

Study of postnatal growth of mule and donkey foals sired by the same jackass



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SUMMARY

Mules have been enrolled for agricultural work in national parks or islands and hard-to-reach places in which the use of machinery is forbidden or not feasible. Despite the importance of this animal, limited information is reported regarding gestation, parturition, and post-natal growth in mule.

To evaluate the effects of the pregnancy length, birthweight, and fetal sex on neonatal health and growth in mule and donkey foals born from Heavy Draft mares (n=15) and Martina Franca jennies (n=18) inseminated with the same jackass until 30 months of age.

This study was conducted on 15 healthy Heavy Draft mares, 4 to 6 years old and weighing 640 to 730 kg, and 18 healthy Martina Franca jennies 4 to 6 years old and weighing 390 to 420 kg. All animals presented normal reproductive history such as cyclicity, pregnancy, and foaling. The semen used for artificial insemination was collected from the same 7 years old 485 kg bodyweight Martina Franca jackass of proven fertility.

Pregnancy length in the mares bearing mule foal (339 ± 9 days) was similar to that reported in the equine gestation and was longer in female mules (347 ± 4.4 days) than males (331 ± 5.46 days) ($P < 0.05$). In donkey, pregnancy length was significantly longer in male foals (373.7 ± 10.4 days) than females (366.9 ± 5.6 days) ($P < 0.01$). The mean birth weight was higher in mules compared to donkey foals (50.6 ± 4.83 vs 33.4 ± 5.7 kg) and was greater in male mules than in females (53.95 ± 3.2 kg and 46.6 ± 2.96 kg, respectively; $P < 0.05$). Mule foal's weight increased faster than donkey foals, despite a similar height at withers at different time points. Pregnancy length affected birthweight in both mule and donkey foals, and birthweight was related to post-natal growth in both mule and donkey foals. The findings reported in this manuscript could optimize the maternal-foal management of this equine hybrid.

KEY WORDS

Horse, mule; Martina Franca donkey, breeding, postnatal growth.

INTRODUCTION

The mule, as the hybrid of the female horse and male donkey, is the most popular equine hybrid, and in the past 5,000 years, millions of mules were reared to sustain human activities¹. The reason for this success could be related to the physical performances, similar to those of the horse, together with the meekness, typical of the donkey. These characteristics have favored the introduction of the mule in agricultural work in developing countries or in delicate ecosystems, such as national parks or islands, where mechanization is not feasible.

Interestingly, Allen reported that at least the horse, the donkey, and the mule (*E. mulus mulus*; $2n = 63$) within the equine family can accept, gestate, carry to term, give birth to, and rear successfully truly xenogenetic extra-specific foals. In that case, the xenogenetic extra-specific pregnancies were obtained

by between-species embryo transfer techniques².

Fetal development is affected by several factors such as genetic, environmental, and maternal factors³. As demonstrated by Allen et al.⁴ and Wilsher and Allen³, the transfer of the nutrients mediated by the placenta is positively correlated with the area of the placenta^{3,4}. This may explain how an increase in foal weight occurs at birth when an embryo of a small breed is transferred to a larger breed. Tischner and his colleagues similarly highlighted the influence of maternal size upon foal growth when they used embryo transfer to gestate Polish Konig pony foals in the uteri of larger draught-type mares⁵.

Several authors have shown how foals of small breed born heavier than usual if gestated in the uteri of larger mares and vice versa, besides this increased size at birth persist to adulthood⁵. In the Arabian horse, the birth height was found correlated with the adult height, suggesting the use of this parameter as a predictor for the adult size⁶.

Differently to other domestic animals, in equid species, the postnatal growth rate received limited attention. In a previous study conducted on Thoroughbred foals, the growth curve from birth

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to weaning was described⁷. At birth, no differences were described between Thoroughbred colts and fillies in birthweight and at weaning, but the birthweight accounted the 16% of the variability of weaning weight, suggesting a relevant role of this parameter on the future foal growth. No information was reported in the literature regarding the growth curve in the mule. The present study aimed at reporting the growth curves for bodyweight, height at withers, chest circumference, and cannon bone circumferences of mule and donkey foals, born after artificial insemination (AI) using only one jackass with proven fertility, between birth and 30 months of age. At the same time, the effect of the foal sex and pregnancy length on the birth weight and the post-natal growth rate were also evaluated.

MATERIALS AND METHODS

Animals and data collection

This study was conducted on $n = 15$ Heavy Draft mares and $n = 18$ Martina Franca jennies. The criteria of inclusion were age between 4 and 6 years, the weight between 630 and 730 kg for the mares, and between 380 to 420 kg for the jennies, the parity (2 previous foalings), the month of ovulation (April to May) and feed management. All animals presented normal reproductive history regarding cyclicity, pregnancy, and foaling. The semen used for the inseminations was collected from the same Martina Franca jackass. The Jackass was of proven fertility, 7 years old, and weighing 485 kg (Figure 1). The animals were housed in the Veterinary Teaching Farm of the University of Teramo (Italy). All the animals were reared in open paddocks with free access to a shed. Daily, mares and jennies received standard hay and water ad libitum and commercial equine fodder (4 and 6 kg for jennies and mares, respectively). Jackass was kept in an individual $5 \times 5 \text{ m}^2$ 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. The procedures described in this study have been performed in agreement with the Italian legislation concerning animal care (DL n.116, 27/01/1992).

Oestrus detection was performed as previously reported⁸. At detection of first signs of estrous in mares and jennies, an uter-



Figure 1 - Martina Franca stallion.

ine sterile swab was performed. Those animals with bacterial growth were excluded from the trial. The ovarian follicular growth was monitored by transrectal ultrasonography twice daily from heat onset until the day of ovulation, with a Concept 2000 ultrasound machine (Dynamic Imaging Limited, Livingston, Scotland, UK) equipped with a 7.5-MHz linear probe. From the visualization of a follicle of 30 mm in size, jennies and mares were subjected to artificial insemination every 48 hours until ovulation. Semen was collected by a Missouri artificial vagina, and sperm concentration and objective progressive motility were measured as previously reported⁹. Insemination doses (15 mL) were prepared by diluting raw semen with INRA 96 extender (IMV Technologies, L'Aigle, France) to achieve 800×10^6 progressive spermatozoa/dose. The ovulation day was defined as the day the dominant follicle disappeared on the ovary cortex, on a daily successive ultrasound monitoring. Pregnancy diagnosis was carried out by transrectal ultrasound on day 14 post ovulation and confirmed on day 45. All the jennies and the mares were monitored monthly throughout gestation to evaluate fetal viability and wellness. At foaling, each foal was clinically examined and birth weight and sex were recorded. In this study, only foals born by spontaneous foaling and without obstetric interventions were included. Within five minutes after birth, the APGAR score was evaluated as previously reported¹⁰. After fetal membrane expulsion, the fetal membranes were weighted. In all cases, pregnancy length (PL), calculated from ovulation to foaling, was recorded.

On the day of foaling, the quality of the colostrum was evaluated with a Brix refractometer (HR-150N, Optika, Bergamo, Italy). Values below Brix score 15 were considered suggestive of poor colostrum quality.

Postnatal growth measurements

Each foal was weighed (W) and measured for height at withers (HW), chest circumference (CC) immediately caudal to the withers (Figure 2), and cannon bone circumference (CAC), measured immediately distal to the metacarpal tuberosity at the proximal end of the cannon bone, at the day of birth and then monthly until 6 months of age, and at 12, 18, 24, and 30 months of age.

Statistical analysis

All morphometric parameters are reported as mean \pm standard deviation (SD). The coefficient of the linear regression was cal-



Figure 2 - Measurement of height in withers and chest circumference in mule foals.

culated for each measurement, to estimate the growth of the specific parameter, namely weight (cW), height at withers (cHW), chest circumference (cCC), and cannon bone circumference (cCAC).

Differences between all the growth measurements and the calculated growth trend for each parameter were compared using the general linear model (GLM) based on the univariate ANOVA. The month and sex were considered as fixed factors. Correlations between the PL, the birth weight, and the cW, cHW, cCC, and cCAC were compared with Pearson's correlation test. Differences were considered significant when $P < 0.05$. Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The number of estrus/pregnancy was 1.8 for Heavy Draft mares, and 1.5 for jennies, without significant differences ($P > 0.05$). The mean duration of the estrus was 5.3 ± 0.8 and 6.5 ± 0.6 days for Heavy Draft mares and Martina Franca jennies, respectively ($P > 0.5$). At ovulation, the mean follicular diameter was 4.8 ± 0.4 cm for Heavy Draft mares and 4.3 ± 0.3 cm for jennies. The pregnancy diagnosis was performed on day 14 post ovulation, and the embryo vesicle was spherical in both Heavy Draft mares and jennies. The mean diameter at diagnosis was 1.6 ± 0.2 cm and 1.5 ± 0.3 cm for mares and jennies. All mules (7 females and 8 males) and donkey foals (7 females and 11 males) were born at term and by spontaneous eutocic foaling. The mean PL was 338.82 ± 9.32 days for mule-bearing mares and 368.4 ± 11.5 days for jennies ($P < 0.01$). The mule sex affected PL in mares since it was longer in females (347 ± 4.4) than males (331 ± 5.46) ($P < 0.05$). Conversely, donkey's PL was significantly longer for males compared to females foals, with mean values of 373.7 ± 10.4 and 366.9 ± 5.6 days, respectively, ($P < 0.01$). In all cases, the APGAR score evaluated within five minutes after birth was 9.3 (range 8 to 10). No differences were found between the APGAR score in mule and donkey foals, nor between males and females ($P >$

0.05). All the newborns followed a normal clinical course throughout the evaluation period. The placental weight for mule foals was 5.6 ± 0.48 kg, without differences between colts and fillies (5.6 ± 0.4 kg and 5.2 ± 0.6 kg, respectively; $P > 0.05$). For donkey foals, the placental weight was 3.1 ± 0.3 kg, without differences between colts, and fillies (3.6 ± 0.4 kg and 3.7 ± 0.2 kg, respectively; $P > 0.05$). Differences between placental weight in mule and donkey foals reflected the difference in birthweight.

The Brix refractometry of the colostrum, collected the day of parturition, was 26.2 ± 2.1 % for Heavy Draft mares and 24.6 ± 3.4 % for jennies.

The mean W, HW, CC, and CAC of mules and donkey foals from birth to 30 months of age were reported in Table 1.

The mean birth weight, evaluated before first suckling, was 50.6 ± 4.83 kg for mules, with differences between males 53.95 ± 3.2 kg and females 46.6 ± 2.96 kg ($P < 0.05$), and 33.4 ± 5.7 kg for donkey foals, with no differences between males (32.4 ± 4.1 kg) and females (33.2 ± 2.5 kg) ($P > 0.05$). The mule foal growth in weight was faster than the donkey foal (Figure 3), as corroborated also by the cW value (14.9 ± 0.6 and 11.8 ± 0.9 for the mules and the donkey foals, respectively).

The HW measured for the mules at foaling was 94 ± 1 cm, with similar values for the males (93 ± 1 cm) and the females (96 ± 3 cm, $P > 0.05$). In the donkeys at foaling the HW was (86.7 ± 4.9 cm) with similar values for the males (84.8 ± 4.3 cm) and female foals (88.3 ± 3.6 cm, $P > 0.05$). The cHW was similar in both the species, as shown in Figure 4.

Although a similar height at withers between the species, the higher weight recorded in the mule foals at each time-point was the result of the higher increase in CC and CAC (Figure 5). The mules' CC measured at foaling was (88 ± 1 cm), with similar values in both males and females (87.4 ± 1.1 cm, and 88.7 ± 1.3 cm, respectively; $P > 0.05$; Figure 6). In the donkey at foaling, the CC was 66.9 ± 4.5 cm with no differences between males (66.3 ± 2.7 cm) and females (67.2 ± 3.4 cm; $P > 0.05$). The coefficient of the linear regression of the chest circumference appeared higher in the mule (3.236) than in the donkey (2.348; $P < 0.05$). Similarly to the CC, the CAC of mules at foaling was

Table 1 - Morphometric parameters (weight - W; height at wither - HW; chest circumference - CC; cannon bone circumference - CAC) measured at the different time-points in donkey and mule foals from the same jackass.

Month	W (kg)		HW (cm)		CC (cm)		CAC (cm)	
	Donkey foal Mean±SD	Mule foal Mean±SD	Donkey foal Mean±SD	Mule foal Mean±SD	Donkey foal Mean±SD	Mule foal Mean±SD	Donkey foal Mean±SD	Mule foal Mean±SD
0	33.4±5.7 ^a	50.6±4.8 ^b	86.7±4.9 ^a	94±4.4 ^a	66.9±4.5 ^a	88.2±4.1 ^b	10.5±1.1 ^a	13.1±0.6 ^b
1	51.1±7.2 ^a	83.2±7.5 ^b	94.2±5.7 ^a	104.1±3.1 ^b	77.9±4.2 ^a	98.5±3.6 ^b	11.6±1.1 ^a	13.5±0.6 ^b
2	71.5±10.6 ^a	111.4±7.8 ^b	100.6±4.8 ^a	110.6±2.8 ^b	88.7±5.1 ^a	106±3.3 ^b	12.6±1.2 ^a	14.3±0.9 ^b
3	88.9±11.8 ^a	140.7±6.4 ^b	107.7±5.3 ^a	114.3±2.6 ^a	95.6±5.2 ^a	112±3.7 ^b	13.8±1.1 ^a	14.7±0.9 ^a
4	102.3±13.1 ^a	166.2±5.8 ^b	112.2±4.7 ^a	117.5±2.8 ^a	101.3±4.7 ^a	116.5±4.9 ^b	15.1±1 ^a	15.2±1 ^a
5	121.7±17.4 ^a	190.7±6.2 ^b	115.4±5.2 ^a	120±2.6 ^a	106.1±4.9 ^a	119.6±5.4 ^b	15.9±1.2 ^a	15.5±1 ^a
6	138.1±17.9 ^a	208.5±5.4 ^b	119.1±5.8 ^a	122.9±3.4 ^a	113.4±5.2 ^a	123.6±6.8 ^b	16.5±1.1 ^a	16±1.1 ^a
12	193.7±24.6 ^a	293.2±11 ^b	132.7±6.3 ^a	127.2±3.8 ^a	127.3±7.1 ^a	147.2±12 ^b	17.8±1.3 ^a	17.1±0.7 ^a
18	240.2±28.1 ^a	373±27.7 ^b	134.2±6.1 ^a	131.6±5 ^a	136.4±7.1 ^a	152.7±13.8 ^b	18.2±1.2 ^a	18.1±0.8 ^a
24	277.3±31.2 ^a	449.7±49 ^b	138.9±6.8 ^a	135.4±6 ^a	143.4±5.2 ^a	157.4±14.5 ^b	18.7±0.9 ^a	18.9±0.9 ^a
30	306.4±23.1 ^a	516.2±59.4 ^b	141.7±6.1 ^a	139.1±6.8 ^a	147.1±4.6 ^a	163.4±15.7 ^b	19.3±1.3 ^a	19.7±1 ^a

For each parameter and at each time, values with different superscript (a/b) differ significantly ($P < 0.05$)

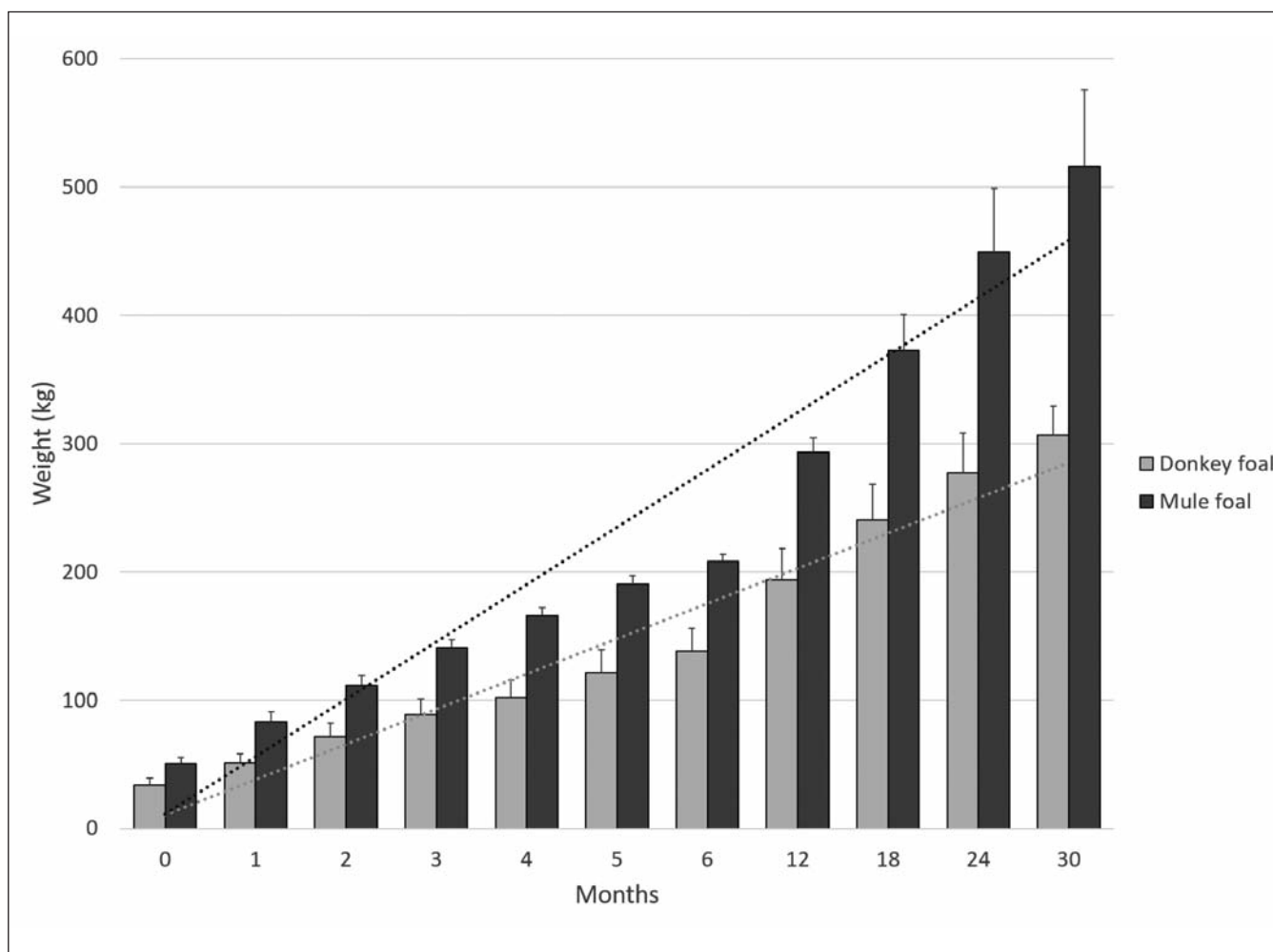


Figure 3 - Graphical representation of the weight (kg) at the different time-points (month) in mule (dark gray) and donkey foals (light gray), and the respective trend lines.

(13.1 ± 0.2 cm), with similar values for the males (13 ± 0.3 cm) and the females (13.3 ± 0.3 cm; $P > 0.05$). The donkey CAC at foaling was 10.5 ± 1.1 cm, with similar values for the males (9.7 ± 1.5 cm) and the females (10.9 ± 1.2 cm; $P > 0.05$). The increase of the parameter, summarized by the coefficient of the linear regression, was higher for mule (0.392) than donkey foals (0.208; $P < 0.05$).

Significant correlations between PL and birthweight in both mule and donkey foals ($R = 0.789$, $P = 0.004$, and $R = 0.723$, $P = 0.008$, respectively) were found. Moreover, PL was significantly correlated with the coefficient of the linear regression of W ($R = 0.813$, $P = 0.002$ and $R = 0.784$, $P = 0.003$ for mule and donkey foals respectively), and the coefficient of the linear regression of the HW ($R = 0.603$, $P = 0.026$, and $R = 0.634$, $P = 0.0022$ for mule and donkey foals respectively). A significant correlation was found between the birthweight and the coefficient of the linear regression of the weight in both mule ($R = 0.639$; $P = 0.001$) and donkey foals ($R = 0.684$; $P = 0.001$).

DISCUSSION

In this study, the pregnancy length calculated from ovulation day to foaling in the mares bearing mule foals was 339 days. The value was similar to those reported in other studies^{11,12}. The PL in the mule was also similar to the values reported for mares¹³⁻¹⁵. The PL in the donkey reported in the present study was

369 days. The PL was within the normal range reported by our group in the Martina Franca donkey^{16,17} and was consistent with that reported in other donkey breeds¹⁸. The data about jennies confirmed the finding that PL is longer in the donkey^{8,16} compared to mares. Few studies evaluated the relationship between PL and birthweight of the equine foal. A previous study found a relationship between PL and placental weight until 6.5 kg, while the correlations were lost after this threshold, suggesting a limiting effect only for low-size placenta on fetal growth¹⁹. In the donkeys and mules considered in the present study, although on a limited number of animals, the effect of the PL appeared more relevant to determine the foal weight at birth, since in both these species significant correlations were found between these parameters. In donkey an increased stereological complexity of the microcotyledons was found, suggesting a reduced efficiency of the placenta in this species¹⁶. This could explain the increased PL recorded in this species, but also the higher dependence of the foal birthweight to the PL. Unfortunately, no information is available in the literature regarding the relationship between mule placenta and mule foal birthweight.

In the present study, the foal sex influenced the duration of PL in jennies, with longer PL in male than in female donkey foals, in agreement with our previous finding in the same donkey breed²⁰. Similar findings were reported in mare bearing a male foal^{13,21,22}. Conversely, in mares bearing mules embryos, the PL was significantly longer for females compared to males. In a pre-

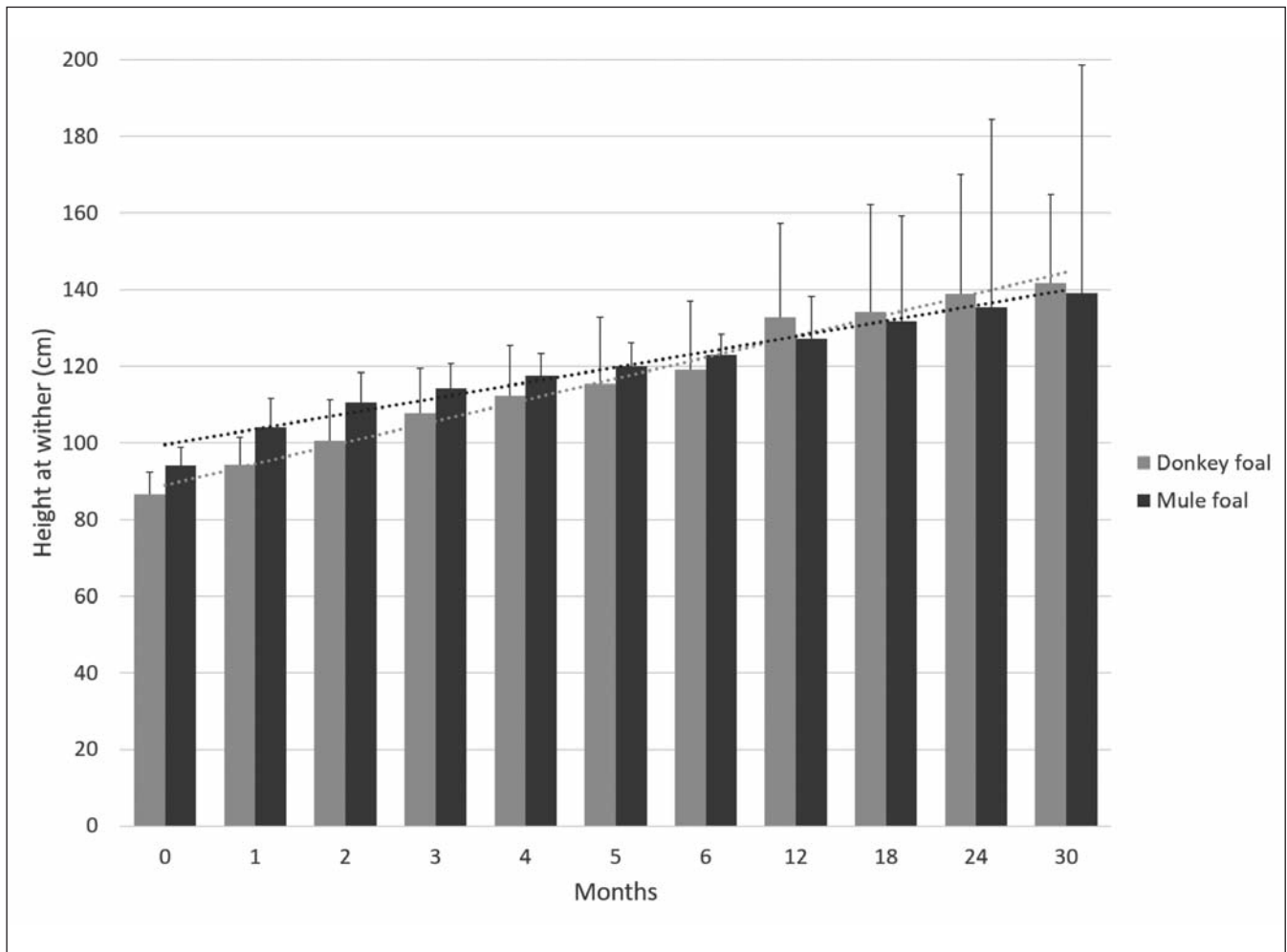


Figure 4 - Graphical representation of the height in withers (cm) at the different time-points (month) in mule (dark gray) and donkey foals (light gray), and the respective trend line.

vious study on mule PL, the gestation was slightly longer in mares bearing female mule, even if the difference was not significant¹². The reason for such effect of fetal sex on the equid PL is unclear. It was hypothesized that the endocrinology of parturition could be affected differently by the hormones produced by the male and female fetus^{21,23}, but the conclusive demonstration was not yet reported in equids. Unfortunately, no information regarding the fetal and foal endocrinology of the mule



Figure 5 - Representative image of a Heavy Draft mare with her mule.

was reported, thus specific studies are needed to elucidate this specific aspect.

Several studies showed that age and parity affect birthweight significantly^{24,26}. Although these parameters are closely correlated, a recent study using a stratified and multivariate approach clarified that the parity rather than the age affects the foal birthweight, in particular between the first and the second foaling¹⁸. Furthermore, Robles et al. reported that primiparous mares showed a reduced foal weight to the placenta ratio compared to multiparous mares²⁶. The reduced chorionic volume associated with the microcotyledon density detected in the placentas



Figure 6 - Representative image of mules.

collected from primiparous mares seems to be responsible for this phenomenon^{19,24}. As in the herein study, we recruited exclusively third foaling mares, foal's birthweight was not affected.

The donkey birthweight recorded in the present study was consistent with previous data reported in Martina Franca donkey foals^{10,20,27}. The mule weight at birth recorded in the present study was consistent with the values reported by Paolucci et al.¹¹, regardless of the different weight of mares considered (Heavy Draft mares in the present study, Italian Standardbred mares in the previous one). In the horse's genus *Equus*, an epitheliochorial non-invasive placenta, diffuse on the entire contact surface is present^{1,28}. As a consequence, the nutrition available for fetal development depends on the volume of the uterus, which is in turn related to the mare's size²⁹. Allen et al., using the embryo-transfer between ponies and Thoroughbreds, showed that the maternal size affects fetal growth and the mass, gross area, and volume of the allantochorion⁴. However, the evident effect on the fetal growth reported in that trials could be due to the large difference in the breeds used. More recently, Peugnet et al. reported no differences between saddlebred embryos developed in draft mares compared with their control developed in saddlebred mares²⁹, similarly to our findings.

In the present study, the foal weight growth after birth was significantly higher in mule than in donkey foal. This higher weight appeared related to the increase in transverse diameters, measured using the chest circumference and the cannon bone circumference, rather than the height at withers, similar in Martina Franca donkey and mule. This difference agrees with the expected morphology of the mule, which express the physical characteristic of the horse parent¹. In the mule, the foal sex affected the bodyweight at birth and the following growth rate. A similar trend was reported previously in a study on 1992 Thoroughbred foals¹³. On the other hand, Morel et al. found no significant differences in weight at foaling and weaning between Thoroughbred colt and fillies, even if males appeared heavier of about 1.7 kg⁷. Although a similar increased weight trend in both sexes, this difference agrees with the evidence that the adult male is heavier than the female one. In the present study, the mule foal weight increased faster than donkey foal, and this difference could be related to several factors. An increased milk availability for the mule foals could be a factor since the milk yield increases with the mare size²⁹. The milk intake, however, could only partially explain this difference, since it was found relevant for the equine growth within the first 4 weeks after foaling, while there was no relationship between milk intake and foal growth between 4 and 8 weeks³⁰. Thus, other genetic or metabolic factors could affect this difference. In both the categories, the birthweight positively affects the weight that the subjects reached at 30 months, confirming previous findings in some studies²⁶.

In conclusion, the mule foal was born after a PL similar to that of the horse foal, with longer PL in the female mule foals. The birthweight was affected by the PL, and it could be considered an important factor to predict the future growth of the mule foal since the birthweight was significantly correlated with the growth rate and the bodyweight at 30 months.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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