Behavior of Martina Franca donkey breed jenny-and-foal dyad in the neonatal period

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Abstract Donkeys display a peculiar social structure, dyad based, different from horses. In particular, scarce in- formation is available on their early life after birth, which was hypothesized to represent the most important period in the development of the social behavior between the jenny and its foal. In the first 24 hours after birth, donkeys develop most of their sensorial, motor, and behavior abilities, typical of the "follower" species. Because this lack of knowledge contrasts with the increasing multifactorial interest for the donkey breeding, the present study was aimed to investigate the jenny-and-foal dyad behavior within 24 hours after birth, with the final purpose to provide the basis for a species-specific ethogram, in the Martina Franca endangered donkey breed. The observed behaviors were simplified in seven clusters to highlight the most representative categories. The most represented behavioral clusters observed during the nighttime and daytime in both jennies and foals were defined, according to the Pareto sta- tistical analysis, "vital few." The results represent a preliminary behavioral database about Martina Franca donkey breed jenny-and-foal dyad in the first 24 hours after delivery, useful for the future ethogram description and for deep investigations of behavior-related maternal or neonatal problems and for the prompt recognition of foals eligible for the Animal-Assisted Activity and Therapy.

Introduction

In the donkeys, the social structure is composed of complex hierarchies within the groups in which the rank does not simply depend on age, sex, body weight, or aggressive behavior (McGreevy, 2012). The social organization of both feral and free-ranging don- keys shows a territorial-based system (Henry et al., 1991; Klingel, 1977; Woodwart, 1979), unlike the harem system of horses (Klingel, 1975; Smith-Funk and Crowell-Davis, 1992). Moreover, the donkey's behavioral repertoire is peculiar in comparison to horses (e.g., social play is infrequent in donkeys [McDonnell and Poulin, 2001]).

Within the donkey social structure, the only permanent union is the one between the mother and

its foal. The bond between each jenny and its foal is strong and represents the key for understanding donkey behavioral development. Jennies may travel over large territories with their foal as dyads, or in small groups of dyads loosely affiliated to each other (McDonnell, 1998; Poindron and Schaal, 1993), or can remain almost resident in a given territory with their foals (McDonnell, 1998). This behavioral trait has a pro- found effect on the interaction between each jenny and its foal (McDonnell and Poulin, 2001). The jenny's activities are related to the foal's response, which decreases as the foals mature, regardless of their sex (French, 1998). Interestingly, in wild contexts, the mother-to-foal separation process begins with weaning (Moehlman, 1998), whereas in domestic contexts, characterized by confinement, unlimited resources, and reproductive inactivity, the dyad does not separate at weaning but reverts to the close one observed between jennies and newborn foals (French, 1998). This finding largely reflects a shift in the jenny's behavior from "leaving" to "approaching" and, to a lesser extent, can be attributable to an increased number of approaches by the foal. Thus, related adult donkeys often stay very close together and are equally active in maintaining contact. Because it can lead to separation anxiety, this close relationship may be undesirable for owners when the pair needs to be separated. On the other hand, it may provide many benefits, including mutual grooming, and the potential for the pair to develop a coalition for future support, for example, in mutual defense (French, 1998; McGreevy, 2012).

In donkeys, as in other domestic mammals, the process of birth and the early phase of development immediately after delivery play a pivotal role in the process of adaptation to the extrauterine life for the newly born individual. The donkey, like other ungulates, is a precocious mammal, because of the rapid motor and sensory development of the foal after birth. In precocious newborns, the active care provided by the mother results in a phase of intense reciprocal stimulation after birth. In turn, maternal responses are elicited by the complex of movements, odors, and vocalizations provided by the foal, so that the mother uses visual, tactual, and vocal stimuli to elicit and direct the foal activities (Crowell-Davis and Houpt, 1986). Because of their economic and commercial importance, far more attention was paid to reproductive physiology in horses than in other Equidae. As a result, much less is known about species-specific aspects of breeding in other Equidae, including the donkey (Canisso et al., 2010; Ginther et al., 1987), which is then often wrongly managed (Perez-Marin et al., 2016).

There is a need to improve the donkey-specific reproductive behavioral knowledge for population and biodiversity programs (Carluccio et al., 2013; Perez-Marin et al., 2016). A large number of donkey breeds are currently considered as endangered and among them is the Martina Franca donkey breed.

The study of jenny-and-foal dyad deserves scientific interest for several reasons: (1) scarce information is, in general, available for donkey dyad behavior, and the authors are not aware of specific data about the Martina Franca endangered donkey breed; (2) the definition of the normal jenny-and-foal dyad behavior will be useful for the prompt recognition of every sign of abnormalities; and (3) foal temperament, excitability, mood, and affective behavior, since birth, could help start the selection of subjects for Animal-Assisted Activity and Therapy, which represents one of the most important present uses of donkeys.

The present study investigated the behavior of Martina Franca jennies and their foals within the first 24 hours after delivery, to provide preliminary basic data for the future description of a Martina Franca donkey breedespecific ethogram and for a better selection of subjects for Animal-Assisted Activity and Therapy.

Materials and methods

Animals and housing

The study was performed on 11 Martina Franca jenny-and-foal dyads.
The jennies, 4-12 (mean SD: 8.8 4.1) years old, 350-370 (mean SD: 360 10) kg body weight, were kept in open paddocks at the Veterinary Teaching Farm, University of Teramo, Italy, and daily fed with 6-8 kg of good-quality polyphite lawn hay and 1.5 kg of commercial food for pregnant or lactating mares.
The jennies were healthy, dewormed before breeding, and regularly vaccinated.

They were reared under a conservation grant project of "Regione Puglia" 2009-14 on endangered Martina Franca breed, in collabo- ration with the "Martina Franca donkey genetic heritage conser- vation center," Crispiano (Taranto, Italy). Institutional and rare breed guidelines for the care and use of animals were followed in full accordance to guidelines for the treatment of animals in behavioral research (ASAB, 2012).

The 11 pluriparous jennies were fully monitored by routine clinical and ultrasonographic examinations from the time of breeding until parturition, to assess the general health status, the normal pregnancy course, and the normal fetal development and well-being.

At approaching parturition, when the udder enlargement was detected, the jennies were moved to individual 3.50 3.50 m delivery boxes and monitored by four infrared cameras (DVMR Kalatel DVMRe Store Safe Digital Video Recorder Multiplexer, Sony, Tokyo, Japan).

Foaling

All the jennies were allowed foaling spontaneously, under sur- veillance, but without interference. Foalings were defined as normal and spontaneous, and donkey foals defined as mature, healthy, and viable, according to the criteria reported for the Mar- tina Franca donkey breed (Carluccio et al., 2008; Carluccio et al., 2015; Panzani et al., 2012b; Veronesi et al., 2011).

Jenny-and-foal dyad behavior

All the dyad behaviors were video-recorded, and images were retrospectively analyzed continuously, during the first 24 hours, by using a focal sampling method, starting at birth, (Altmann, 1974), (Figure 1).

Mutually exclusive behaviors were analyzed, and the following behavioral clusters were considered in both daytime and night- time: interaction, including lactation, licking, grooming and touching, playing together; activity: the total movement (excluding the interaction ones); and resting were all considered as static behaviors.

Sensorial behaviors were also evaluated, such as olfaction (smell: itself or the body of the counterpart, excretions, straw, and environment, characterized by clear sniffing movements) and observing (watch: itself or the body of the counterpart, surrounding environment, characterized by clear head movements and eye pointing). Other behaviors, such as autogrooming (scratch and licking itself), and excretion (urine and feces) were also considered. All the behavioral clusters were expressed as rates, considered as the total numbers of events, divided by the duration expressed in seconds.

Data were expressed as means T SD Statistical analysis

After a preliminary analysis by multivariate analysis of variance, significant data were analyzed by repeated measure analysis of variance and one way post hoc analysis of variance.

One of the useful mathematical formulas that could be applied in behavioral studies to identify the most important events in a group of data is represented by Pareto analysis (Beirlant et al., 2006; Carluccio et al., 2013). In particular, 70% of the total cumulative percentage of the behavioral categories represents the most important behavior observed. These categories are termed the "vital few" according to Pareto formula. In the present study, this analysis was used to highlight the most represented behavior in the first 24 hours after birth in the dyad.

The data analysis was performed with Excel, Origin, SPSS and MATLAB software.

Statistical significance was set for a < .01 or < .05.

Figure 1. Jenny-and-foal dyad. A selection of images describing some events and behaviors during the neonatal period (in the sequence from left to right: birth and placenta rupture; first sight; first stand up attempt with mother's assistance; playing; jenny liking the foal; jenny smelling the foal; foal suckling). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Results

Clinical findings

All the jennies foaled spontaneously and unassisted, at the physiologic term of pregnancy. All the eutocic deliveries occurred between 10:00 pm and 04:00 am, and in all cases, the fetal and placental expulsion times fulfilled the criteria previously reported for normal parturition in Martina Franca donkey breed (Carluccio et al., 2008; Carluccio et al., 2015; Panzani et al., 2012b; Veronesi et al., 2011).
The 11 foals were mature, healthy, and viable, with Apgar score, body weight, time for standing, and for first suck within the ranges previously reported for this donkey breed (Carluccio et al., 2008; Carluccio et al., 2015; Panzani et al., 2012b; Veronesi et al., 2011).
The clinical findings, expressed as mean SD, about the jennies, foaling, and neonatal characteristics of the 11 dyads examined, are summarized in Table.

Behavioral data

Behavioral data are shown in Figures 2e8. Pareto analysis allowed us to highlight the most representative behavioral cate- gories in jennies, foals, and dyads, the so-called “vital few” behaviors.
In jennies, the most representative behavioral categories were “observing” and “olfaction.” Interestingly, these sensorial behaviors reached the 70% of the total displayed behavior, associated with “autogrooming” during the daytime and associated with “excre- tion” during nighttime.
In foals, the most representative behavioral categories were “observing” and “autogrooming.” The 70% of the total behavior was represented by “observing” and “autogrooming,” associated with “activity” during the daytime and with “olfaction” at nighttime.
In the dyads, the most represented behavioral categories during daytime were “observing” and “autogrooming,” followed by “olfaction” for jenny and “activity” for foal.
During nighttime “observing” and “olfaction” represented the 70% of behavioral categories in both jennies and foals, but in jennies, they were associated with “excretion,” whereas in foals they were associated with “autogrooming.”

Discussion

In precocious animals, the neonatal period is recognized as a phase of intense interactions between the mother and the infant, important for bonding, and characterized by all behaviors expressed in the 24 hours after delivery, allowing the achievement of the newborn autonomy, including motor, sensorial, and cognitive processes. Early in life, these relationships are characterized by close proximity

(Barber and Crowell-Davis, 1994; French, 1998), a common pattern of spatial distribution seen in draft mares with mule foals (Smith-Funk and Crowell-Davis, 1992), Welsh ponies (Crowell-Davis, 1986), and New Forest ponies (Tyler, 1972). In fact, problems in behaviors and in social interactions were reported in several ungulates when newborns are separated from their mothers for two and a half hours after birth (Rossdale, 1968; Scott, 1945). In some species, such as the donkey, horse, and wild boar, these interactions could continue beyond weaning and are responsible for the social organization, whereas these are uncommon in feral asses (French, 1998; McDonnell, 1998; Waring, 1970).

Table

Mean (TSD) clinical data about jennies, foaling, and neonatal characteristics in the 11 dyads examined in the present study

	Jennies age (years)	Pregnancy length (days)	Foal expulsion time (min)	Placental expulsion time (min)	Apgar score	Foal birth weight (Kg)	Time for standing (min)	Time for first suck (min)
Mean T SD	10.2 T 3.84	368 T 4.37	18.3 T 5.87	40.4 T 16.56	9.1 T 0.89	30.3 T 2.95	62.3 T 25.07	87.1 T 29.15

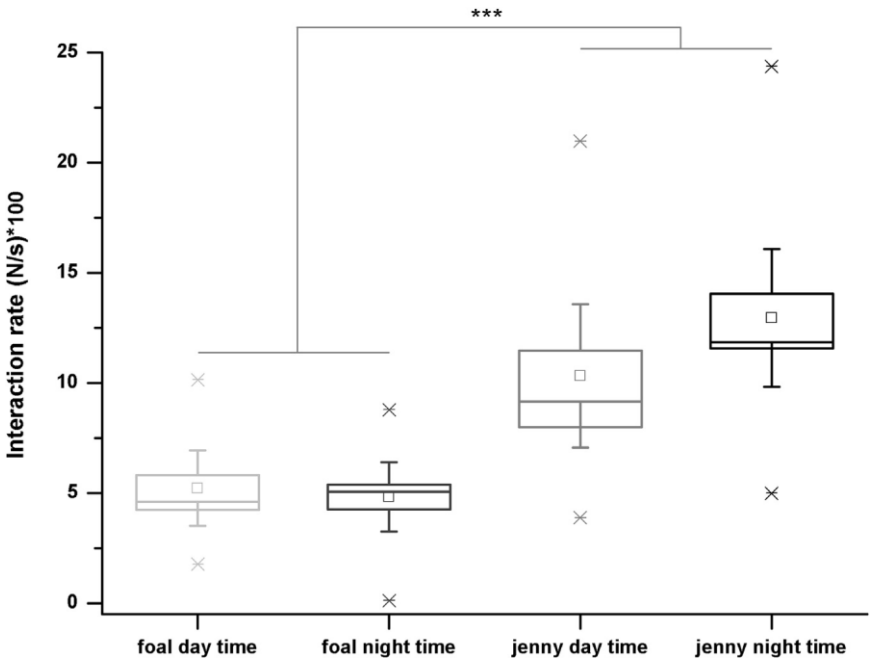

Figure 2. Interaction rate. It represents all social behaviors in the dyad, significantly ($^{***}P < 0.001$) higher in jennies, in particular during nighttime. N: total number of events observed in the 24 hours of behavioral sampling; s: the sum in seconds of the specific behavioral event duration along the 24 hours.

However, studies investigating the early maternal or neonatal normal behaviors must take into consideration the physiologic characteristics of the process of foaling, from both the maternal and the neonatal point of view. About 50 years ago, Rossdale (1967) reported some maternal and neonatal physiologic parameters in horses, such as the gestational length, the duration of the second stage of delivery in multiparous and primiparous mares, the time of delivery in recumbent position, the foals time to stand. Those parameters provided the first reference data for normal parturition and neonatal early physiology in the horse, followed by numerous articles that completed those reference data in the subsequent decades.

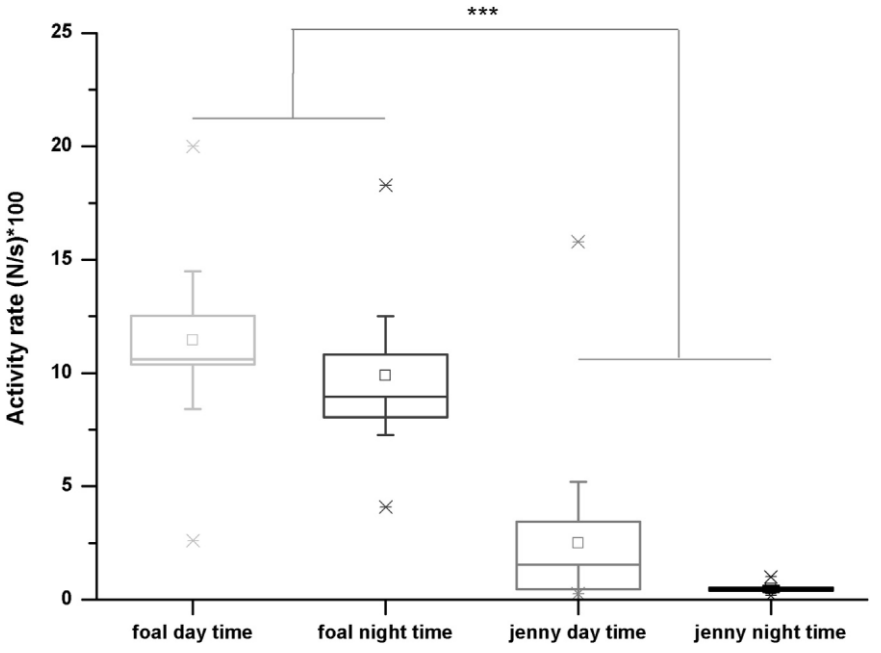

Figure 3. Activity rate. It is the rate of all movement, excluding the social ones, significantly higher in foals, mainly during daytime ($^{***}P < 0.001$). N: total number of events observed in the 24 hours of behavioral sampling; s: the sum in seconds of the specific behavioral event duration along the 24 hours.

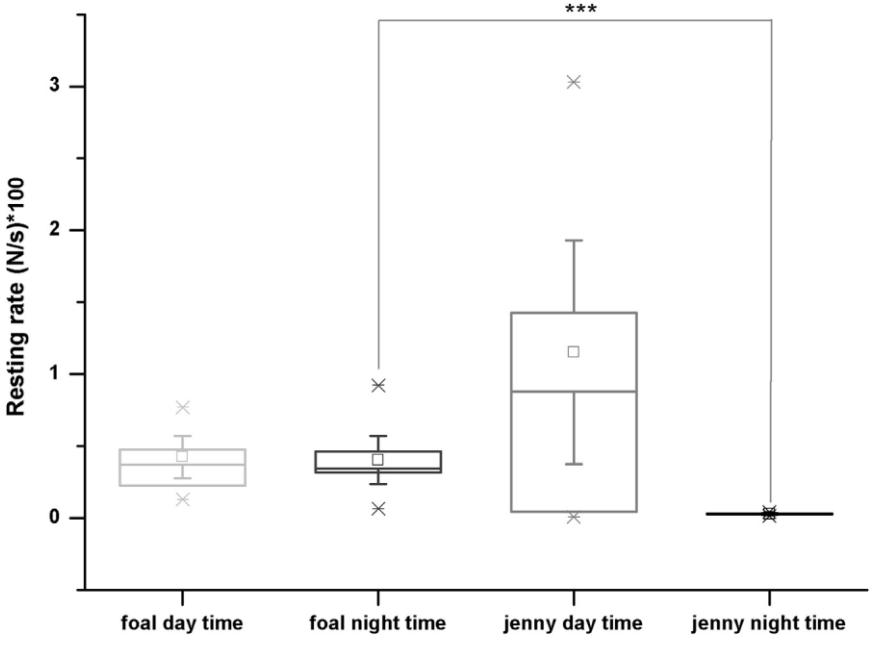

Figure 4. Resting rate. It embraces all static behaviors, almost constant in the dyad, excluding foal particularly inactive at nighttime in comparison to jenny ($^{***}P < 0.01$). N: total number of events observed in the 24 hours of behavioral sampling; s: the sum in seconds of the specific behavioral event duration along the 24 hours.

Similar data in donkeys were lacking until about one decade ago, when donkey-related research was performed and data about normal foaling and neonatal physiologic behaviors for the Martina Franca donkey breed were provided (Carluccio et al., 2008; Carluccio et al., 2015; Panzani et al., 2012b; Veronesi et al., 2011). The present study aimed to describe, for the first time, the behav- iors of Martina Franca donkey breed jenny-and-foal dyad in the first 24 hours after birth under normal maternal and neonatal physio- logic conditions.In the present study, because all the 11 jennies and their 11 foals fulfilled

the requirements for normal pregnancy length, normal spontaneous, unassisted eutocic vaginal delivery, giving birth to mature, healthy, and viable foals (Carluccio et al., 2008; Carluccio et al., 2015; Panzani et al., 2012b; Veronesi et al., 2011), data obtained from all the 11 dyads were analyzed and reported. In detail, when the foals' physiologic characteristic. Similar data in donkeys were lacking until about one decade ago, when donkey-related research was performed and data about normal foaling and neonatal physiologic behaviors for the Martina Franca donkey breed were provided (Carluccio et al., 2008; Carluccio et al., 2015; Panzani et al., 2012b; Veronesi et al., 2011). The present study aimed to describe, for the first time, the behaviors of Martina Franca donkey breed jenny-and-foal dyad in the first 24 hours after birth under normal maternal and neonatal physio- logic conditions.

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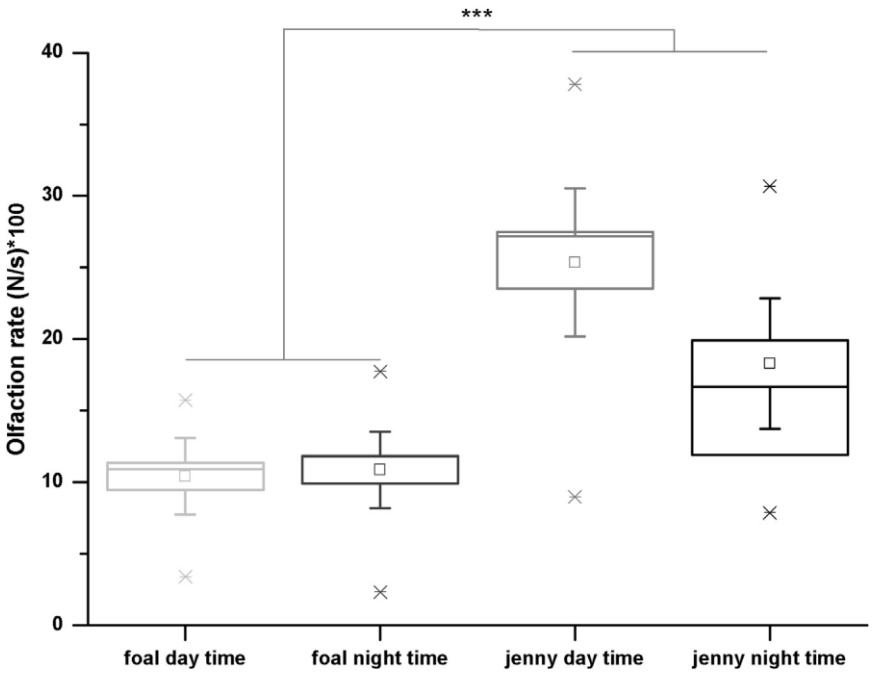

Figure 5. Olfaction rate. It includes smell itself, smell the counterpart, smell urine and feces, straw, and environment. It was higher in jennies ($^{***}P < 0.001$). N: total number of events observed in the 24 hours of behavioral sampling; s: the sum in seconds of the specific behavioral event duration along the 24 hours.

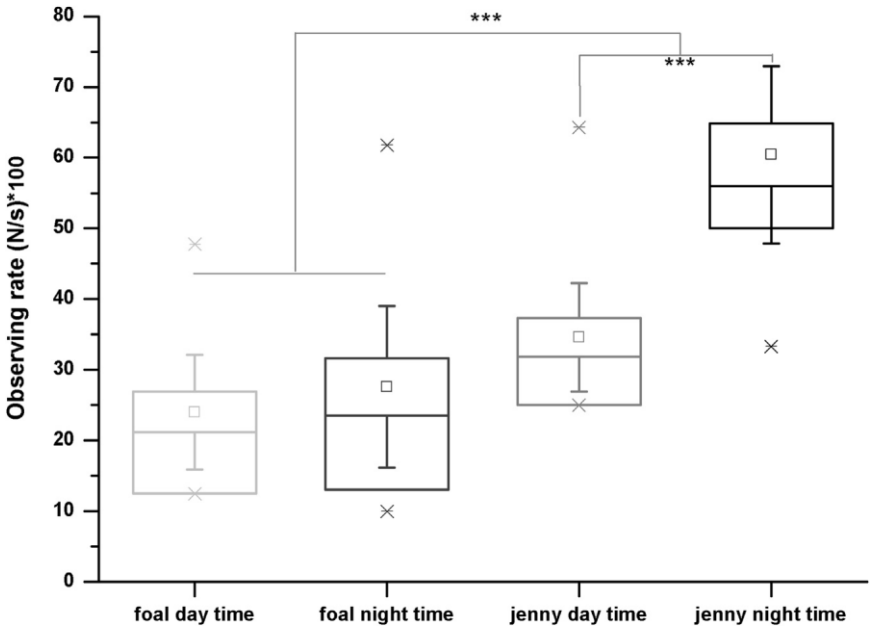

Figure 6. Observing rate. This rate includes watch itself or the counterpart and the surrounding environment, and it was higher in jennies ($^{***}P < 0.001$). N: total number of events observed in the 24 hours of behavioral sampling; s: the sum in seconds of the specific behavioral event duration along the 24 hours.

These data were in agreement of those by Rossdale et al. (1984) and Koterba (1990), and with the 62.4 42.63 min observed for the horse foals by Panzani et al. (2012a). The time to first suck (87.129.15 min) was in agreement with the time (101 40.8 min) pre- viously reported for the same donkey breed by Carluccio et al. (2015). Data from donkeys were similar to those reported for the horse foals (94.7 53.01) (Panzani et al., 2012a) and in agreement with literature on horse foals (Koterba, 1990; Rossdale et al., 1984). Although 11 jenny-and-foal dyads could seem a small number, it must be underlined that the Martina Franca donkey breed is an endangered population, so that 11 dyads can be considered a suitable and representative sample size, especially for a preliminary study.

As a first consideration, in the present study, all the births occurred during the nighttime. This night synchrony is well known among ungulates (Estes, 1976) and has been supposed to be useful for maximizing the neonatal survival by reducing the risk of pre- dation (Estes and Estes, 1979). The times for foals standing, walking, and sucking observed in the present study agreed with the ranges reported for wild equids (Klingel and Klingel, 1966), suggesting that, in donkeys, domestication does not seem to have affected the foal's motor development in the neonatal period. In the Martina Franca breed, this finding could be supposed to be also related to the characteristic environment, the Italian South East very rural area, in which this donkey breed was raised.

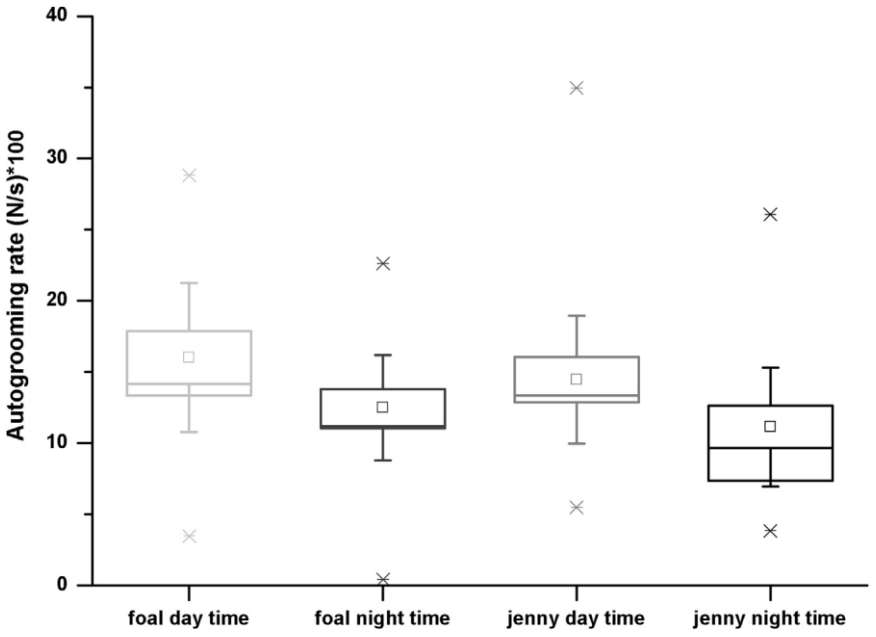

Figure 7. Autogrooming rate. It is represented by scratch and licking itself. No statistical differences between jennies and foals were found. N: total number of events observed in the 24 hours of behavioral sampling; s: the sum in seconds of the specific behavioral event duration along the 24 hours.

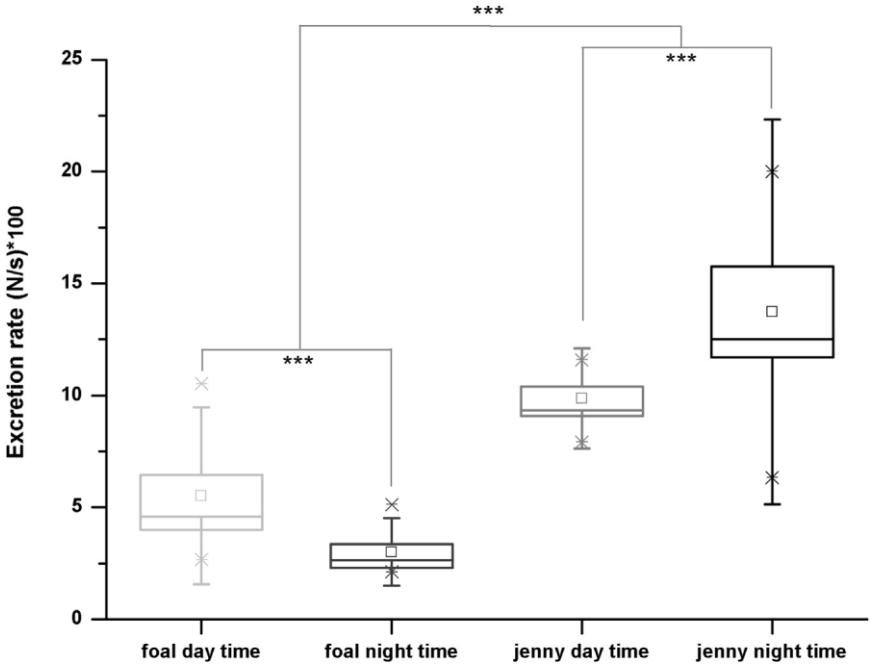

Figure 8. Excretion rate. It was significantly lower in the foal ($^{***}P < 0.001$), especially at night ($^{***}P < 0.001$). N: total number of events observed in the 24 hours of behavioral sampling; s: the sum in seconds of the specific behavioral event duration along the 24 hours.

The dyad's behaviors were clustered into seven main categories. Among them, significant differences were found between jennies and foals for all kind of reciprocal interactions, such as licking, lactation, grooming and touching, and dyad playing. When compared with horses and Welsh ponies (Crowell-Davis 1985), no differences in lactation behavior were observed during the first

24 hours after delivery. We do not know if there is a relationship between lactation reduction and aggression, previously described in horses (Crowell-Davis 1985). In jennies, the interaction rate was higher than that for foals, and it resulted as the fourth most representative behavioral parameter (see Pareto analysis), remaining almost constant during the neonatal period. In foals, at this stage of development, the interaction rate was expressed slightly more during the nighttime. This finding could probably reflect a behavior retained from the predomestication state. In fact, in the wild environment, nighttime is highly risky for the newborn foal, and the interactions with mother contribute in providing safety to the foal, keeping it calm. Furthermore, equids, some bovine species, sheep and related genera, the musk ox and the caribou, are defined as "follower" species. Those species tend to be associated with grassland or tundra habitats. Usual "follower" species are characterized by great seasonal movements. In contrast to hider species such as cervids, gazelles, and antelopes living in forested or bush habitats, which are characterized by infant isolating hiding behavior to avoid predation, the "follower" species do not display this ontogenetic phase. In the "follower" species, in fact, mothers and infants generally maintain close spatial relationships and frequent communication after delivery. In horses and ponies, maternal bonding decreases with time because it reflects a different social structure, herd based (Barber and Crowell- Davis, 1994; Crowell-Davis, 1986; Tyler, 1972). The donkey's strat- egy provides defense against predation by maternal protection and allows extensive movements of mother and infant groups during the early stages of infancy (Lent, 1974). Immediately after birth, maternal licking increases the neuroexcitability of the foal, promoting rapid motor development. Thus, there may be a specu- lative association between the range of cellular events, for instance neurogenesis, controlled in a time-of-day edependent fashion by the endogenous circadian clocks (Hastings et al., 2003). The lack of reciprocal interaction has been reported to affect the post parturient
behavior when stillbirths occur in caribous (Lent, 1966), goats (van der Hammen and Schenk, 1963), and merino ewes. In those cases, the absence of the newborn stimulation leads the mothers to adopt the young of other mothers (Alexander, 1960).

The first neonatal activity seemed to be represented by the struggles of the foal to coordinate itself. The foal activities during the first 24 hours after birth were distinctive for the "follower" species (Lent, 1974). The activity rate was higher in foals than in jennies, both during nighttime and daytime. However, the present study analysis of data did not allow representation of the real circadian distribution of the activity rate of the foals. In the Pareto analysis, activity was found to be the third most represented behavior during daytime, and among the last ones during the nighttime in foals, whereas in jennies, it was almost constant during the first 24 hours after delivery. Hence, this interpretative bias must be taken into account when statistical results about the evaluation of "vital few" behaviors of foals during the first 24 hours of age are considered.

Resting, which includes all kind of static behaviors, was significantly higher in foals than in jennies during nighttime, in agreement with the resting time budgets of Welsh ponies' foals (Crowell-Davis, 1994). In ponies, in fact, foals were more likely to engage in recumbent rest than the adults, whereas upright resting was not commonly observed in foals during their first days of life, which is typical of adult behavior (Crowell-Davis, 1994). Increased resting in foals during the nighttime is in accordance with paradoxical sleep function maturation of the nervous system, as suggested by Crowell-Davis (1994).

Furthermore, the lower resting behavior of jennies in the nighttime could be related to the requirement of foal protection. Olfaction, including smelling itself or the body of the counterpart, smelling urine and feces, straw and environment, were significantly
different within the dyad. In jennies, it was the second most rep- resented behavior, while in foals, it was the third at nighttime and the fourth during the daytime. Interestingly, the olfactory behavior toward maternal feces was also observed in horses, with foals able to discriminate between maternal or another female's feces (Crowell-Davis and Caudle, 1989). Those data are in agreement

with the imprinting of the olfactory mechanism. Within few minutes after delivery, jennies rapidly built a close bond with their foals, and this time is usually referred as the "critical period." The maternal bonding, in mammals, is essentially driven by olfactory cues; odors provide the basis for individual recognition, responsible for the deep changes of the neural structures of the main olfactory bulb, involved in memorization and long-term maternal experience (Klopfer et al., 1964, Lévy et al., 2004). Conversely, foals are considerably slower in building a stable bond and in fixating their behavior on their mother (Lent, 1974). Foal bonding to the jenny seems to be more opportunistic and less linked to the olfactory cues (Leon, 1992; Moriceau and Sullivan, 2004).

The observing behavior, considered as watching itself or the counterpart and the surrounding environment, was the highest rated in the dyad. Jennies displayed observing behavior more than foals, in particular, at nighttime. This difference could be explained as the effect of the foal visual stimulation on the jen- ny's condition of maternal awareness and activity. Moreover, this behavior was considered as vital in the "follower" species, such as the donkey, because it is used by jennies and foals to maintain reciprocal responses and reinforce bonding. Visual recognition was reported to be used in ungulates to identify the mother (Tschanz, 1962); although it is not clear whether this identifica- tion is based on individual physical characteristics or instead on the perception of specific maternal behavioral patterns (Schloeth, 1958).

The autogrooming rate, scratching and licking itself, showed no statistical differences within the dyad during the neonatal period. However, it was the second most represented behavior in foals, while in jennies, it was the third during daytime and the fifth during nighttime. This behavior in foals may help develop body cognition, which could be useful for spatial orientation.

Excretion of urine and feces, interestingly, showed an inverted significantly different rate within the dyad: at nighttime, it was lower in foals and higher in jennies. This finding could be explained by two different hypotheses: the reduction of odor release at night by the foals could decrease cues for predators or it could be the simple consequence of decreased activity. According to the Pareto analysis, excretion was the third most represented behavior during nighttime in jennies and second to last during daytime. A speculative interpretation of this behavior could be related to the foal protection during nighttime, when predators recognize preys by smelling. For this reason, an intense excretion activity and odor production by the mother could represent a defense mechanism based on the possibility to confound predators by covering the odors of the foal with the maternal ones. Conversely, the 24-hour periodicity has been reported to influence some parameters linked to urine formation, such as renal blood flow, glomerular filtration, tubular reabsorption, and tubular secretion. The origin of these rhythms has been attributed to reactive response to rest/activity and feeding/fasting cycles or circadian clock self-sustained mechanism, because renal excretory rhythms persist for long periods of time, even in the absence of periodic environmental cues (Firsov and Bonny, 2010).

In precocious "follower" mammals, motor, sensory, and learning processes are rapidly set (Leuba, 1955; Lent, 1974). In the particular social structure of donkeys, the dyad represents the only one, and basic, social system. In this system, jennies provide maternal cares such as nutrition, thermoregulation, passive immunity transfer, and protection, as well as appropriate stimuli, education, and socialization to their foals. This system was probably induced by predation, which has modeled maternal and social behavior. Consequently, there must have been a strong selection for rapid development and frequent reinforcement of close jenny-to-foal contact within the dyad.

Conclusions

In conclusion, the description of the "vital few" donkey dyad behaviors in the neonatal period provides useful information to the behavioral studies of precocious "follower" species, making

avail- able first data for a future donkey ethogram description. The first behavioral base for the tight jenny to its foal dyad bonding, pivotal for their long-lasting interaction as demonstrated in previous studies (French, 1998), was recorded. The depiction of the normal behavioral patterns is useful for the future early detection of abnormal behaviors in both mother and foal dyad components. Moreover, the assessment of foals' behavior in the first 24 hours after birth could be useful for selecting subjects for Animal-Assisted Activity and Therapy.
At last, the tight bond between jenny-and-foal dyad as soon as the first 24 hours after delivery could be also useful under an animal welfare perspective, reducing the stress of dyad's separation by postponing it or developing focused species-specific strategies.

Acknowledgments

All the authors contributed equally to study design, data recording, discussion of results, and presentation and manuscript writing.

Conflict of interest

The authors have no competing interests to declare. No conflict of interest exists.

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