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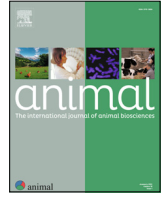
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Weaned horses, especially females, still prefer their dam after five months of separation



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ABSTRACT

Under natural conditions, foals stop nursing from their dam at approximately 9 months old, but their bond persists until 1.5–2.5 years of age. In contrast, in horse breeding, foals are generally artificially weaned and totally separated from their dam at 5–7 months. However, it is not known whether the bond between the dam and her foal is maintained after artificial weaning. The aim of this study was (1) to assess whether foals still recognise and prefer their dam over other familiar mares several months after weaning and (2) to evaluate whether the preference for the dam is more pronounced in fillies or colts. Fifteen fillies and 19 colts were weaned at the age of 7 months old (complete separation from the mother). At the age of one year (i.e., 5 months after the separation), they underwent a test evaluating their preference for their dam or a familiar mare from their natal group. Significantly more foals first approached their dam; they also sniffed and tended to look more often at her. This finding indicates that artificially weaned horses remember and still exhibit a preference for their dam, suggesting that the bond persists even after 5 months of separation. Moreover, fillies exhibited a stronger preference for both mares than colts: they looked at them more frequently, sniffed them for a longer duration and spent more time in proximity to both mares than colts. This suggests that fillies generally have an even stronger attachment to their dam as well as to other mares from their natal group. This study calls into question the practice of artificial weaning at 5–7 months of age.

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Implications

Our study shows that foals weaned at 7 months old show a preference for their dams 5 months later, at the age of one year; this was especially true for fillies. Thus, despite separation, dams may continue to provide social support. Our study suggests that in breeding practice, foals should remain with their dams for longer.

Introduction

Social animals form stable groups that change in composition according to reproductive status, age or season. The ultimate goal of banding together is to increase survival by achieving earlier detection of predators (Mendl and Held, 2001). Since the group provides social support, i.e., the benefits of social partners when coping with challenges (Rault, 2012), social separation may be per-

ceived as a life-threatening situation. Thus, the urge of domestic horses (*Equus caballus*) to remain in proximity to conspecifics is very strong and constitutes a temperamental trait (Lansade et al., 2008).

In social species, individual recognition of group members is essential. In horses, the bonds among group members, especially among mares, are very strong and sometimes lifelong (Jaworska et al., 2020). Feral horses distinguish between conspecifics from their own and other groups (Krueger and Flauger, 2011). Although it has been confirmed that horses remember humans from 6 months prior (Lansade et al., 2020a), it remains unknown how long horses remember their social partners after a period of separation. A previous study showed that after 18 months, stallions were unable to discriminate between members of their natal group and another group (Berger and Cunningham, 1987). That finding poses questions about the duration of the memory for conspecifics, especially when the conspecifics are kin, as in mare-foal pairs.

The bond between a dam and foal is very strong in the foal's early life (Haupt, 2002). Under natural conditions, dams generally refuse to nurse foals over 9 months old (Fraser, 1992), but the

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mare-foal bond persists until young horses emigrate to form their own social groups, as late as 1.5–2.5 years of age (Boyd and Keiper, 2005). In traditional breeding practice, foals are artificially weaned at a younger age, usually at 4–6 months old (Waran et al., 2008). For practical reasons, they then live separately from their dams and the natal group of broodmares. Artificial weaning is a stressful event, for both foals (Waran et al., 2008; Górecka-Bruzda et al., 2015; Lansade et al., 2018) and mares (Malinowski et al., 1990; Houpt, 2002). This practice has numerous behavioural and physiological consequences, as reviewed by Henry et al. (2020). However, it remains unknown whether foals still recognise their dams several months after artificial weaning and whether the mare-foal bond is maintained despite this separation. Moreover, it is unknown if foals would benefit from further social support from their mother.

Furthermore, whether both sexes exhibit similar preference for their dam is unknown. In sheep, a possible mechanism for social segregation according to the sex of the animal was proposed given the maternal affinity of female lambs and greater independence of male lambs (Gaudin et al., 2015). However, in horses, strong relationships between females are crucial for the stability and durability of natural social groups (Stanley et al., 2018; Jaworska et al., 2020); thus, fillies might be more motivated than their male counterparts to remain in closer contact with mares from their natal group, especially their mothers. The present study had two aims: (1) to assess whether foals still recognise and prefer their dam over familiar mare 5 months after artificial weaning and (2) to evaluate whether preference for the dam is more pronounced in fillies than in colts. Therefore, we compared the performance of one-year-old horses of both sexes on a test evaluating their preference for their dam or a familiar mare 5 months after weaning.

Methods

Mares and foals

This study involved 34 Welsh ponies foals (15 fillies and 19 males) and their dams at the Animal Physiology Experimental Unit PAO, INRAE (<https://doi.org/10.15454/1.5573896321728955E12>). From birth to weaning, the foals lived with their dams in a pasture; when the weather conditions worsened (during autumn and winter), mare-foal pairs were housed in stalls with adjacent paddocks. Artificial weaning took place in December, at 7 ± 0.4 months of age. Foals and dams were immediately separated and moved to two different barns (separated by 3 km) with free access to outdoor paddocks. All horses were fed hay (*ad libitum*) and concentrated pellets twice a day. In spring, the horses were moved to two different pastures (mares and foals three km apart). Thus, after weaning, mares and foals were unable to visually or vocally interact. Foals and dams were handled regularly to provide basic care (feeding with hay, deworming, and administering vaccines).

Preference test

The test was conducted 5 months after weaning, when the foals were 1.0 ± 0.03 years old. Each foal was tested only once, but two consecutive days were needed to test all animals. The day before testing, foals and their dams were moved to two separate stables with paddocks 100 m apart, still visually separated. The test arena was located in a paddock close to both stables and consisted of a rectangular area (10×9 m) made of metal hurdles 1.4 m high. The arena contained two holding pens 3 m apart: one for the dam and the other for a mare that was familiar to the foal (from the natal broodmare group). The arena also contained a pen in

which the foal was tested (Fig. 1). Two 3 m square areas were drawn on the floor next to each mare to mark contact zones.

The familiar mare was part of the natal broodmare group; thus, the tested foal was acquainted with her for the same duration (7 months) as the foal spent with their dam. At the time of testing, the two mares were led to the holding pens in the arena. To standardise the test, both mares were tied to the front barrier so that they always faced the foals during the tests. The pens permitted tactile contact between the mares and the focal foal. Immediately after the mares were relocated, the foal was encouraged to move from its stall to the testing pen (i.e., not led, but driven in that direction). The test began immediately after the foal entered the pen and lasted for 180 s. The entire test was recorded by three cameras (Fig. 1). Five behavioural items were recorded: (a) first mare approached (first contact zone entered), (b) total time spent in each contact zone, (c) time spent sniffing a mare, (d) time spent snapping at a mare (extension of the neck towards her and smacking the lips; specific juvenile, submissive behaviour (Crowell-Davis et al., 1985), and (e) occurrence of looks at a mare (foal faces towards the mare, points both ears at her, and looks at her with both eyes) regardless of the position of the foal in the testing area. We also recorded the vocalisations of both foals and mares: whinnies (neighs: loud sounds emitted to regain contact with conspecifics); greeting nickers, a low sound associated with the arrival of an associate or food; and squeals, a high-pitched sound produced during agonistic interactions with conspecifics (McGreevy 2004). However, these vocal behaviours were absent or rare in both mares and foals, and the medians of these vocal behaviours were 0; therefore, they were not analysed further. A foal was considered to enter a contact zone once both its front hooves were inside the zone. Each mare served as a stimulus in the preference test two times: once as a dam when her own foal was tested and once as the familiar mare when another foal was tested. The order in which the mare participated in these two contexts (with her own foal or with a familiar foal) was randomly determined. The positions of the dam and the familiar mare (right or left) were semi-random. After testing, the foal was removed from the testing pen and returned to the foal group. An observer blinded to the category of the mare (i.e., dam or familiar mare) and to the sex of the foals analysed the video recordings.

Statistical analysis

The proportion of foals that first approached their dam rather than the familiar mare was compared to the null hypothesis (no preference; a theoretical threshold of 50%) with a two-tailed Z test. Because the behavioural data were not normally distributed, non-parametric statistical tests were used. Comparisons of the foal behaviours directed towards their dam and the familiar mare were analysed by a Wilcoxon signed-rank test, which is used to analyse related ordinal data. The effect of foal sex (male vs female) was tested with the Wilcoxon two-sample test for non-related samples (equivalent to a Mann-Whitney U test). The analyses were performed in XL Stat (Addinsoft, 19.4) and SAS statistical package (SAS 9.4., SAS Inst., Inc., Cary, NC).

Results

Comparison of foal behaviour towards their dam vs the familiar mare

More foals first approached their dam rather than the familiar mare (dam, $N = 25$; familiar mare, $N = 9$; $z = 2.70$, $P < 0.01$, Fig. 2A). This pattern persisted when fillies and colts were analysed separately (fillies: dam, $N = 11$, familiar mare, $N = 4$, $z = -2.06$, $P = 0.04$, Fig. 2B; colts: dam, $N = 14$, familiar mare, $N = 5$; $z = 2.29$,

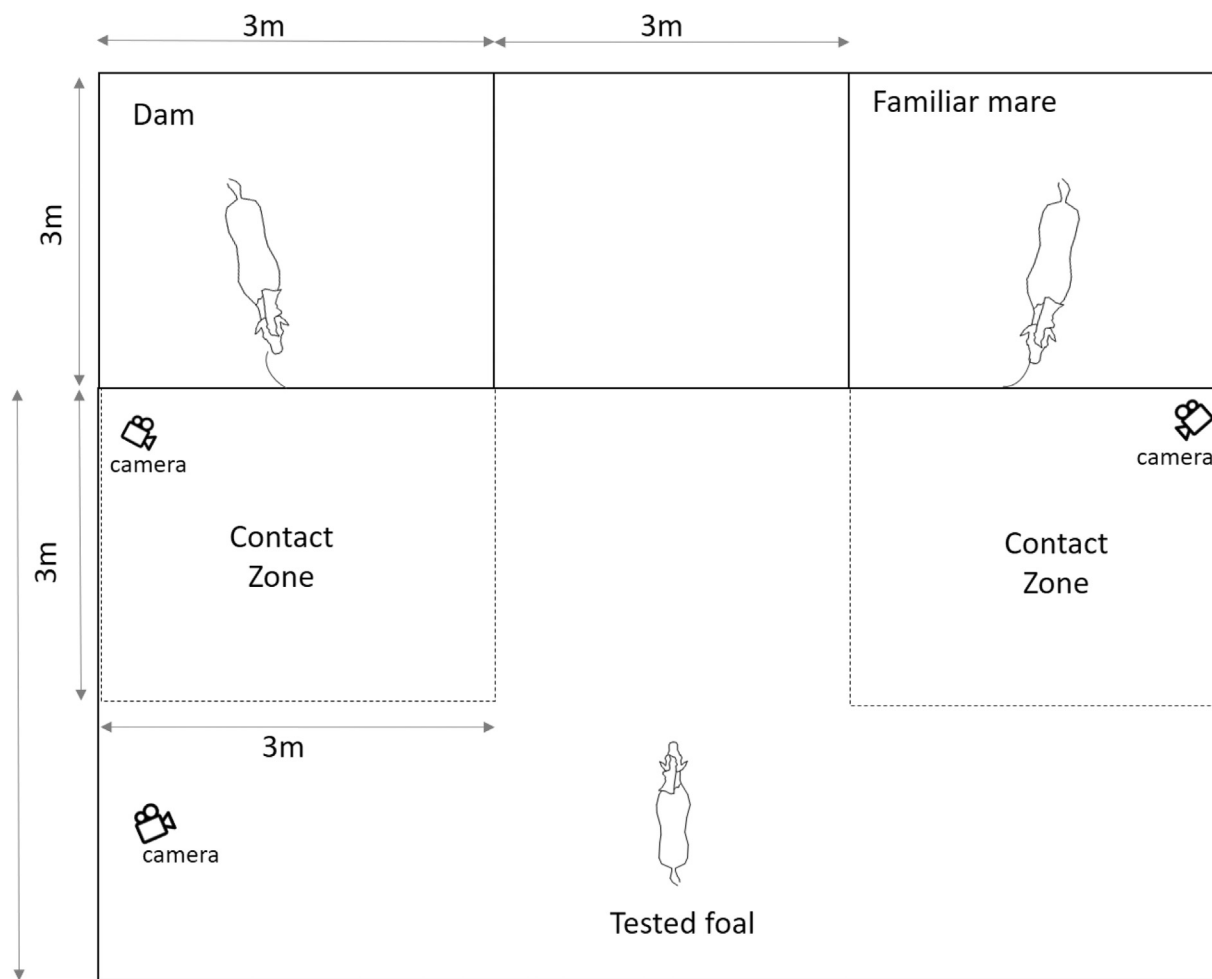


Fig. 1. Diagram of the preference test of horse foals. — represents metal hurdles; - - - - represents the line drawn on the floor. “Dam”: dam of the tested foal; “Familiar mare”: broodmare from the natal group (dam of another foal; familiar to the tested foal).

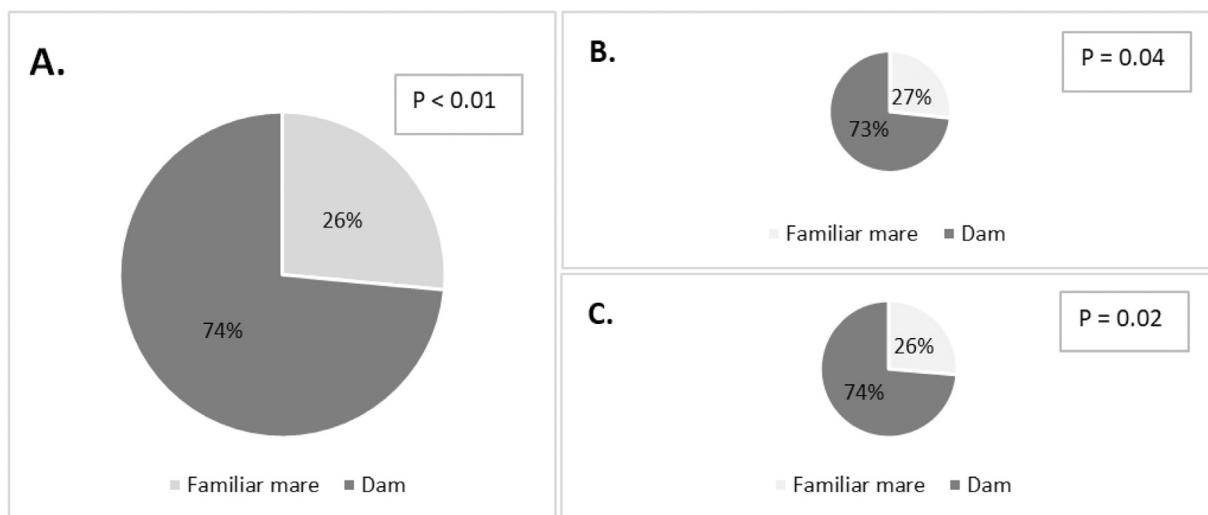


Fig. 2. Percentage of horse foals in preference test that first approached their dam instead of the familiar mare, (A): percentage of fillies (B) and colts (C). In both sexes, the dam was more likely to be approached first (Z test).

$P = 0.02$, Fig. 2C). The foals spent on average 117 s (median = 117; Q1 = 85, Q3 = 157) in proximity to both mares. They sniffed their dam longer than the familiar mare ($S = 151$, $P < 0.01$) and tended to look more often at their dam ($S = 94.5$; $P = 0.08$, Table 1). When

the sexes were analysed separately, both fillies and colts sniffed their dam longer than the familiar mare (fillies: $S = 37$, $P = 0.02$, Fig. 3B; colts: $S = 42$, $P = 0.03$, Fig. 3C). Fillies snapped less often at their dam than at the familiar mare ($S = 30.5$, $P = 0.02$, Fig. 3C),

Table 1
Horse foal behaviours directed towards their dam or a familiar mare in preference test.

Behaviour	Behaviour directed towards		S	P value
	Dam	Familiar mare Median [Q1, Q3]		
Looks (occurrence)	33.5 [21.0, 46.0]	27.5 [10.0, 44.0]	94.5	0.07
Sniffing (s)	5.50 [2.00, 9.00]	3.00 [1.00, 4.00]	151	<0.01
Snapping (s)	1.00 [0.00, 5.00]	1.00 [0.00, 6.00]	41	0.16
Time in contact zone (s)	45.0 [33.0, 89.0]	51.0 [31.0, 77.0]	22.5	0.71

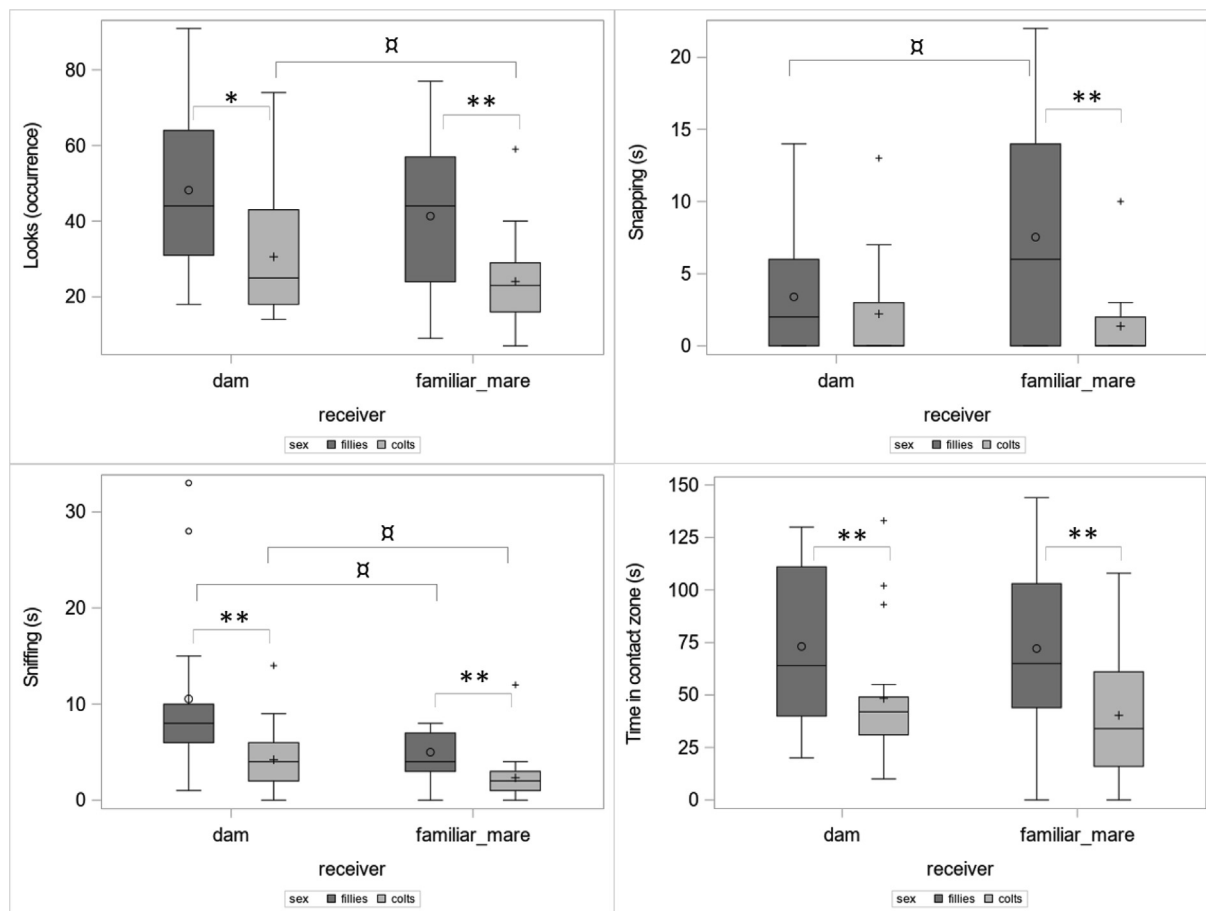


Fig. 3. Horse foal behaviours in preference test directed towards their dam or a familiar mare. Footnote: (A) occurrence of looks, (B) sniffing duration, (C) snapping duration, (D) time in contact zones; comparison of behaviours between fillies and colts. ** $P < 0.01$; * $P < 0.05$; comparison of behaviours directed towards their dam or the familiar mare within each sex: □ $P < 0.05$.

and colts looked at their dam more frequently than at the familiar mare ($S = 41, P = 0.05$, Fig. 3A). The descriptive statistics and detailed comparisons are presented in Supplementary Table S1.

Sex differences in foal behaviour

The sex of the foal had a significant effect on all behavioural variables. Compared to colts, fillies looked more frequently at both their dam and the familiar mare (dam: $z = 2.31, P = 0.03$; familiar mare: $z = 2.57, P = 0.02$, Fig. 3A). Fillies also sniffed both mares for longer (dam: $z = 3.05, P = 0.01$; familiar mare: $z = 3.26, P = 0.01$, Fig. 3B) and spent more time in close proximity (dam: $z = 2.01, P = 0.05$; familiar mare: $z = 2.76, P < 0.01$, Fig. 3D). Fillies also snapped at the familiar mare for a longer duration than colts ($z = 30.5, P = 0.02$, Fig. 3C). The descriptive statistics of detailed comparisons are presented in Supplementary Table S1.

Discussion

The results of the present study show that artificially weaned horses remember their dam and display a preference for her over a familiar mare even after 5 months of separation. Attraction to the dam, but also to the familiar mare, was even stronger in fillies than in colts. This shows that horses have long-term memories of conspecifics and suggests that, despite separation, the mare-foal bond remains strong, especially in fillies.

Foals still recognise and prefer their dam 5 months after weaning

Three-quarters of the foals approached their dam first rather than the familiar mare, indicating that the foals recognised their dam even though they had not been in contact after weaning 5 months earlier. This result confirms that horses have strong mutual recognition capabilities, as shown in a study of cross-modal recog-

dition of conspecifics (Proops et al., 2009). Additionally, our study further demonstrates that horses remember their conspecifics, or at least their dam, for periods lasting several months, similar to recognition of the face of their keeper (Lansade et al., 2020a; 2020b). Since the mares were tied during the test, their behaviours were quite limited. However, they were free to vocalise but the vocalisations were scarce. So, there is no evidence that the mares have attracted their foals.

Since the foals were weaned several months ago, both mares (their dam and the familiar mare) may have provided equivalent social support in the unfamiliar context of the test (transient separation from their peers). However, in this context, the foals preferentially approached their dam, sniffed her more and tended to look at her more frequently than the familiar mare. Similar preferences of pre- and postweaning foals for their dam over a familiar mare were observed by Henry et al. (2012, 2020). They found that before weaning, the foals remained within close proximity of their dams 50–92% of the time (Henry et al., 2012). A study on spontaneous weaning in Icelandic ponies showed that mares and their foals were nearest neighbours for the majority of the time before and after weaning (Henry et al., 2020). This finding is in line with other studies and practical observations showing that during natural weaning, the mare-foal bond lasts beyond the birth of a new sibling (Crowell-Davies and Weeks, 2005; Feh, 2005). We note that although the first approach, as well as the number of sniffs, differed between the two mares, the duration in the contact zone did not differ. The size of the contact zone may have been too large to reveal any differences and may be modified in further studies. This difference may also have been more pronounced if the other mare was unfamiliar rather than familiar. The fact that foals still show a preference for their dam, even after several months of separation, calls into question the practice of artificial weaning, which is generally performed at the age of 5–7 months and is known to be stressful (Weary et al., 2008; Mach et al., 2017; Henry et al. 2020). Thus, foals prematurely deprived of the social support of their mothers and the adult members of their familiar group, notwithstanding their purported adaptation to the new situation (living in same-sex and same-age groups without adult companions), may still be in need of the support of such an age-diversified familiar group (Henry et al. 2012).

Fillies more strongly prefer their dam but also exhibit a greater preference for the familiar mares than colts

Fillies also demonstrated a greater preference for both mares than colts did, in terms of looking, sniffing, and spending time in proximity. They also differentiated between their dam and the familiar mare by exhibiting shorter bouts of snapping towards their dam than the familiar mare (Crowell-Davis et al., 1985). These results are in line with Gaudin et al. (2015), who found that preweaning female lambs exhibited stronger preference for their dam compared to a familiar ewe in a similar free-choice test. These authors proposed that sex segregation, i.e., the tendency of ungulates to stay in sex-specific social groups outside the breeding season, is rooted in infancy and strengthens during development. Although some studies have shown that mares favour colts in terms of allowing longer suckling bouts or exhibiting more affiliative behaviour (Heitor and Vicente, 2008; Stanley and Shultz, 2012), other observations have not confirmed this preference (Crowell-Davis, 1986; Barber and Crowell-Davis, 1994). However, even if dams favour colts, fillies seemed inclined to stay closer to their dam. Our results are in line with Heitor and Vicente (2008), who found that during the dependency period (1st month of life), fillies are closer to their dam than colts. While colts have been observed to groom with other yearlings, fillies prefer to groom with their dams (Rho et al., 2007). Additionally, Duncan

et al. (1996) observed that fillies preferred to direct affiliative behaviour towards their dams at the time of weaning.

Although horses do not segregate into same-sex groups after the breeding season (Boyd and Keiper, 2005; Feh, 2005; Bowyer et al., 2020), young stallions form same-sex bachelor groups. This tendency may explain colts' reduced interest in affiliating with their dams in our preference test. In contrast, although young, migrating mares may change social groups, they do not exhibit spatial dispersion, thus showing philopatric tendencies (Linklater and Cameron, 2009). Young mares have also been found to move to groups with familiar females, mostly with those that dispersed in previous seasons from their natal harems (Monard and Duncan, 1996). Thus, stronger mare-foal attachments may favour future affiliations with other females (Gaudin et al., 2015), constituting the core of social familial groups in horses (Boyd and Keiper, 2005; Stanley et al., 2018; Jaworska et al., 2020).

Conclusions

Overall, our study highlights that one-year-old artificially weaned foals remember and prefer their dams even after a 5 month separation. This finding suggests that horses form strong memories of conspecifics, able to recognise them even after a long period of separation. This finding also indicates that the mare-foal bond remains strong even when foals reach one year of age and when they have not seen their dam for a long time. This study also revealed an effect of sex of the foals. Fillies exhibited a stronger preference for their dam but also for the familiar mares than colts. This suggests that fillies have an even greater need for social support. Overall, our study indicates that young individuals who are prematurely deprived of their mothers need social support at one year old and calls into question the practice of artificial weaning at 5–7 months of age.

Supplementary materials

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.animal.2022.100636>.

Ethics approval

The protocol was approved by the Ethics Committee of Val de Loire 20161124174134. Animal care complied with the French and European guidelines for the accommodation and care of animals used for scientific purposes (European Union Directive 2010/63/EU) and was performed under authorisation and with the supervision of official veterinary services (agreement number F371752 delivered to the UEPAO animal facility by the veterinary service of the Département d'Indre et Loire, France).

Data and model availability statement

None of the data were deposited in an official repository but are available upon request.

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Author contributions

Conceptualisation, L.L. and F.L.; methodology, L.L., F.L. and A.G.-B.; software, A.G.-B.; formal analysis, L.L. and A.G.-B.; investigation, L.L., F.L. C. P. and F.R.; resources, L.L.; data curation, L.L.; writing—original draft preparation, A.G.-B., L.L. and F.L.; writing—review and editing, A.G.-B., L.L., and F.L.; visualisation, A.G.-B.; supervision, L.L.; project administration, L.L. All authors have read and agreed to the published version of the manuscript.

Declaration of interests

None.

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