Factors affecting pregnancy length and phases of parturition in Martina Franca jenny

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Abstract

The knowledge of normal pregnancy length, duration of parturition stages, and neonatal early adaptation are mandatory for a rationale management of birth, especially in monotocous species with long gestations. This study reports data obtained from a large number of Martina Franca jennies with normal, healthy pregnancies, and spontaneous eutocic delivery of a mature, healthy, and viable donkey foal. Pregnancy lasts, on average, 371 days and only the fetal gender significantly determines pregnancy length, with longer gestations observed in jennies bearing male fetuses. Other factors, such as the year of foaling, month of ovulation, month of parturition, birth weight of the foal, and age of the jenny did not influence pregnancy length. The first stage of foaling lasted on average 65 min, the second stage 19 min, and the third stage 58 min. The umbilical cord ruptured on average within 16 min after birth, the foal stood up in 61 min, suckled the colostrum for the first time within 10 min after birth and again after 143 min of birth; meconium passage occurred, on average, 86 min after birth. Although times reported for the process of foaling are similar to data reported for the horse, the times for early neonatal donkey foal adaptation are longer as compared to the horse foal.

Keywords: Donkey; Pregnancy length; Foaling stages; Early neonatal adaptation

1. **Introduction**

In farm animals, many breeds are recognized as important components of world biodiversity, and, in the developing countries, the genes and the combinations of genes that convey these breeds could prove vital for agriculture in the future [1]. Among several endangered species and breeds, the Italian Martina Franca (MF) donkey breed is classified as endangered by the Food and Agriculture Organization [2]. The recent interest for this large-sized donkey breed is based on several aspects, such as the production of milk used as hypoallergenic substitute for children affected by cow milk, protein allergies, or multiple food intolerances [3–5], for the so-called “onotherapy”, and also to produce hybrids (mules) for agricultural labor in national parks, where agricultural machineries are banned. All these interests enforced prompt efforts to increase the existing donkey population, leading, in the last few years, to an increasing number of researches concerning MF female and male reproduction features, as well as researches on the donkey foal [6–11]. However, some aspects of MF reproduction still need elucidation. Among them, the duration of pregnancy, reported to be highly variable in donkeys, was not evaluated on a large number of MF jennies. Therefore, a reliable range of normal length of pregnancy for this breed and the factors that could influence pregnancy length, were not investigated. Similarly, the course of parturition, with description of foaling stages duration, still remains incompletely defined.

The knowledge about normal pregnancy length and parturition stages duration are mandatory for a rational management of birth in every animal species, and even more for a monotocus species, characterized also by a long gestation, such as the donkey.

In the horse, several factors were reported to influence pregnancy length, including not only the breed [12], maternal age [12,13], and parity [14] but also foal gender [14,15] and birth weight [16]. Some environmental factors, such as the month of conception within the breeding season [17], the month of foaling [12,18,19], and the year of foaling [12] were also found to influence pregnancy length in the mare.

Although some studies reported a longer pregnancy length in MF jennies as compared to the horse, [6,7,20], to the authors knowledge, only one study reported factors influencing pregnancy length in donkeys [21]. In the study from Galisteo and Perez-Marin, pregnancy length was influenced by the time of foaling within the breeding season, but not by donkey breed, age of the jenny, foal gender, or year of birth [21].

The knowledge of the normal duration of parturition is crucial to promptly detect abnormalities that could endanger the health of the mother and/or the fetus and to allow a correct management of dystocias. In the horse mare, the normal duration of the second and third stages of foaling (expulsion of fetus and fetal membranes, respectively), were defined [22], but in the jenny, these information are scarce. A few studies [6,21,23] conducted on MF jennies reported foaling stages durations documented by studying a small numbers of animals, but, for a more correct definition of normal parturition, consistent data from a large number of animals are necessary.

In the horse, the time interval between birth and the manifestation of specific reflexes are used to evaluate the health of newborns [24]. The main parameters reported for the horse newborn are the time from birth to standing and the time from birth to the first suck. These events are mandatory for the maintenance of metabolic homeostasis and for the correct establishment of the bond between the mare and the foal [24]. It is generally assumed that horse foals with inability to stand and nurse within 2 h after birth are considered potentially abnormal [25]. In the donkey foals, only one study reported some indications about these parameters obtained from small groups of newborns [23].

For these reasons, the aims of the present article were to depict, retrospectively, on a large number of MF jennies with normal, healthy pregnancies and spontaneous eutocic delivery of a mature, healthy, and viable donkey foal: a) the mean and the range of pregnancy length, and to assess possible influence of some maternal, fetal, or environmental parameters on pregnancy length; b) the mean and the range of normal foaling stages duration; c) the mean and range of time for neonatal adaptation behavioral appearance, possible correlations among foaling stages and neonatal parameters, and also possible correlations among each neonatal parameter.

2. Material and methods

2.1 Management of the animals

The present study was performed on 142 Martina Franca jennies 4 to 12 years old and 310 to 390 kg in weight, housed in the Veterinary Teaching Farm of the University of Teramo. The data were collected from January 2002 to March 2014. Jennies recruited for this study showed regular cycles and showed normal pregnancy and parturition. The pregnant jennies were kept in open paddocks for most of the pregnancy, and subjected to the natural atmospheric conditions. Every day, the jennies received standard hay ad libitum and commercial equine fodder (4 kg). The body condition score of all the jennies was between 3/5 and 4/5 and remained constant throughout the trial. Jennies were inseminated with doses prepared with semen of 8 jackasses of proven fertility, 4 to 15 years old and 365 to 412 kg in weight. Jackasses were kept in an individual 5 x 5 m^2 box with access to an outdoor paddock and received 10 kg of standard hay supplemented with 3 kg of commercial balanced stallion fodder twice daily.

2.2 Estrus detection, insemination, and pregnancy length estimation

The estrus detection was performed as previously reported [10]. The follicular growth was monitored by transrectal ultrasonography every 12 h from heat onset until ovulation with a Concept 2000 ultrasound machine (Dynamic Imaging Limited, Livingston, Scotland, UK) equipped with a 7.5-MHz probe. From the visualization of a follicle of 30-mm in size, the jennies were artificially inseminated every 48 h until ovulation. Semen was collected by a Missouri artificial vagina, sperm concentration and objective progressive motility were measured as previously reported [26]. Insemination doses (15 ml) were prepared diluting raw semen with INRA 96 (IMV Technologies, L'Aigle, France) to achieve a 800 x 10^6 progressive spermatozoa/dose. The pregnancy was diagnosed by transrectal ultrasound (at day 14 and confirmed at day 45 after ovulation. All the jennies were monitored throughout gestation to check for the normal course of pregnancy and normal development and viability of the fetus. At parturition, the foal was clinically examined and birthweigth and gender were recorded. The pregnancy length (PL), defined as the time (days) between ovulation and the day of parturition, was recorded after foaling. This study considered only normal pregnancies and parturitions without obstetric intervention, and pregnancy with stillbirth, clinically abnormal foals, and death within day 21 postpartum were excluded.

2.3 Monitoring of parturition

In 42 out of the 142 jennies, the parturition was video-monitored to verify the normal foaling stages duration and the neonatal adaptation at the extrauterine life. Jennies were kept outdoors during the pregnancy, and were moved into individual 4.5 x 4.5 m boxes equipped with four closed circuit TV cameras when udder enlargement was detected [27]. The records were evaluated by the same operator, and the following phases and parameters were considered: a) stage one of parturition (dilatation - D), characterized by uterine contractions not visible externally with restlessness and agitation of the animal that ended with the chorioallantois rupture; b) stage two of parturition (expulsion - E), as the time between rupture of the chorioallantois and the complete passage of the fetus (birth); c) stage three of parturition (fetal membrane expulsion (FME), as the time between birth and fetal membrane expulsion; d) umbilical cord rupture time, as the time between birth and spontaneous umbilical cord rupture (UCT).

2.4 Foal evaluation

At parturition, each foal was clinically examined for maturity, viability, and absence of gross malformation [23]. The foal standing time, as the time between birth and stand up (ST), the meconium expulsion, as the time between birth and the start of the meconium expulsion (MET), the time of the first suckle, as the time between birth and first suckle (TFS), and the time of the second suckle, as the time between birth and the second suckle (TSS) were recorded. Each foal was weighed before nursing and gender was also recorded.

2.5 Statistical analysis

All the parameters measured in this study were reported as the mean ± the standard deviation (SD). The factors affecting the pregnancy length in the jennies were examined. To this aim, the PL was analyzed by a General Linear Model (GLM) based on the one way Analysis of Variance (ANOVA), in which the year of the foaling, the month of ovulation, the month of parturition, the gender of the foal, the birth weight of the foal, and the age and weight of the jenny were considered fixed factors. Where appropriate, the post hoc evaluation was performed using the Scheffè method.

Possible correlations between the different parameters of parturition and the relationship between the weight of the jenny, the PL, the birth weight, and the gender of the foal and the different parturition parameters (D, E, UCT, ST, MET, FME, TFS, and TSS) were compared with Pearson's correlation test.

In all cases, the differences were considered significant with $p < 0.05$. All data were analyzed using the software PASW (SPSS) version 18 for Windows (IBM, SPSS Inc., Chicago, USA).

3. Results

The mean pregnancy length recorded in 142 healthy Martina Franca jennies with spontaneous eutocic foaling of a mature and viable foal was 371 ± 12 d (range 333 to 395 d). The male foals were 57.04% (81/142) and the female foals were 42.96% (61/142). The foal gender seemed the most relevant factor affecting the PL, with longer PL for males (376.4 ± 12.1 d) than for females (369.8 ± 4.1 d) ($P < 0.01$) (Fig. 1). The birth weight of the 142 donkey foals considered in this study was 30.4 ± 4.3 kg, with no differences between males (30.3 ± 5.1 kg) and females (30.7 ± 4.6 kg). The birth weight of the foal did not significantly affect the PL, indeed no correlations were found between the PL and the birth weight of the foal ($R = 0.143$, $P = 0.487$). The other factors, such as the year of foaling, the month and season of ovulation and parturition, and the age of the jenny marginally affected the pregnancy length ($P > 0.05$).

The duration of the different phases and parameters of the parturition in MF donkey are summarized in the Table 1. Parameters recorded in male and female foals were similar. The Perason's correlation test showed weak but significant correlations between D and MET ($R = 0.475$, $P = 0.014$), D and TSS ($R = 0.576$, $P = 0.004$). Umbilical cord rupture time was significantly correlated with the ST ($R = 0.680$, $P = 0.0001$), and with the MET ($R = 0.420$, $P = 0.033$). The standing time was positively correlated with TFS ($R = 0.507$, $P = 0.008$), and the TFS correlated with the TSS ($R = 0.671$, $P = 0.0001$). Finally, the TSS correlated with the FME ($R = 0.416$, $P = 0.043$).

4. Discussion

In the present study, the normal pregnancy length, the phases of the normal parturition, and the factors that could affect these parameters were reported on 142 normal pregnancies from MF endangered jennies. The pregnancy length, calculated from ovulation to foaling, was of 371 days, confirming previous studies on a limited number of animals [6,7,10,23,27]. The pregnancy length showed a wide range of distribution (333 to 395) that, however, was narrow compared with a previous study involving three Spanish donkey breeds, in which the pregnancy length was reported from 331 to 421 days (mean 362 d) [21]. The data reported in this article confirmed the general finding that the pregnancy length in donkey is longer [20,27,28] than the values reported in the horse [29–33].

The pregnancy length in MF donkey seemed affected by the gender of the foal, with longer PL for male donkey foals (376 d) compared with female (371 d). This finding is in agreement with most of the previous articles in mares [14,15,17,18,29,30,34], but not in jennies [21]. Contrary to the previous reports on the mare [35], the data reported in the present study did not find an association between PL and foal birth weight, suggesting a different endocrine activity for male and female foals [36] or a sex chromosome-linked effect [37].

In this study, neither the year of foaling nor the month of ovulation and the month of parturition influenced PL significantly. In some studies performed on mares in the Northern hemisphere [18,38], shortest gestation was reported when foaling occurs in January and the longest in April [18] or thereafter, as foaling months progress [38]. On the opposite, when the month of conception was related to PL, longer pregnancies were observed for conceptions that occurred at the beginning of the season, and decreasing thereafter [12,18,38]. In Spanish jennies, Galisteo and Perez-Marin found that gestation lengths were longer for jennies covered during the early breeding season [21]. The lack of the possible effect of seasonality on PL in MF jennies was not surprising. Some studies previously demonstrated negligible effect of seasonality on the reproductive characteristics in MF jennies [10] or jackasses [9,39].

In the present study, no significant influence of age on PL was observed in MF jennies, which is in agreement with the results obtained in Spanish jennies [21]. In the mare, data concerning the effect of age on PL are conflicting. Some studies [17,18,38] report the absence of significant effect of age on pregnancy duration, whereas others found pregnancies significantly longer [13,40] in young as compared to old mares. The wide range in the MF donkey PL (333 to 395 d) suggested that other factors than gender could affect this parameter. Some of these factors could be related to the stallion or the jenny, however these genetic parameters were not evaluated in the present study mainly due to the limited number of pregnancies per jackass or per jenny.

The knowledge of the normal duration of each parturition stage is crucial to improve the chances of foal and the mare health.

In the present study, the first stage of foaling in jennies lasted on average 65 min (20 to 135 min). Although the exact timing of the first stage of parturition could not be always clear because jennies, similar to mares [41], may not show obvious signs of first stage labor; this is a useful data for the first study reporting detailed timing of parturition in MF jennies. The second stage of the parturition, called expulsion, started with the rupture of the allantochorion and the expulsion of the allantoid fluid. In the MF jenny, this phase was completed between 10 and 30 min, in agreement with the data previously reported for the MF jennies [6-7,11,23]. The stage two of the parturition was also consistent with the timing observed in the horse, in which the complete expulsion can range between 5 and 60 min and the foals were expelled on average at 17 min [42] or 20 min [43]. In the horse, the lack of an evident fetus within 20 min after allantoid fluid expulsion suggested an evaluation for dystocia [22].

In normal eutocic jennies, the stage three of parturition was completed in 58 min (from 10 to 175 min), a value consistent with previous data reported for MF donkey [6,20] and in the mare [44–47]. The range of the fetal membrane expulsion was wide (10 to 175 min) in agreement with the data reported for the horse [48].

The standing time of the donkey foal was on average 61 min (25 to 190 min), very similar to the value (60 to 65 min) previously reported for MF donkey [11,23]. The mean standing time in the horse foal ranged from 32 to 34 min [24,44] and 57 min [25].

The first suckle occurred at 101 min (55 to 162 min), very close to the value previously reported by Koterba et al. [25]. Although no specific data are reported for the donkey, the value was lower than the limit reported for the equine over which a reduced passive immunity transfer was described [22,49]. In the mare, the concentration of the immunoglobulins in the mammary secretion decline rapidly in the first hours after the foaling [50, 51], with a significant reduction between 0 to 3 h and 4 to 8 h after foaling [52]. Thus, a rapid and frequent suckle is considered crucial for the immunological protection of both equine and donkey foal.

The MF donkey foal expelled the meconium at 86 min (32 to 180 min), a value longer than the data reported in the horse foal [24], and for crossbred pony foals [44]. No differences were found between

The significant positive correlations found between the time of umbilical cord rupture and the time to stand up, the time to stand up and the time for first suck, and also between the time of first suck with the time of second suck, suggest that the efficiency of early neonatal adaptation is closely related to the timed umbilical cord rupture and subsequent rapid stand up and colostrum intake. Lastly, the positive significant correlation found between the time of second suck and placental expulsion time could be related to a cumulative effect of udder suckling by the foal on oxytocin release and subsequent placental expulsion.

5. Conclusion

The data reported in this study showed that the pregnancy length in healthy Martina Franca jennies was 371 ± 12 days, and this parameter is affected by the foal gender only with longer values in jennies bearing male fetuses. Other factors, such as the year of foaling, the month of ovulation and parturition, the birth weight of the foal, and the age of the jenny did not significantly influence pregnancy length. Normal foaling stages duration were defined and appeared to be similar to previous finding reported for the jenny, and also in agreement with data reported for the horse. On the contrary, in the normal, mature, and viable donkey foal, the average times to stand up, for first suck, and for meconium passage were longer as compared to data found in the horse foal.

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Table 1. Mean (±SD) duration of parturition phases and parameters in 42 Martina Franca jennies.

	Female foals Mean±SD	**Male foals Mean±SD**	**Merged Mean±SD**
D (min)	69.8±27.5	60.6±20.3	65.2±24.3
E (min)	18.3±5.5	19.6±5.7	18.8±5.5
FME (min)	70.7±52.6	44.9±32.7	57.8±45.8
UCT (min)	16.1±6.5	15.7±3.7	15.9±5.2
ST (min)	64.7±39	57.4±38.8	61.2±38.4
MET (min)	94.3±40.2	78.1±26.7	86.2±34.4
TFS (min)	104.5±43.6	97.5±39.34	101±40.8
TSS (min)	159.5±99.5	127.3±34.9	143.4±74.8

D - stage one of parturition; E - stage two of parturition; FME - stage three of parturition; UCT - umbilical cord rupture time; ST - standing time; MET - meconium expulsion time; TFS - time of the first suckle; TSS - time of the second suckle.

Figure legend

Figure 1. Distribution of pregnancy length in jennies bearing male and female donkey foals according to parturition range.