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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-17β (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.

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$\times$ 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 diestrous 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 ($P < 0.05$).