follicles was 44.1 ± 0.6 mm (range 35–55.4 mm), and in single ovulations 46.3 ± 0.9 mm (range 37–60 mm) ($P > 0.05$) (Table 4). The preovulatory follicles involved in synchronic and asynchronic double ovulations had mean diameters of 44.1 ± 0.9 and 44.0 ± 1.0 mm, respectively ($P > 0.05$).

The growth rate of the preovulatory follicle was 3.7 mm per day over days −5 to −1, but this fell over the last 24 h before ovulation (Table 5).

Only in one jenny (in 2 of 14 oestrus cycles - in January and May) did the preovulatory follicles fail to reach the ovulation stage; these follicles were transformed into what seemed to be hemorrhagic follicles. The latter follicles reached 46 and 61 mm in diameter and showed an interior fibrin network; the follicular wall was also thicker than normal (4–7 mm). The next ovulation occurred after 23 and 46 days, respectively (Fig. 1).

Table 4
Diameters of preovulatory follicles at 1 day before ovulation in single and double ovulations

Mean diameter of preovulatory follicle (mm)				
Jenny	Single ovulation	*n*	Double ovulation	*n*
1	52.3 ± 2.9	4		0
2	39.5 ± 2.5	2	40.3 ± 1.8	7
3	48.6 ± 5.4	2	43.2 ± 1.2	11
4	46.7 ± 2.4	3	44.9 ± 1.3	10
5	43.6 ± 1.5	4		0
6	44.3 ± 3.4	3	46.1 ± 1.8	7
7	48.3 ± 2.5	6	48 ± 1.2	8
8	47.4 ± 2.4	7	48 ± 5.0	2
9	41	1	41.6 ± 1.2	11
10	43.1 ± 0.7	4	44.4 ± 3.7	4
Mean	46.3 ± 0.9	36	44.1 ± 0.6	60

Results are expressed as means ± S.E.M.

Table 3
Percentage of single, double and triple ovulations

Jennies	Single ovulations (%)	*n*	Double ovulations (%)	*n*	Triple ovulations (%)	*n*	Total ovulations
1	62.50	5	37.50	3			8
2	33.33	2	66.67	4			6
3	50.00	7	50.00	7			14
4	64.29	9	35.71	5			14
5	88.89	8	11.11	1			9
6	44.44	4	55.56	5			9
7	66.67	8	33.33	4			12
8	91.67	11	8.33	1			12
9	8.33	1	75.00	9	16.67	2	12
10	40.00	4	60.00	6			10
Total	55.66	59	42.45	45	1.89	2	106

Fig. 1. Hemorrhagic follicle (diameter 46 mm).

Table 5
Growth rate of preovulatory follicles over days −5 to −1

Day before ovulation	Follicle growth rate (mm/day)	*n*
−5 to −4	4.3 ± 0.4	24
−4 to −3	3.8 ± 0.2	63
−3 to −2	3.9 ± 0.2	94
−2 to −1	3.3 ± 0.2	73
−5 to −2	3.9 ± 0.1	181
−5 to −1	3.7 ± 0.1	254

Results are expressed as means ± S.E.M.; day 0: ovulation.

A corpus luteum (CL) with a homogeneous echogenic texture was observed in the majority of jennies the first day after ovulation; the remainder showed a CL with a non-echogenic central area (Fig. 2).

3.4. Prediction of the ovulation

3.4.1. Changes in other variables over the days before ovulation

Table 6 shows the changes in the variables measured. Oestrous behaviour scores increased over oestrus as the jennies showed more interest in and greater receptivity towards jacks; significant differences were seen between day −5 and days −1, −2 and −3 ($P < 0.05$). Follicle size increased significantly on

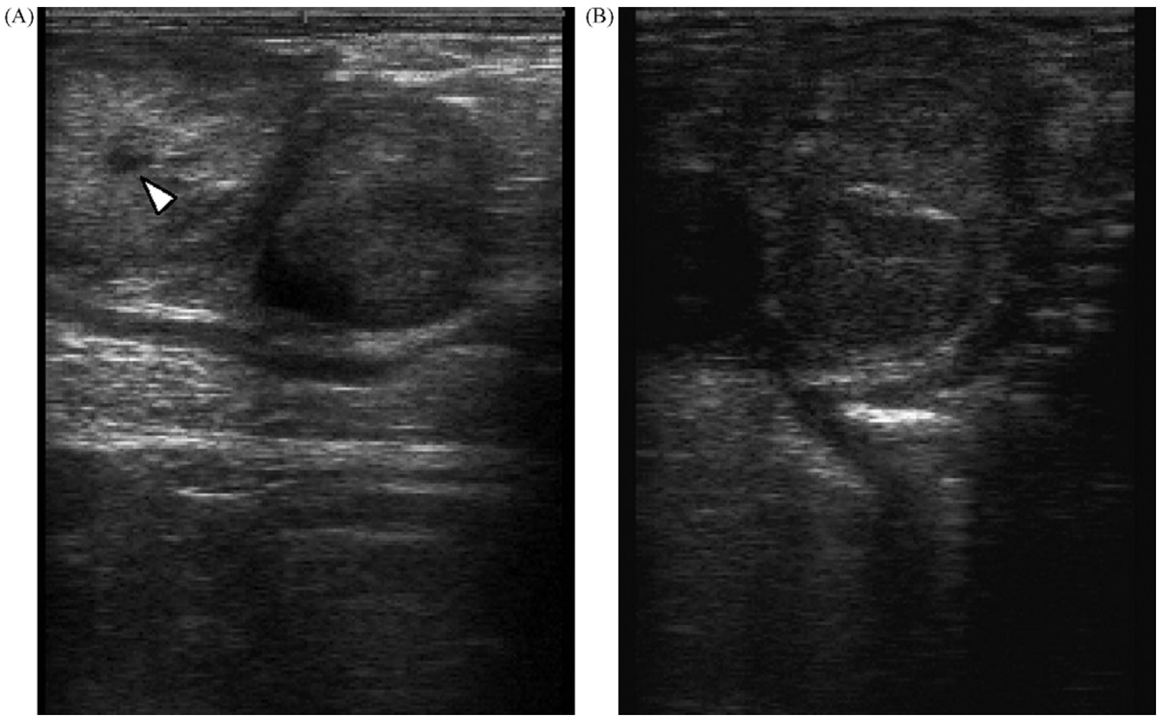

Fig. 2. Corpus luteum with non-echogenic central area (white arrow) (A), and another with a homogeneous echogenic texture (B).

Table 6
Values of the measured variables on different days before ovulation

Variables	Days before ovulation				
	5	4	3	2	1
Oestrous behaviour	1.24 ± 0.22^{c}	$1.66 \pm 0.20^{b,c}$	$1.89 \pm 0.15^{a,b}$	$2.03 \pm 0.14^{a,b}$	2.15 ± 0.11^{a}
Follicle size	0.36 ± 0.17^{c}	0.48 ± 0.10^{c}	1.23 ± 0.17^{b}	1.85 ± 0.13^{a}	2.36 ± 0.08^{a}
Follicle texture	1.69 ± 0.19^{c}	1.68 ± 0.11^{c}	$1.99 \pm 0.10^{b,c}$	2.23 ± 0.12^{b}	2.69 ± 0.08^{a}
Echographic follicular app.	0.14 ± 0.08^{b}	0.16 ± 0.06^{b}	0.47 ± 0.13^{b}	0.48 ± 0.10^{b}	1.05 ± 0.13^{a}
Uterine tone	0.81 ± 0.14^{b}	$0.66 \pm 0.14^{a,b}$	$0.59 \pm 0.11^{a,b}$	$0.55 \pm 0.12^{a,b}$	0.35 ± 0.08^{a}
Echographic uterine app.	0.95 ± 0.15^{b}	$1.03 \pm 0.10^{a,b}$	$1.14 \pm 0.11^{a,b}$	$1.30 \pm 0.09^{a,b}$	1.33 ± 0.09^{a}
N	22	31	39	41	66

Results are expressed as means ± S.E.M. Different superscript letters (a–c) within the same row indicate significant differences ($P < 0.05$). app. = appearance.

days −4, −3 and −2 ($P < 0.05$); on the day before ovulation further growth was slow. The consistency of the preovulatory follicle, measured by rectal palpation, decreased from the third day prior to ovulation. Echographic follicular appearance scores increased particularly on the day before ovulation ($P < 0.05$). The preovulatory follicles tended to elongate and took on a triangular shape over oestrus. Uterine tone, evaluated by rectal palpation, decreased between day −5 and −1 ($P < 0.05$), but day-to-day reductions were not significant. During oestrus the uterus appeared echographically as a series of black spaces arranged in a regular, radial fashion.

3.4.2. Probability of ovulation

Follicle size, oestrous behaviour and follicle palpation characteristics were revealed by stepwise logistic regression as the only variables to influence the probability of ovulation occurring in the following days.

Four intercept estimates corresponding to each to the cumulative distribution probabilities of the $n - 1$ response categories (1, 2, 3 and 4 days to ovulation) were determined: these were −5.39, −3.85, −2.36 and −0.67, respectively. The slopes for oestrous behaviour, follicle texture and follicle size were 0.40, 0.55 and 1.40, respectively. By introducing these values into the logit link function, the cumulated probabilities were

Table 7
Probability of ovulation within 24 h (>50% probability) determined from follicle size, follicle texture, and oestrous behaviour scores

Oestrous behaviour score	Follicle size score	Follicular texture score	Cumulative probability of ovulation (%)			
			−24 h	−48 h	−72 h	−96 h
3	3	4	90.21	97.72	99.48	99.90
3	3	3	84.16	96.11	99.09	99.83
3	3	2	75.39	93.43	98.44	99.71
3	3	1	63.85	89.14	97.33	99.50
3	3	0	50.46	82.55	95.46	99.14
3	2	4	69.47	91.36	97.91	99.61
3	2	3	56.75	85.90	96.44	99.33
2	3	4	86.01	96.62	99.22	99.86
2	3	3	78.00	94.27	98.65	99.75
2	3	2	67.15	90.47	97.68	99.57
2	3	1	54.10	84.55	96.05	99.25
2	2	4	60.29	87.58	96.91	99.42
1	3	4	80.40	95.01	98.83	99.78
1	3	3	70.28	91.66	97.99	99.63
1	3	2	57.69	86.36	96.57	99.35
1	2	4	50.32	82.47	95.43	99.13
0	3	4	73.24	92.71	98.26	99.68
0	3	3	61.21	87.99	97.02	99.44
0	3	2	47.64	80.86	94.94	99.03

determined for all possible combinations of values for the three variables selected. Table 7 shows the combinations predicting ovulation within 24 or 48 h, etc., with a probability of at least 50%.

4. Discussion

At the start of pre-copulatory interactions, the jacks vocalized and mounted without erection. They also nibbled the jennies head, neck and extremities to make sure they were submissive. These findings have been reported in other studies [7–10].

The main signs of oestrus detected were similar to those described by Trimeche and Tainturier [11] and Blanchard et al. [5] for domestic jennies. Mouth clapping was a clear sign of submission, as other authors have indicated for zebra, young mares and foals [11–13].

The present jennies showed heterotypical behaviour such as mounting, the Flehmen response and chasing one another, as described Henry et al. [14]. This type of behaviour (particularly mounting) is common among cows in oestrus, but not in mares [14].

Only one jenny showed a permanently aggressive attitude towards the jacks, although she did ovulate. Similar findings of altered oestrous behaviour have been reported for domestic jennies [3] and mares [12].

Nine of the studied jennies ovulated regularly throughout the year, only one jenny had two anovulatory periods (54 and 35 days) during the winter. It was generally observed, however, that the oestrous cycle was not affected by the season of the year. Ginther [12] indicates that the reproductive cyclicity of jennies is less affected by season than that of horses or ponies. In a study of different breeds in southern Wisconsin, Ginther et al. [15] reported that 64% of jennies ovulated in December and 82–100% during the other months. However, Asdell [16] found the breeding season of donkeys to be similar to that of horses.

The length of the oestrus cycle (24.9 ± 0.26 days) was similar to that indicated by Miró et al. [17], Vandeplassche et al. [18] and Trimeche and Tainturier [11], although Blanchard et al. [5] and Nishikawa and Yamazaki [2] have described shorter cycles (22.8 and 23.3 days, respectively). The mean length of oestrus in Catalonian donkeys is clearly longer than in mares (21 days) [19] but similar to that in ponies (25 days) [12].

The duration of oestrus in the present jennies was similar to that reported for other breeds of donkey [4,5], but shorter than the 7.9 ± 2.5 days reported by Henry et al. [3] for Pega and crossbreed jennies in Brazil, and the 7.5 ± 1.2 days reported by Trimeche and Tainturier [11] for Poitou jennies in France. Oestrus was longer during the winter months (December–February) (6.75 ± 0.25 days) compared to the rest of the year (5.52 ± 0.22 days). Ginther et al. [15] also described different durations of oestrus depending on the time of year (May–October: 5.7–6.9 days and November–April: 7.4–15.2 days).

The incidence of multiple ovulations in jennies has been reported to range from 5.3 to 61% [3,5,11,18]. In the present study the incidence of multiple ovulations was high (44.3%), with 45% occurring in just three jennies. Other authors have reported similar findings in individual mares and jennies [3,5,11,19]. In horses, breed is a determining factor in the occurrence of multiple ovulations. Large breeds have a higher incidence than small breeds and ponies (25% in thoroughbreds compared to 2% in ponies) [19,20]. The Catalonian and Mammoth breeds have the highest incidence of double ovulations (61 and 44.3%) recorded for donkeys. In fact, Catalonian donkeys were involved in the development of the Mammoth breed, the main characteristic contributed being their large size [1]. This suggests that double ovulations in jennies are affected by breed.

Henry et al. [3] indicate a high frequency of ovulation for the left ovary (61%). In the present study a slightly greater frequency of ovulation from the left ovary was seen, but the difference was not significant.

The mean interval between multiple ovulations in the present jennies was 1.44 ± 3.98 days (range 1–9 days). Ginther et al. [15] and Blanchard et al. [5] reported larger intervals of 3.1 and 2 days respectively in other jennies.

The mean diameter of the ovulating follicle in the present jennies was about 45 mm, a size similar to that described for mares by other authors [19,21,22]. Carluccio et al. [23] reported the mean diameter at ovulation to be 42.9 mm in Martina Franca jennies, similar to the mean preovulatory diameter seen in the present Catalonian jennies. However, in other donkey breeds the mean diameter of preovulatory follicles is reported to be smaller at around 36–41 mm [4,18,24]. These differences may be associated with the large size of Catalonian donkeys.

Pierson and Ginther [22] noted that the diameter of preovulatory follicles in double ovulations was smaller than in single ovulations. However, no significant differences were seen in the present work.

The growth rate of the preovulatory follicle was found to be 3.7 mm/day, although a reduction in this rate was seen on day -1 (in fact the follicle sometimes became smaller). Ginther [12] observed the same growth rate in ponies, but a lower rate in mares (2.7 mm/day).

One jenny suffered a likely hemorrhagic follicle in two cycles—one in January and one in May. Carnevale [25] described ponies to more frequency suffer hemorrhagic follicles in autumn (21%) than in the April–August period (1%). In contrast, Ginther [12] concluded that season has no influence on the appearance of hemorrhagic follicles.

In the present work, most of the CLs observed showed a homogeneous echogenic texture; in contrast, 50–70% of mares have CLs with a non-echogenic central area [26].

Of the six variables (oestrous behaviour, follicle size, follicle texture, echographic follicle and uterine appearance) examined to determine which best predicted impending ovulation, stepwise multiple regression showed follicle size to be the most reliable. Koskinen et al. [27] concluded the same in mares. Signs of oestrous increased as ovulation approached, as recorded by other authors [13,28]. This variable was the second best predictor. As observed in the present study, many researchers have described the softening of the follicle prior to ovulation; in mares 80–90% of preovulatory follicles show a change in consistency from turgid to soft during oestrous [12,27,29]. However, some authors report that this does not always occur [19,30]. These differences may be related to the interval between examinations. In the present study the jennies showed a soft follicle on day −1 when the examination interval was 24 h. Thus, follicle texture also appeared as a predictor of ovulation, although it was the least reliable of those selected.

In mares, Miró et al. [31] reported only follicle size and texture to be selected by the same procedure; oestrous behaviour was not predictive of ovulation. This difference may be connected with the social organization of horses and donkeys. Wild horses breed in a harem group of one stallion to several mares, while donkeys are territorial with each male guarding a territory and breeding with jennies passing through or residing in that territory [32]. It may be that jennies undertake more obvious sexual behaviour than mares to attract the attention of males.

Neither the echographic appearance of the uterus and follicle, nor the uterine tone, were selected as predictors of ovulation. In mares, Pierson and Ginther [22] classified 85% of follicles as non-spherical from day −1 to ovulation. It is possible that the softening of follicles is related to their elongation into the ovulation fossa. In the present study the follicles showed a tendency to elongate, but this was not sufficiently marked to predict ovulation. When the follicle population was very large the smaller follicles took on a similar shape due to the pressure they exerted on one another, a consequence of the lack of free space. During oestrous, the uterus loses tonicity since the endometrial folds enlarge and become oedematous [33], but there are no important day-to-day changes during the oestrous period; uterine tone does not, therefore, help predict ovulation. Finally, echographic evaluation of the uterine appearance showed hypoechogenic regions linked to oedema, but without variation over the oestrus period [34]; it is therefore not able to predict ovulation.

In conclusion, the combination of oestrous behaviour, follicle size and follicle texture, measured daily, is able to predict the onset of ovulation. A jenny that shows mouth clapping, urination and immobility, that has a follicle larger than 45 mm, and that has very soft follicle texture, is likely to ovulate within 24 h with a probability of 90%. Different combinations of these three variables predict ovulation within 48 h with a probability of >70%.

Acknowledgements

The authors would like to thank Mr. José Manuel Fernandez Castro, owner of some of the jennies, for his help in this study. This work was funded by Grant RZ-03-021 (*Instituto Nacional de Investigaciones Agrarias*, Spain).

References

[1] Jordana J, Folch P. The endangered Catalonian donkey breed: the main ancestor of the American ass or mammoth. J Eq Vet Sci 1996;16:436–41.
[2] Nishikawa Y, Yamazaki Y. Studies on reproduction in asses. I. Breeding season, oestrous cycle and length of heat. Jpn J Zootech Sci 1949;19:119–24.
[3] Henry M, Figueiredo AEF, Palhares MS, Coryn M. Clinical and endocrine aspects of the oestrous cycle in donkeys (*Equus asinus*). J Reprod Fert Suppl 1987;35:297–303.
[4] Meira C, Ferreira JCP, Papa FO, Tornero MTT, Bicudo SD. Study of the estrous cycle in donkeys (*Equus asinus*) using ultrasonography and plasma progesterone concentrations. Biol Reprod Mono 1995;1:403–10.
[5] Blanchard TL, Taylor TS, Love CL. Estrous cycle characteristics and response to estrus synchronization in Mammoth Asses (*Equus asinus americanus*). Theriogenology 1999;52:827–34.
[6] Pukazhenthi BS, Wildt DE. Which reproductive technologies are most relevant to studying, managing and conserving wildlife? Reprod Fertil Dev 2004;16:33–46.
[7] Lobo VL. Comportamiento sexual y normalización de las características seminales del burro catalan. Universitat Autónoma de Barcelona. Facultat de Veterinària/Unitat de Reproduccció. Treball Master; 2003. p. 9–11.

[8] Henry M, Gastal EL, Meira C, Diaz AP. Caracteristicas do semen de jumentos da raça nordestina. In: Proceedings of VII Braz Cong Anim Reprod, vol. 72; 1987 (Abstr.).
[9] Gastal MO, Henry M, Beker AR, Gastal EL, Gonçalves A. Sexual behavior of donkey jacks: influence of ejaculatory frequency and season. Theriogenology 1996;46:593–603.
[10] Kreuchauf A. Reproductive physiology in the jackass. Anim Res Dev 1984;20:51–78.
[11] Trimeche A, Tainturier D. Étude échographique de la cinétique folliculaire des baudettes du Poitou pendant l'oestrus au cours du printemps et de l'été. Revue Méd Vét 1995;146:743–8.
[12] Ginther OJ. Reproductive biology of the mare: basic and applied aspects, 2nd ed., Wisconsin: Cross Plains, Equiservices Publishing; 1992. p. 75–232.
[13] Asa CS. Sexual behaviour of mares. Vet Clin N Am: Eq Pract 1986;2:519–34.
[14] Henry M, McDonnell SM, Lodi LD, Gastal EL. Pasture mating behaviour of donkeys (*Equus asinus*) at natural and induced oestrus. J Reprod Fert Suppl 1991;44:77–86.
[15] Ginther OJ, Scraba ST, Bergfelt DR. Reproductive seasonality of the jenney. Theriogenology 1987;27:587–92.
[16] Asdell SA. Patterns of mammalian reproduction. Ithaca, NY: Cornell University Press; 1964. p. 530.
[17] Miró J, Lobo V, Medrano A, Rigau T. Dinámica folicular en la burra catalana. In: Comunication, IV Congreso Ibérica de Reproducción Animal; 2003.
[18] Vandeplassche M, Wesson JA, Ginther OJ. Behavioral, follicular and gonadotropin changes during the estrous cycle in donkeys. Theriogenology 1981;16:239–49.
[19] Hughes JP, Stabenfeldt GH, Evans JW. Clinical and endocrine aspects of the oestrous cycle of the mare. Proc Annu Conv Am Assoc Eq Pract 1972;18:119–51.
[20] Ginther OJ, Gastal EL, Gastal MO, Bergfelt DR, Baerwald AR, Pierson RA. Comparative study of the dynamics of follicular waves in mares and women. Biol Reprod 2004;71: 1195–201.
[21] Lindeberg H, Kuntsi-Vaattovaara H, Katila T. Predicting ovulation in the mare. In: Communication, 12th International Congress on Animal Reproduction; 1992. p. 144–46.
[22] Pierson RA, Ginther OJ. Ultrasonic evaluation of the preovulatory follicle in the mare. Theriogenology 1985;24:359–68.
[23] Carluccio A, Panzani S, Tosi U, Faustini M, De Amicis I, Veronesi MC. Efficacy of hCG and GnRH for inducing ovulation in the jenny. Theriogenology 2007;68:914–9.
[24] Dadarwal D, Tandon SN, Purohit GN, Pareek PK. Ultrasonographic evaluation of uterine involution and postpartum follicular dynamics in French Jennies (*Equus asinus*). Theriogenology 2004;62:257–64.
[25] Carnevale EM. Folliculogenesis and ovulation. In: McKinnon AO, Rantanen NW, editors. Equine diagnostic ultrasonography. Williams & Wilkins; 1998. p. 201–11.
[26] Pierson RA, Ginther OJ. Ultrasonic evaluation of the corpus luteum of the mare. Theriogenology 1985;23:795–806.
[27] Koskinen E, Kuntsi H, Lindeberg H, Katila T. Predicting ovulation in the mare on the basis of follicular growth and serum oestrone sulphate and progesterone levels. J Vet Med A 1989;36:299–304.
[28] Squires EL. Estrous detection. In: McKinnon AO, Voss JL, editors. Equine reproduction. Philadelphia/London: Lea & Febiger; 1993. p. 186–95.
[29] Parker WG. Sequential changes of the ovulating follicle in the estrous mare as determined by rectal palpation. In: Proceedings of Annual Conference of Veterinarians, College of Veterinary Medicine and Biomedical Sciences. Fort Collins (CO): Colorado State University; 1971. p. 1–25.
[30] Witherspoon DM, Talbot RB. Nocturnal ovulation in the equine animal. Vet Rec 1970;87:302–4.
[31] Miró J, Muñoz F, Piedrafita J, Quintero-Moreno A. Prediction of the day of ovulation in mares through physiological parameters measured during estrous. Revista Científica FCV-LUZ 2004;XIV(1):54–8.
[32] McDonnell SM. Reproductive behavior of stallions and mares: comparison of free-running and domestic in-hand breeding. Anim Reprod Sci 2000;60/61:211–9.
[33] England G. Allen's fertility and obstetrics in the horse, 2nd ed., Oxford, London: Blackwell Science Ltd.; 1996. p. 9–12.
[34] Ginther OJ. Ultrasonic Imaging and Reproductive Events in the Mare. Cross Plains, WI: Equiservices Publishing; 1986.