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Effect of the season on some aspects of the estrous cycle in Martina Franca donkey

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ABSTRACT

The Martina Franca (MF) donkey breed, with 48 jackasses and 515 jennies, is considered an endangered breed according to the data from the Monitoring Institute for Rare Breeds and Seeds in Europe. The knowledge of the estrous cycle characteristics has a great impact for assisted reproduction, especially in endangered species. In this study, the estrous cycle characteristics were investigated in 12 MF jennies throughout the year. Estrous cycle, estrous and diestrous lengths, follicular growth and ovulation, and estradiol-17 β (E2) and progesterone (P4) plasma concentrations were monitored in MF jennies and compared in different seasons. In all jennies (100%) estrous cycle was detected during the whole year, with no differences in the estrous cycle length among seasons. However, a significant increase of estrous length in spring and summer compared with autumn and winter was found. Diestrus was shorter in summer than in the other seasons. Estrous behavior was always shown and characterized by rhythmic eversion of the vulvar labia (winking) with exhibition of the clitoris, urination, male receptivity and clapping, with sialorrhoea, neck and head extension, and back ears. Estrus was characterized by the ovulation of a larger follicle in spring and summer than in autumn and winter. The pattern of E2 and P4 plasma concentrations during the estrous cycle were similar to that reported for the mare, but without differences among the four seasons, so that a negligible effect of environmental conditions on ovarian E2 and P4 secretion was hypothesized, despite the larger diameter of the ovulating follicle in spring and summer.

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1. Introduction

In the last years, a growing importance in donkey (*Equus africanus asinus*) medicine has been observed as the result of the interest for hypoallergenic milk production and onotherapy. Furthermore, several donkey breeds are considered as endangered because of their small population size and serious risks of consanguinity [1]. To increase donkeys' population and breed's biodiversity preservation, several research projects on donkey reproduction have

been performed, and the knowledge on reproductive aspects of this species and breed-specific characteristics arose [2–7]. The Martina Franca (MF) breed consists of about 48 approved-for-breeding jackasses and 515 jennies, and it is therefore considered an endangered breed according to the data from the Monitoring Institute for Rare Breeds and Seeds in Europe. For these reasons, several studies addressed to the reproductive characteristics of MF jennies and jackasses [6,8–12].

The knowledge of the estrous cycle has a great impact on reproduction management because of the possible control of ovulation or diestrus, and the simple transposition of knowledge obtained in the horse to the donkey is often ineffective [13,14].

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In the donkey, in example, the reproductive activity seems to be less affected by the season in comparison with the horse. Henry, et al. [15] reported that only 40% of the monitored jennies showed a seasonal anestrus, and a limited effect of the season was recently reported in the jackass [6,16]. In the jenny, also the estrous cycle seems to be different in comparison with the horse. The diestrus seems, in fact, to be longer in the jenny than in the mare, whereas the estrus results were of comparable length [15,17–19].

However, the possible role of the environmental conditions (temperature and natural lighting) on estrous cycle length and reproductive endocrinology of the jenny and in seasonal anestrus onset, is still not completely investigated.

In the purpose to provide new knowledge for the optimization of breeding management in the endangered MF donkey breed, the aim of this study was the evaluation of the possible seasonal effect on some reproductive aspects in the MF jenny.

2. Materials and methods

2.1. Animals and location

The study was conducted in the province of Teramo (Italy), latitude of 42°43'34.351" N and longitude of 13°46'21.539" E, at around 270 m on sea levels, from January, 2008 to January, 2009. The study involved 12 healthy MF jennies, 4- to 8-year old and weighing 396 to 420 kg. Jennies recruited for this study showed regular cycles and foaled without obstetric assistance in the previous parturition, occurred at least 60 days before the start of the study. The jennies were kept in open paddocks and subjected to the natural atmospheric conditions for the whole trial. Daily, jennies received standard hay ad libitum and commercial equine fodder (2 kg). The body condition score of all the jennies was between 3/5 to 4/5 and remained constant throughout the trial.

Calendar-based seasons were considered: winter (22 December–20 March), spring (21 March–20 June), summer (21 June–22 September), and autumn (23 September–21 December). The environmental conditions such as the amount of light hours (LH), the light ratio, as the percentage of the light hours in 24 hours (LHR), and the lower, the higher, and the mean environmental temperature (LET, HET, and MET respectively), were recorded.

2.2. Estrus detection and ultrasonography

Each jenny was teased daily from Day 15 after the previous ovulation with a fertile and mature jackass for at least 3 to 4 minutes [20] to verify the presence of behavioral signs of estrus. The day of the behavioral willingness to mating (heat) was considered the Day 1 of the estrus (Ed1). The day when the jenny refused the jackass was considered the Day 1 of the diestrus (Dd1). Thus, the estrus time was defined as the time, in days, between Ed1 to Dd1-1; the diestrus time was defined as the days between Dd1 to the subsequent Ed1-1. At Ed1, the behavioral characteristics of the heat were recorded and the jennies in estrus were submitted to gynecological and ultrasonographic

examination. The ultrasound examinations of the reproductive tract were performed transrectally using an ultrasound Concept 2000 (Dynamic Imaging Limited, Livingston, Scotland, UK) equipped with a 7.5-MHz linear transducer. Uterus was monitored for the diameter of both horns at Ed1. Jennies with anechoic content of the uterus were excluded from the trial. The follicular dynamics was followed by ultrasonographic examinations repeated every 12 hours between Ed1 and the visualization of a follicle of 30-mm diameter, then every 6 hours until ovulation. The day at which the dominant follicle disappeared on the ovary was defined as the day of ovulation, and the diameter of the dominant follicle at the last examination before ovulation was recorded. Estrous cycle was considered as the time elapsing between two consecutive ovulations, detected using ultrasonography.

Blood samples were daily collected from Ed1 until Ed1 of the following estrus in each jenny by venipuncture in heparinized tubes, centrifuged at 1000× g for 20 minutes and plasma stored at –20 °C until E2 and P4 analysis. This complete monitoring protocol was used for each animal and repeated during the four year-round seasons.

2.3. Hormone assays

Plasma E2 concentration was assayed using radioimmunoassay (RIA). The intra- and interassay coefficients of variation were 4.8% and 7.6%, with a sensitivity of 1.1 pg per tube. Plasma P4 concentration was measured using radioimmunoassay, with an intra- and interassay coefficients of variation of 4.6% and 6.9%, respectively and a sensitivity of 2.3 pg per tube. Both the methods were previously validated [21].

2.4. Statistical analysis

The normal distribution of data, reported as mean and standard deviation, about the estrous cycle and estrous and diestrus lengths were tested by the Kolmogorov–Smirnov test. No deviation from normality was detected.

For the hormonal analysis, the day of blood collection was normalized for ovulation (Day 0): preovulatory days considered from Day 6 to 1 and postovulatory days from Day 1 to 18. Length of estrous cycle, estrus, and diestrus were analyzed using a two-way ANOVA, in which the season and the cycle within each season were used as factors. The follicle size at Day 5 and at ovulation was analyzed using a one-way ANOVA, in which the season was considered the factor. The plasma concentrations of E2 and P4 were analyzed using a two-way ANOVA, in which the season and the day (pre- or postovulation) were considered as factors. In all cases, the post hoc evaluation was performed using the Scheffè's test. The differences were considered significant with P-value less than 0.05. All data were analyzed using the software PASW (SPSS) version 18 for Windows platform.

3. Results

The number of light hours/day was higher in spring and summer compared with autumn and winter ($P < 0.05$), such as the percentage of light hours in the day ($P < 0.05$).

All the temperature parameters were different in different seasons (Table 1).

In the present study a total of 120 estrous cycles from 12 jennies were considered. The estrous cycles examined were 32 in spring, 32 in summer, 29 in autumn, and 27 in winter.

Before ovulation, the heat behavior of the jennies in the presence of the jackass was characterized by rhythmic eversion of the vulvar labia (winking) with exhibition of the clitoris, urination, male receptivity, and clapping, with sialorrhoea, neck and head extension, and back ears. These behavioral signs lasted, during the whole year, from 5.66 ± 0.51 to 7.8 ± 0.44 days.

The duration of estrus cycle, estrus, and diestrus were summarized in Table 2. The mean estrous cycle in the MF jennies consisted of 23.3 ± 0.8 days during the whole year, with similar values throughout different seasons.

On average, the estrous length was 6.5 ± 0.6 days and it was significantly longer ($P < 0.01$) in summer (7.1 ± 0.4 days) compared with autumn (6.2 ± 0.6 days) and winter (6.1 ± 0.4 days). Estrus in spring was significantly longer than in winter ($P < 0.01$).

The mean duration of the diestrus during the whole year in MF jennies was 16.8 ± 0.6 days. A significant reduction in diestrus length ($P < 0.01$) was reported in the summer compared with the other seasons. The diestrus was not significantly different among the other seasons.

In the MF jenny, the follicle size measured in the last examination close to ovulation was on average 43.7 ± 0.13 mm, and was fluctuant, with a decreased turgidity of the wall, and a modest amount of hyperechoic particles fluctuating within the follicular fluid. The follicular diameters at Day 5 and at ovulation (Day 0) in the different seasons were reported in Table 3. The ovulation occurred 0.68 to 0.73 days before the end of the estrous behavior.

Plasma E2 concentrations showed a progressive increase from Day 6 to 2, the days of hormone peak in spring and autumn. In summer and winter, the peak seemed slightly delayed and detected on Day 1. Then, a progressive and significant ($P < 0.05$) reduction was found between Day 2 to 1 and from Day 1 to 0. After Day 1 in autumn, Day 2 in spring, summer, and winter, E2 basal levels were found and remained unchanged until the beginning of the subsequent heat (Fig. 1). Plasma P4 concentration was at basal level during the estrus, with no significant differences between Day 6 to 0. Afterwards, it increased significantly ($P < 0.01$) from Day 1 to 3, and showed a plateau between Day 6 to 11 (spring, summer, and autumn) or Day 12 (winter).

Table 1

Environmental characteristics during the different seasons of the trial.

Environmental characteristic	Winter	Spring	Summer	Autumn
LH (h)	10.1 ± 0.7^a	13.9 ± 1.3^b	13.1 ± 1.2^b	10.3 ± 0.8^a
LHR (%)	41.8 ± 3.3^a	58.2 ± 3.9^b	57.1 ± 5.3^b	42.6 ± 3.4^a
HET (°C)	8.2 ± 5.1^a	21.6 ± 3.9^b	27.8 ± 4.3^c	13.6 ± 5.1^d
LET (°C)	1.5 ± 2.4^a	12.9 ± 3.9^b	18.4 ± 3.8^c	6.2 ± 4.1^d
MET (°C)	3.8 ± 2.9^a	17.3 ± 4.1^b	22.7 ± 3.6^c	9.8 ± 4.3^d

^{a,b,c,d} Different superscript alphabets in the same row mean significant differences ($P < 0.05$).

Abbreviations: HET, the higher day-temperature; LET, the lower day-temperature; LH, number of light hours; LHR, percentage of the light hours in 24 hours; MET, the mean daily temperature.

Table 2

Mean \pm SD of the estrous cycle, estrous, and diestrus lengths in different seasons in 12 Martina Franca jennies.

Season	Estrous cycle (days)	Estrous (days)	Diestrous (days)
	Mean \pm SD	Mean \pm SD	Mean \pm SD
Spring	23.6 ± 0.7	$6.7 \pm 0.4^{a,b}$	16.9 ± 0.5^a
Summer	23.4 ± 0.7	7.1 ± 0.4^a	16.1 ± 0.6^b
Autumn	23.1 ± 0.9	$6.2 \pm 0.6^{b,c}$	17.1 ± 0.6^a
Winter	23.1 ± 0.7	6.1 ± 0.4^c	17 ± 0.3^a

^{a,b,c} In the same column, values with different letters in superscript differ significantly ($P < 0.05$).

Abbreviation: SD, standard deviation.

After this plateau, the plasma level of the hormone decreased progressively (Day 13–17) returning to basal levels (Fig. 1).

4. Discussion

In this study, 12 jennies were evaluated for the estrous characteristics in a whole year-round time of observation. All jennies (100%) showed estrous cycles along the whole year; heats were observed also in winter, and always coupled to ovulation. This percentage was greater than data reported in literature, even if the presence of a year-round cyclicity was previously reported [18,22]. Henry, et al. [15] observed a seasonal anestrus in about 40% of the jennies monitored. Thus, opposite to the mare, the whole year-round reproductive activity detectable in jennies could suggest a different seasonal effect on the hypothalamic–pituitary–gonadal axis in donkeys. In the mare, in the northern hemisphere, two transitional phases and a winter anestrus are reported and characterized by a partial or complete stop of reproductive activity because of a partial or complete stop of the hypothalamus–pituitary–gonadal axis [23]. This reproductive “seasonality” in the mare seems to be driven by the progressive decrease of the daylight in winter via a pineal-melatonin mechanism [24,25]. The lack of a seasonal anestrus in all MF jennies suggested a reduced effect of the seasonality on reproductive characteristics, as previously demonstrated in the MF jackass [6,16].

In the present study, the mean (23.3 ± 0.8 days) estrous cycle length was similar in the different seasons, in accordance with the data reported by Blanchard, et al. [22] and Meira, et al. [19]. The mean estrous and diestrus lengths (6.5 ± 0.6 and 16.8 ± 0.6 days, respectively) were

Table 3

Mean \pm SD diameter of the dominant follicle at Day 5 before and at ovulation in different seasons in 12 Martina Franca jennies.

Season	Dominant follicle size on Day -5 (mm)	Dominant follicle at ovulation (mm)
	Mean \pm SD	Mean \pm SD
Spring	31.5 ± 0.18	45 ± 0.23^a
Summer	30.6 ± 0.04	44.2 ± 0.32^a
Autumn	32.8 ± 0.02	41 ± 0.54^b
Winter	32.4 ± 0.12	42 ± 0.49^b

^{a,b} In the same column, values with different letters in superscript differ significantly ($P < 0.05$).

Abbreviation: SD, standard deviation.

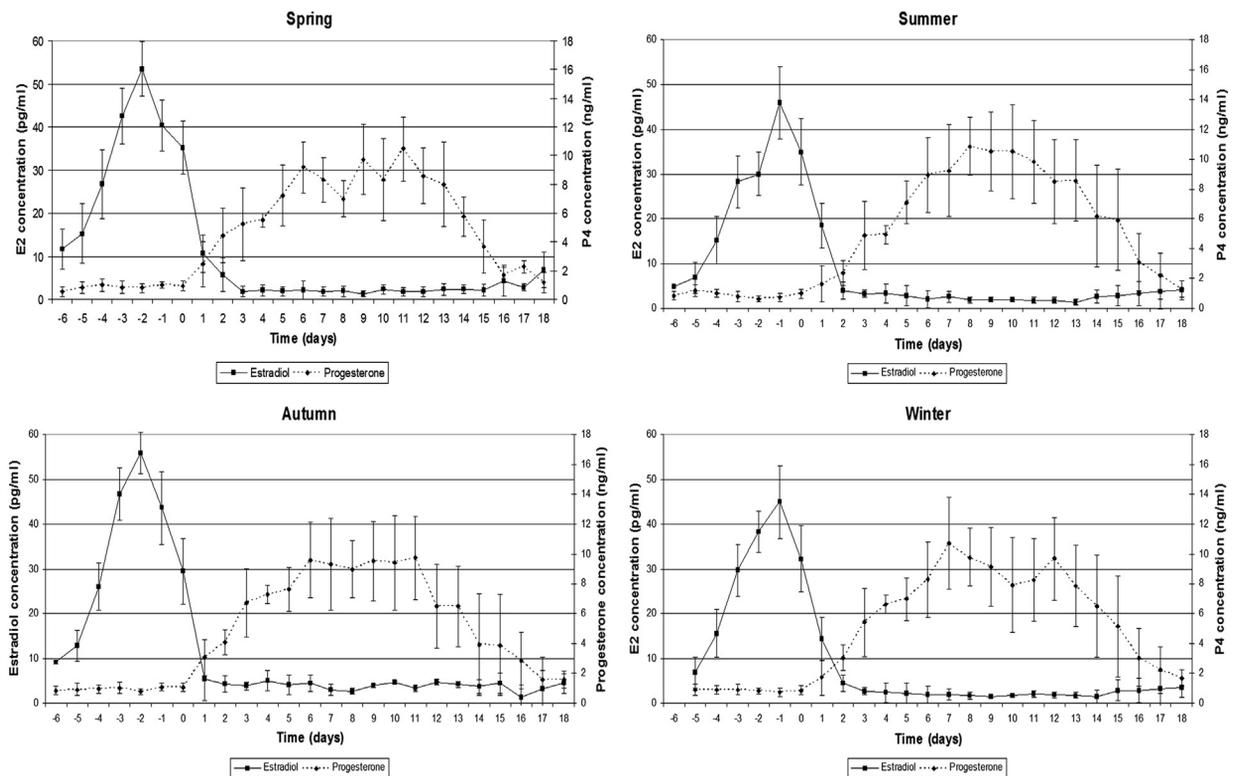


Fig. 1. Mean circulating patterns of estradiol-17 β (E2) and progesterone (P4) along the estrous cycle of different seasons in Martina Franca jenny.

comparable with data reported in the jenny by Blanchard, et al. [22] (5.9 ± 2.1 and 17.4 ± 2.6 days), but a different effect of the season was detected. Our data showed an increased estrous duration in summer compared with autumn and winter, and a reduced diestrus in summer compared with the other seasons. Blanchard, et al. [22] reported a variable but similar estrous length, and a significant reduction in diestrus length between October and March. These differences could be because of a different environmental conditions in which these studies were performed. The estrous length resulted comparable with the value reported in the mare [17,18]. However, the longer heat observed in summer in MF jennies was in contrast with the data reported in the mare, in which the estrus was shorter in summer than in autumn, as the result of an increased frequency of LH secretion during summer [26].

The behavioral signs of estrus in the jenny are very obvious. Clapping represents a typical estrous sign in the jenny and in the zebra [17], but it is not reported in the mare. This sign, together with winking and urination, is very easy to be observed at teasing with the jackass. Clapping could be observed, although less strongly, in the same jennies, also in the absence of the jackass, but when they are kept in group. Therefore, when this sign is observed, probably the jennies are in estrus.

Although criteria for impending ovulation estimation seems similar to that reported for the mare by Carnevale, et al. [27], the follicle size recorded in the last 6 hours before ovulation in the MF jennies in spring (45 ± 0.23 mm) and summer (44.2 ± 0.32 mm) were similar to that

reported in the horse at the same time of the year (May–June) [28].

The pattern of E2 and P4 plasma concentrations during the estrous cycle was similar to that reported for the mare. Estrogens increased during estrus, as the consequence of an increased growth and activity of the granulosa cells within the dominant follicle. The sharp reduction in the circulating estrogens from 2 days before ovulation, and a following slower decrease between Days 1 and 4 is a well-known pattern in the horse [29,30] and could be involved in the concomitant increase of the circulating LH. The effect of season on ovarian steroids plasma concentrations seems to be negligible, because E2 peak was slightly delayed (Day 1) only in summer.

The pattern of P4 plasma concentrations during the estrous cycle was comparable with what reported in the mare [31], and the basal estrous level of P4 (<1 ng/mL) detected in the jenny was similar to that classically reported in the mare [26,32].

4.1. Conclusions

The jennies monitored in this study showed ovulatory heats in all seasons of the year, with estrous cycles' length significantly longer in summer compared with autumn and winter. On the other hand, diestrus was significantly reduced during summer compared with the other seasons. The estrus was characterized by an increase of the circulating E2 with slight difference between seasons, with a peak 2 days (spring and autumn) or 1 day (summer and

winter) before ovulation. Diestrus was characterized by an increase in plasmatic P4, that reaches a plateau at Day 6 to 11 (spring, summer, and autumn) or Day 12 (winter), then decreased until values below 2 ng/mL at the beginning of the following estrus. The heat behavior could be easily evocable by jackass teasing and was characterized by unequivocal signs, different to those reported in the mare. The jennies seemed to ovulate follicles with similar size and characteristics in the four seasons of the year, thus the criteria used for ovulation monitoring could be the same during the whole year.

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