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Research article

Effect of age and of reproductive status on reproductive indices in horse mares carrying mule pregnancies

A. Carluccio [a], R. Bucci [a], J. Fusi [b,*], D. Robbe [a], M.C. Veronesi [b]

[a] Faculty of Veterinary Medicine, Veterinary Teaching Hospital University of Teramo, 64100 Teramo, Italy
[b] Department of Veterinary Medicine, Università degli Studi di Milano, 26900 Lodi, Italy

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ABSTRACT

Mules (Equus mulus) are interspecific hybrids derived from crossing horse (Equus caballus) mares with jackasses (Equus asinus). In Italy, the production of mules is very important for the special environmental areas, where they are used as working animals, instead of the forbidden agricultural machines. Although many horse mares carry mule pregnancy successfully at term, low fertility of mares used for producing mules was reported, but limited data about reproductive efficiency in mares carrying mule pregnancy, especially when age and reproductive status are concerned, are available. Therefore, the present study aimed to assess the effect of age and of reproductive status on some reproductive indices of horse mares carrying mule pregnancy, in the final purpose to better clarify factors affecting reproductive performances in the valuable production of mules as working animals under special environmental areas in Italy.

The study, performed on 96 draft mares inseminated with Martina Franca donkey semen, showed a satisfactory 89.6% 14 days PO pregnancy rate, lower pregnancy losses (19.8%) than those previously reported for mule pregnancies, leading to satisfactory foaling rates (71.9%), similar to those reported for intraspecific horse pregnancies. A clear, significant detrimental effect of mare's age on pregnancy losses, foaling rate, and on number of estrous cycles needed to achieve pregnancy, was found. Reproductive status also affected the reproductive indices, with significantly higher pregnancy losses and lower foaling rate in barren mares, reopening the question about the possible lower reproduction efficiency in horse mares carrying mule pregnancy. The present study results, although mostly descriptive, add useful information about some factors affecting reproductive performances in the valuable production of mules as working animals under special environmental areas in Italy.

1. Introduction

Mules (Equus mulus) are interspecific hybrids derived from crossing horse (Equus caballus) mares with jackasses (Equus asinus). Because of their physical characteristics, combining the best features of horses and donkeys, mules, although sterile, are known and appreciated since 5000 years.

In Italy, mules were used as working animals during the Great War for the transportation of cannons, people and food provisions, and were also used in agriculture, especially on the mountains, until the development of mechanization. Agricultural mechanization created conditions leading to the almost complete disappearance of mule's populations. However, recently, the usefulness of mules as working animal under special environmental areas (marginal mountain agricultural works, wood transportation within National parks, etc.), was reevaluated.

In the Abruzzo region, National park areas cover 36% of the whole regional territory and mules are largely requested for multiple uses, such as tourists excursions and for woods transportation. For this last purpose, heavy mules are much appreciated and requested by breeders, farmers and forestry, so that heavy horse mares are used to produce mules. Among the many Italian donkey breeds, the Martina Franca is one of the heaviest, appreciated for robustness and resistance at work. Thus, the use of Martina Franca jackasses for mule producing is requested.

Although many horse mares carry mule pregnancy successfully at term, low fertility of mares used for producing mules was reported [1] and subsequently suggested to be partly due to low eCG concentrations leading to a greater occurrence of abortions [2], even if low levels of eCG were found also in mares carrying mule pregnancy at term [2]. A subsequent study [3] demonstrated that abortions in mares carrying mule pregnancies is associated with diverse kinds of luteal malfunction. Those

* Corresponding author.
E-mail address: jasmine.fusi@unimi.it (J. Fusi).

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authors reported a similar percentage of embryo mortality (losses occurring before 40 days after ovulation) in mares carrying horse and mule pregnancies, but a 6 times higher incidence (30%) of abortions (after day 40) in mares carrying mules than in mares carrying horses. That very high abortion rate was reported to be in agreement with previous studies [2, 4].

Apart from the suggested more sensitivity to abortions in mares carrying mule pregnancy, and the recent establishing of reference values for gestational length in mares pregnant with male and female mules [5], information about reproductive indices of horse mares impregnated by donkeys are limited, and need further investigations.

In equine reproduction, many reproductive indices can be used as a toll to measure reproduction efficiency, some of them related to males, semen characteristics and mating/insemination management. However, most of the indices are measured on females: number of artificial inseminations or mating/estrous cycle (AI/EC); number of artificial inseminations or mating needed to achieve pregnancy (AI/Pregnancy); number of estrous cycles needed to achieve pregnancy (EC/Pregnancy); pregnancy achievement per estrous cycle (Pregnancy/EC); embryo mortality (pregnancy losses occurring <40 days post ovulation); abortions (pregnancy losses occurring ≥40 days post ovulation); total pregnancy losses (sum of the previous two); number of mares foaling at term. Beside pregnancy/EC rate, the foaling rate (number of foaling/numbers of mated or inseminated mares) was reported to be useful to assess reproductive efficiency [6], because it reflects the best economical measure.

In horse husbandry, the role of advancing age in mares on decreased fertility and pregnancy rate has been investigated [7, 8, 9, 10, 11], even though different cut-off ages have been suggested [10], as well as different criteria for grouping mares in age classes [6, 8, 9]. Foaling rate was also reported to be influenced by mares' age, with predictable lower live-foal rate in mares aged >18 years old than younger mares [7].

Mares can also be grouped according to their reproductive status as maiden (never mated/inseminated), barren (mated/inseminated but loosing pregnancy, no conception), foaling (mares with foal). Beside the mares age, the reproductive status was also reported to influence fertility and pregnancy rate, with a tendency for higher pregnancy rate in maiden and foaling mares in comparison to barren mares [12, 13]. Foaling rate is also influenced by mare's reproductive status [6, 13].

Moreover, the relation between follicular diameter and oocyte quality/corpus luteum function has been investigated and suggested to play a role in reproductive efficiency [14, 15, 16]. Davies-Morel et al [16] found that mares aged >19 years old had significantly smaller preovulatory follicles in comparison to mares aged 2–4 years old, but the relation between preovulatory follicle diameter and pregnancy occurrence was not investigated. A previous study, however, failed to show a significant association between follicle area and pregnancy occurrence in mares [17].

Because of the scarce information about the reproductive indices of mares carrying mule pregnancy and especially taking into consideration the effect of age and reproductive status, the present study was aimed to assess the effect of age and of reproductive status on some reproductive indices of horse mares carrying mule pregnancy, with the final purpose to better clarify factors affecting reproductive performances in the valuable production of mules as working animals under special environmental areas in Italy.

2. Materials and methods

2.1. Ethics

The study was performed in accordance with the ethical guidelines provided by the animal welfare committee and all the procedures were carried out according to the Italian legislation about animal care (DL 116, 27/01/1992) and to the European Guidelines on Animal Welfare (Directive 2010/63/EU). A written informed consent was signed by the owners, giving the permission to record and use for research purposes the clinical data.

2.2. Animals

The study, conducted during 2012–2018 breeding seasons (March–August), enrolled 96 healthy horse mares belonging to private owners, of different breeds (32 Italian draft horse, 64 Half-blood draft mares), with bodyweight ranging between 580-650 kg and 560–620 kg, respectively, admitted to the Veterinary Teaching farm of the University of Teramo, Italy, located in Chiareto di Bellante, for producing mules.

According to age, mares were grouped in three classes (Bosh et al, 2009, modified): young (YN), <10 years old (n = 68); aged (AG), 10–16 years old (n = 18); old (OD), >16 years old (n = 10). According to the reproductive status, 20 mares were maiden, 20 were barren, and 56 were foaling.

At admission, mares were clinically examined to confirm the health status according to legislation in force in Italy and the absence of reproductive disorders.

Animals were kept in open paddocks, with free access to water and hay. Five kg of commercial equine fodder was provided to each mare.

All the owners signed an informed consent to allow all the procedures related to the routine reproductive management and the use of data for research purposes.

2.3. Estrus monitoring and AI

Mares were daily observed for estrous signs, and from the beginning of heat, teased with a stallion. At positive teasing, a transrectal ultrasonographic (US) (Mindray DP-2200 Vet, Mindray DS USA Inc.; V2, General Electric Healthcare, 7.5-MHz probe) monitoring of follicular growth was performed every 12–24 hours until the detection of a follicle diameter of at least 3.0 cm. At the detection of a follicle with diameter ≥3.0 cm, a first AI was performed, with fresh donkey semen collected from jackasses of proven fertility. AI doses providing 800×10^6 progressive spermatozoa/dose in 15 ml were used. A second ultrasonography was performed 24 h later to detect the occurred ovulation. When ovulation did not occur, a second AI was performed, and ovulation occurrence rechecked 24 h later. The diameter of the follicle measured at the last ultrasound exam before ovulation, was recorded, and considered as the "preovulatory follicle diameter". According to their diameter, preovulatory follicles sizes were moreover grouped in four classes: 3.0–4.0 cm; 4.1–5.0 cm; 5.1–6.0 cm; >6.0 cm.

2.4. Pregnancy diagnosis, pregnancy length and foaling

Pregnancy was firstly checked by US at 14 days post ovulation (PO) and confirmed at 40 days PO, when mares were discharged. By follow-up, data about mares losing pregnancy >40 days PO and about mares foaling at term, were recorded. According to [3], embryonic mortality was considered when pregnancy loss occurred between 14- and 40-days PO, while abortion, when losses occurred >40 days PO.

Pregnancy length was measured from ovulation to the date of foaling, and recorded.

At birth, birth weight and sex of the foals were also recorded.

2.5. Reproductive indices

The following reproductive indices were considered:

14 days PO Pregnancy rate (n pregnant mares at 14 days PO/n inseminated mares);

embryonic mortality rate (n pregnancy losses between 14- and 40-days PO/n pregnant mares at 14 days PO);

abortion rate (pregnancy losses >40 days PO/n pregnant mares at 40 days PO);

total pregnancy losses: sum of embryonic mortalities + abortions

foaling rate (n of foaling/n inseminated);
AI/estrous cycles (EC);
EC/14 days PO Pregnancy;
14 days PO Pregnancy/EC rate;
AI/14 days PO Pregnancy.

2.6. Statistical analysis

One-way ANOVA was used to assess possible differences in birthweight of mule foals between the overall males and overall females, between males and between females born by Italian draft and Half-blood draft mares, and also between males and females within Italian draft and Half-blood draft mares.

Fisher exact test was used to assess possible differences between age classes and between reproductive status for the following indices: pregnancies at 14 days PO, foaling, embryonic mortality, abortions, total pregnancies losses.

One-way ANOVA test was used to assess possible differences between classes of age and between reproductive stratus for the following indices: pregnancy length, AI/EC, AI/14 days PO pregnancy, EC/14 days PO pregnancy, 14 days PO pregnancy/EC.

One-way ANOVA test was also used to assess possible differences between age classes and between reproductive statuses on preovulatory follicle diameter in 14 days PO pregnant and not pregnant mares.

The χ^2 square test was used to assess possible differences between 14 days PO pregnant and not pregnant mares according to preovulatory follicle diameter classes.

For all statistical procedures, significance was set for $P < 0.05$.

3. Results

Eighty-six out of 96 (86.9%) mares become pregnant and 69 carried a successful pregnancy (range 312–362 days) to term, giving birth to 30 (43.5%) male and 39 (56.5%) female healthy and viable mule foals.

The results about the birthweight in the overall male and female mule foals born by 69 mares, and about the birthweight in the male and female mule foals, born by the 24 Italian draft mares and by the 45 half-blood draft mares are reported in Table 1.

The results about the 14 days PO pregnancies, pregnancy lengths, and foalings (all expressed as number and %) in the overall mares and in the same mares grouped according to age classes, are reported in Table 2, while the same results in the overall mares and in the same mares grouped according to their reproductive status, are reported in Table 3.

The results about embryonic mortality, abortions and total pregnancy losses (all expressed as number and %) in the overall 86 pregnant mares and in the same mares grouped according to age classes and their reproductive status are reported in Tables 4 and 5, respectively.

The results about AI/EC, EC/14 days PO pregnancy, AI/14 days PO pregnancy (all expressed as number, and mean ± SD), and 14 days PO pregnancy/EC and (expressed as number and %) in the overall mares and in the mares grouped according to age classes and their reproductive status are reported in Tables 6 and 7, respectively.

The results about the mean ± SD preovulatory follicle diameter in 14 days PO pregnant and not pregnant mares, grouped according to age classes and reproductive statuses, are reported in Tables 8 and 9, respectively, while the results about the distribution of 14 days PO pregnant and not pregnant mares, according to preovulatory follicle diameter classes, are reported in Table 10.

4. Discussion

In the present study 69 mares carried successfully at term the mule pregnancy, with pregnancy lengths ranging between 312 and 362 days (mean ± SD 340.4 ± 11.57), without differences according to the age class and reproductive status. The result is very similar to the 341 ± 1.6 days gestation length (range of 307–360 days) reported for mule pregnancy by [5], with 315–369 days reported by [18], and with 342.3 ± 10.8 days reported by [19]. When pregnancy length was compared to the pregnancy length observed (author's unpublished data) in Half blood draft (339.9 ± 9.18 days) and Italian draft (339.3 ± 11.68 days) mares, the results showed superimposable findings, while it resulted very different when compared to Martina Franca donkey pregnancy length of 333–395 days [20, 21, 22, 23, 24, 25, 26, 27].

The birthweight of the foal was significantly higher in the overall male than female mules (54.8 ± 5.69 vs 48.8 ± 5.38 kg, respectively) ($p < 0.0001$), as well as when males and females where compared within each horse breed ($p < 0.001$ for mules born by Italian Draft, and $p < 0.05$ for those born by Half blood draft), but no significant differences were found between the two horse breeds. This finding is similar to the 52.4 ± 4 kg reported by [19] in mule foals.

The overall 14 days PO pregnancy rate was 89.6%, a bit lower than 93.3% found by [19] in mares carrying mule pregnancies, but in agreement with the range of 68.3–92.1% previously reported for intraspecific horse pregnancy [9, 28, 29]. When age class was considered, 14 days PO pregnancy rate was higher in YN than AG, and OD mares, although no significant differences were found among classes of age. The comparison with bibliography is a bit difficult, because of different age class grouping in the diverse studies. However, a significant higher pregnancy rate was reported in mares carrying horse pregnancy, 2–13 years old, in comparison to older mares [9, 28]. According to reproductive status, high 14 days PO pregnancy rates were obtained in all reproductive status mares, without significant differences among groups. When compared to data reported for intraspecific horse pregnancy, the 100% pregnancy rate in maiden mares was higher than the reported 72.2–74.9% [28] and 89.6% [29], but very close to the 96.2% reported by [9]. In foaling mares, the 85.7% 14 days PO pregnancy rate was higher than 67.4–69.1% reported by [28], and slightly lower than the 90.5% [29], and 91.3% reported by [9]. In barren mares the pregnancy rate was 90%, higher than the 66.6–69.5% reported by [28], but close to the 90.9% reported by [29]. A significant higher pregnancy rate in maiden than barren and foaling mares was reported only by [28] in Thoroughbred mares.

The overall 71.9% foaling rate was a bit higher than the 66.7% reported by [19] in mares carrying mule pregnancies, lower in comparison to the 78.3–82.7% reported for intraspecific horse pregnancy [9, 13, 29], but higher than 68% reported by [6] in mares with horse pregnancies. When mares were grouped according to age classes, in the present study, YN mares showed 81% foaling rate, AG 67%, and OD 20%, with significant differences between YN and OD ($p < 0.001$), but also between AG and OD ($p < 0.05$). For what concerning foaling rates, in mares carrying mule pregnancies [19], reported 71.4% in mares 5–10 years old, 62.5% in mares 11–15 years old and 66.7% in mares >15 years old, with lower rates in young mares, but higher in old mares. Although a different age

Table 1. Birthweight (kg), expressed as mean ± SD and range, in the overall male and female mule foals born by the 69 mares, and in male and female mule foals born by the 24 Italian draft mares and by the 45 half-blood draft mares.

	Males mule foals (n = 30)	Females mule foals (n = 39)	Total (n = 69)
Italian draft (n = 24)	57.5 ± 3.81* (53–62)	48.5 ± 3.01* (44–53)	52.3 ± 5.60 (44–62)
Half-blood draft (n = 45)	53.5 ± 6.07♥ (40–61)	48.9 ± 6.36♥ (39–58)	50.9 ± 6.59 (39–61)
Total (n = 69)	54.8 ± 5.69♣ (40–62)	48.8 ± 5.38♣ (39–58)	51.4 ± 6.26 (39–62)

Within row *$p < 0.001$; ♥$p < 0.05$; ♣$p < 0.0001$.

Table 2. Data about 14 days PO pregnancies, pregnancy lengths, and foalings (all expressed as number and %) in the overall 96 mares, and in the same mares grouped according to age classes.

	YN (n = 68)	AG (n = 18)	OD (n = 10)	Overall (n = 96)
14 days PO Pregnancy	62/68 (91.2%)	16/18 (88.9%)	8/10 (80%)	86/96 (89.6%)
Foaling	55/68* (80.9%)	12/18♥ (66.7%)	2/10*♥ (20%)	69/96 (71.9%)
Pregnancy length	340.1 ± 11.03	340.9 ± 15.01	346.5 ± 0.71	340.4 ± 11.57

Within row *p < 0.001; ♥p < 0.05.

Table 3. Data about 14 days PO pregnancies, pregnancy lengths, and foalings (all expressed as number and %) in the overall 96 mares, and in the same mares grouped according to their reproductive status.

	Maiden (n = 20)	Barren (n = 20)	Foaling (n = 56)	Overall (n = 96)
14 days PO Pregnancy	20/20 (100%)	18/20 (90%)	48/56 (85.7%)	86/96 (89.6%)
Foaling	18/20♥ (90%)	9/20♥[a] (45%)	42/56[b] (75%)	69/96 (71.9%)
Pregnancy length	337.9 ± 14.12	348.0 ± 13.48	341.5 ± 10.38	340.4 ± 11.57

Within row ♥p < 0.05; [a,b]p<0.01.

Table 4. Data about embryonic mortality, abortions and total pregnancy losses (all expressed as number and %) in the overall 86 pregnant mares and in the same mares grouped according to age classes.

	YN (n = 62)	AG (n = 16)	OD (n = 8)	Total (n = 86)
Embryonic mortality (<40 days PO)	2/62[a] (3.2%)	2/16 (12.5%)	4/8[b] (50%)	8/86 (9.3%)
Abortion (>40 days PO)	5/60♥ (8.3%)	2/14 (14.3%)	2/4♥ (50%)	9/78 (12.5%)
Total pregnancy losses	7/62 (11.3%)	4/16♥ (25%)	6/8♥ (75%)	17/86 (19.8%)

Within row [a,b]p<0.01; ♥p < 0.05.

class distribution was used [9], reported significantly higher foaling rates in mares carrying horse pregnancies of 2–13 years old, in comparison to older mares, while [7] found a higher foaling rate in 2–8 years old mares than mares aged >14 years old.

According to the reproductive status, the foaling rate was higher in maiden (90%) than in barren (45%) mares ($p < 0.05$), and also in foaling (75%) than barren mares ($p < 0.01$). Because of the lack of data from mule pregnancies, when compared to data reported for intraspecific horse pregnancy, except for barren mares, the foaling rates were very similar to the 85.9–86.5% reported by [9, 29] in maiden mares; the foaling rates were also very similar to the 76.5–79.5% [9, 29] in lactating mares, but different from the reported 75.9–78.5% in barren mares [9, 29]. Bosh et al [9] found a significant higher foaling rate in maiden in comparison to lactating mares.

The overall embryonic mortality was 9.3%, higher than the 6.2% previously reported in mares carrying mule pregnancies [3], and the 7.1–10.39% reported by [9, 13, 28] for intraspecific horse pregnancies. Nath et al [28] found a significant higher embryo loss rate in mares aged >18 years in comparison to 2–13 years old mares in Standardbreds, and lower embryo loss rate in mares 2–8 years old than all the older in Thoroughbreds. Morris and Allen [13] reported a significant lower rate of embryo losses in mares aged 3–8 vs 9–18 years old mares. Scoggin [7] reported a significant lower embryo mortality rate in mares aged 2–8 than in mares older than 14 years. However [8], did not find significant differences in embryo mortality rate among age classes. According to age, a lower embryonic mortality in YN (3.2%) than OD (50%) ($P < 0.01$). When the embryonic mortality is evaluated according to mare reproductive statuses, 5% was found in maiden, 22.2% in barren and 6.2% in foaling mares, with no significant differences among groups, differently to [9] that found lower embryo losses in maiden in comparison to barren and foaling mares, and to Morris and Allen, reporting lower embryo losses in maiden than foaling mares.

Pregnancy losses beyond 40 days PO, accounted to an overall value of 12.5%, were lower than the reported 30% abortion rate [2], occurring between 56 and 98 days of gestation, in mares carrying mule pregnancies, but superimposable to the 12.9% reported by [9] for mares carrying intraspecific pregnancies. This lower abortion rate reopens the hypothesis about the higher risk for abortions in mares carrying mule pregnancies, formerly related to possible problems due to eCG production and endometrial cups function in horse mares impregnated with donkeys. Abortion rate was higher ($p < 0.05$) in OD mares (50%) than YN (8.3%). Although a comparison with bibliography is not possible, it is to note the higher rate for OD mares (50%) in comparison to the 20% reported by [9] for mares carrying horse pregnancies aged >18 years. The effect of increasing age on fetal death was reported to be significantly higher in mares >13 years old [30] for intraspecific pregnancies. According to the reproductive status, abortion rate was higher ($p < 0.01$) in barren (42.8%) than maiden (5.3%) and foaling (4.4%) mares. Those rates were lower than those reported for intraspecific horse pregnancies by [9] for maiden (10%) and foaling (13.5%) mares, but higher for barren (13.1%) mares.

Table 5. Data about embryonic mortality, abortions and total pregnancy losses (all expressed as number and %) in the overall 86 pregnant mares and in the same mares grouped according to their reproductive status.

	Maiden (n = 20)	Barren (n = 18)	Foaling (n = 48)	Total (n = 86)
Embryonic mortality (<40 days PO)	1/20 (5%)	4/18 (22.2%)	3/48 (6.2%)	8/86 (9.3%)
Abortion (>40 days PO)	1/19[a] (5.3%)	6/14[b] (42.8%)	2/45[a] (4.4%)	9/78 (11.5%)
Total pregnancy losses	2/20 (10%)	10/18* (55.5%)	5/48* (10.4%)	17/86 (19.8%)

Within row [a,b]p<0.01; *p < 0.001.

Table 6. Data about AI/EC, EC/14 days PO pregnancy, AI/14 days PO pregnancy (all expressed as number, and mean ± SD), and 14 days PO pregnancy/EC and (expressed as number and %) in the overall mares and in the mares grouped according to age classes.

	YN (n = 68)	AG (n = 18)	OD (n = 10)	Total (n = 96)
AI/EC	154/88 (1.7 ± 0.90)	53/40 (1.2 ± 0.51)	39/26 (1.5 ± 0.92)	246/154 (1.5 ± 0.78)
EC/14 days PO Pregnancy	88/62*♣ (1.2 ± 0.44)	40/16♣ (2.1 ± 0.81)	26/8* (2.0 ± 0.71)	154/86 (1.8 ± 0.65)
14 days PO 14 days PO pregnancy/EC	62/88 (70%)	16/40 (40%)	8/26 (31%)	86/154 (56%)
AI/14 days PO Pregnancy	154/62♥ (2.2 ± 1.41)	53/16 (2.8 ± 1.64)	39/8♥ (3.7 ± 0.47)	246/86 (2.9 ± 1.17)

Within row *p < 0.001; ♣p < 0.0001; ♥p < 0.05.

The overall pregnancies loss rate was 19.8%, lower than the 28.6% found by [19] in mares carrying mule pregnancies, but similar to the 17.38% reported by [13] in mares carrying intraspecific pregnancies. Overall pregnancies losses were higher in OD (75%) than YN (11.3%) (p < 0.001), and than AG (25%) (p < 0.05) mares. This finding evidence, once more, the detrimental effect of old age in reproductive efficiency in mares, that seems, from the present study results, more important than the interspecific horse-donkey pregnancy. Overall pregnancies losses were higher in barren (55.5%) than maiden (10%) (p < 0.01), and foaling (10.4%) (p < 0.001) mares. Morris and Allen [13] reported lower overall pregnancy losses rate in mares carrying horse pregnancies 3–8 years old than all the older mares. Barren mares are recognized as "problem mares", in which a wide variety of causes can be responsible for pregnancy loss. In the present study barren mares showed the highest rate of pregnancy losses, confirming the low reproductive efficiency in this category of mares. It should be noted that very often barrens are also old mares, so that the "problem" can be addressed to the old age. In the present study, a statistical evaluation of the cumulative effect of age and reproductive status was not possible because of the number of mares. It is to note, however, that 14/18 barren mares were 10–16 years old, only 4 were >16 years old, and none was young.

Table 7. Data about AI/EC, EC/14 days PO pregnancy, AI/14 days PO pregnancy (all expressed as number, and mean ± SD), and 14 days PO pregnancy/EC and (expressed as number and %) in the overall mares and in the mares grouped according to their reproductive status.

	Maiden (n = 20)	Barren (n = 20)	Foaling (n = 56)	Total (n = 96)
AI/EC	46/24 (1.7 ± 0.68)	59/42 (1.2 ± 0.66)	141/88 (1.5 ± 0.69)	246/154 (1.5 ± 0.78)
EC/14 days PO Pregnancy	24/20[a] (1.2 ± 0.45)	42/18*b (2.0 ± 0.76)	88/48* (1.3 ± 0.48)	154/86 (1.8 ± 0.65)
14 days PO Pregnancy/EC	20/24 (83%)	18/42 (43%)	48/88 (55%)	86/154 (56%)
AI/14 days PO Pregnancy	46/20 (2.4 ± 1.71)	59/18 (2.9 ± 1.81)	141/48 (2.2 ± 1.47)	246/86 (2.9 ± 1.17)

Within row *p < 0.001; [a,b]p<0.01.

The overall 14 days PO pregnancy/EC rate was 56%, similar to the 59.9% reported by [13], but lower than the 64% reported by [9]. No significant differences were found among reproductive status and also among age classes, although best rates were observed in maiden and YN mares. In intraspecific horse pregnancies [9], did not found significant differences among reproductive status, but higher rates in mares aged 2–13 years than mares >18 years old, while [13] found better rates in mares aged 3–13 years than in mares 14–18 years old.

The overall number of inseminations per cycle was 1.5, similar to the 1.12–1.43 reported for mares mated/inseminated with horses [13, 28], with no differences among reproductive statuses and age classes, similar

Table 8. Data about the mean ± SD preovulatory follicle diameter in 14 days PO pregnant and not pregnant mares, grouped according to age classes.

	YN (n = 68)	AG (n = 18)	OD (n = 10)	Overall (n = 96)
Days14 PO pregnancy	4.7 ± 0.59	4.8 ± 0.44	3.9 ± 0.61	4.7 ± 0.61
Days 14 PO No pregnancy	4.3 ± 0.55	4.3 ± 0.21	4.7 ± 0.14	4.4 ± 0.45

Table 9. Data about the mean ± SD preovulatory follicle diameter in 14 days PO pregnant and not pregnant mares, grouped according to the reproductive status.

	Maiden (n = 20)	Barren (n = 20)	Foaling (n = 56)	Overall (n = 96)
Days14 PO pregnancy	4.7 ± 0.44	4.4 ± 0.76	4.8 ± 0.65	4.7 ± 0.61
Days 14 PO No pregnancy	-	4.4 ± 0.21	4.6 ± 0.40	4.4 ± 0.45

Table 10. Data about the distribution of 14 days PO pregnant and not pregnant mares, according to preovulatory follicle diameter classes.

	Preovulatory follicle diameter classes				
	3–4 cm	4.1–5 cm	5.1–6 cm	>6 cm	
Days14 PO pregnancy	14 (16.3%)	47 (54.6%)	24 (27.9%)	1 (1.2%)	86 (100%)
Days 14 PO No pregnancy	1 (10%)	8 (80%)	1 (10%)	0	10 (100%)
Total	15	55	25	1	96

to the findings reported by [13]. However, in the present study higher number of AI (2.9) were necessary to achieve 14 days PO pregnancy, in comparison to data (1.88) reported by [13], that also found lower number of matings with horses necessary to achieve pregnancy in mares aged 3–13 years than in mares 14–18 years old. In mares carrying mule pregnancies [19], reported an average 1.6 AI/conception, obtained on a total of 30 mares inseminated with donkeys, without significant differences among age classes. It is to note that, in the present study, AI started when a follicle of 3.0 cm in diameter was found, based on previous experience with mares ovulating with follicles smaller than 3.5 cm. This could at least partly explain the observed high number of AI/conception. At last, the overall number of estrous cycles needed to achieve pregnancy was 1.8. A lower number of estrous cycle/pregnancy was found in YN (1.2) than AG (2.1) ($p < 0.0001$), and than OD (2.0) ($p < 0.001$); an higher number of cycles were needed to achieve pregnancy in barren (2.0), in comparison to maiden (1.2) ($p < 0.01$), and in comparison to foaling (1.3) mares ($p < 0.001$).

When the follicle diameter was considered, there were no significant differences in the overall mean preovulatory follicle diameters between 14 days PO pregnant (4.7 cm) and not pregnant mares (4.4 cm), as well as no differences were found when the preovulatory follicle diameter classes were considered. These results are in agreement with the absence of significant differences in pregnancy rate according to follicle area classes reported by [17]. No significant differences were also found in preovulatory follicle diameter among class age and reproductive status of the mares. The absence of significant differences in preovulatory follicle diameter between young and old mares disagree with previous data reported by [16], that found significantly smaller follicles in old than in young mares.

5. Conclusions

In conclusion, data from the present study reported the reproductive indices of horse mares carrying mule pregnancies and evidenced not only a satisfactory 14 days PO pregnancy rate, but also pregnancy losses lower than those previously reported for mule pregnancies, leading to satisfactory foaling rates, similar to those reported for intraspecific horse pregnancies. A clear, significant, detrimental effect of age of the mares on pregnancy losses and foaling rate, and on number of estrous cycles needed to achieve pregnancy was found. Reproductive status also affected the reproductive indices, with significantly higher pregnancy losses and lower foaling rate in barren mares, reopening the question about the possible lower reproduction efficiency in horse mares carrying mule pregnancy. The present study results, although mostly descriptive, add useful information about some factors affecting reproductive performances in the valuable production of mules as working animals under special environmental areas in Italy.

Declarations

Author contribution statement

Carluccio A, Robbe D: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Bucci R: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Fusi J: Conceived and designed the experiments; Wrote the paper.

Veronesi MC: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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