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Relation between fearfulness level and maternal behaviour in Arab mares

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The study aimed at testing the hypothesis that mares' fearfulness is a potential factor differentiating the level of their maternal behaviour. Twenty purebred Arab mares were used. In part one of the study fearfulness of mares without foals was assessed by their behavioural reactivity and heart rate in response to rotating black-white squares (fearfulness test). On that basis the mares were classified into three groups: (1) fearless, n=8, (2) medium-fearful, n=7 and (3) fearful, n=5. In part two, maternal behaviour of mares was assessed in two sessions of separation test: 3 min dam's partial separation from foal (mare out of box, but with visual, auditory and olfactory contact with foal) and 3 min full separation (mare out of stable, no contact with foal). Locomotor activity, vocalization and defecation frequency as well as heart rate in mares were recorded during both separation tests. The results showed a relation between mares' fearfulness and their maternal behaviour. Fearless dams vocalized more (10.63±5.32, mean rank 13.00, P<0.05) and showed higher heart rate (138.00±22.66 beats/min, mean rank 15.40, P<0.05) when totally separated from their foals, compared to fearful dams (4.00±3.74 calls, mean rank 6.10; 116.00±20.32 beats/min, mean rank 7.23, P<0.05). Thus, fearless dams generally displayed higher maternal ability which is vitally important in successful breeding. In conclusion, selecting for low fearfulness ensures appropriate maternal behaviour of the brood mares.

KEY WORDS: fearfulness / heart rate / mare / maternal behaviour / vocalization

Studies of maternal behaviour traits are becoming increasingly important to improve animal productivity and welfare in many species. The behaviour of the dam has a strong influence on the survival and growth of offspring during the preweaning

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period [Grandinson 2005]. It is known that in the practice of breeding work on stud farms, the mares, showing extreme behavioural patterns such as aggression towards the offspring or foal rejection, are rarely allowed to breed again. Thus, the traits indicating maternal success should be considered in selection programmes to ensure a high survival, growth and development rates in the offspring. However, behavioural traits of the dam are often difficult to identify, record and use on a large scale (require observations over a long period of time).

In terms of the dam-offspring relationship, the horse is often described as a follower species and it is thought that the mare becomes bonded to the foal shortly after parturition [Fraser 1985, Waran *et al*. 2008]. Mare and foal bonding behaviour is stimulated by visual, auditory and olfactory factors and these kinds of perception are important in dam-foal recognition [Wolski *et al*. 1980]. Breeding practice at many horse farms often includes withdrawal of mares for mating or for the other farm procedures. On some farms, the foal is left alone in its stall for a short time, while on the others the foal accompanies the dam to the covering shed. In testing the dam-foal bond, Houpt [2002] found that both mare and foal demonstrated higher motor activity and vocalization even when the separation was short. Moons *et al*. [2005] found the increase in frequency of mares' contact behaviour (grooming and watching the foals) following short-term isolation. This procedure may be successful in testing the strength of dam's behaviour for maternal care evaluation. Juarbe-Diaz *et al*. [1998] reported that poor maternal behaviour resulting in foal rejection was more frequent in Arab than in the other horse breeds.

The expression of maternal behaviour in the dam may be affected by several factors such as breed, temperament or nutrition during the pregnancy [Grandinson 2005, Dwyer 2008]. Equine temperament may influence the horse manageability and usefulness for specific tasks [McCall *et al.* 2006]. Generally, studies on horse's temperament have made use of several methods that can be classified into two main types: (1) analysing a horse's reactivity and (2) using a questionnaire survey completed by caretakers or handlers who are familiar with the horses [Momozawa *et al*. 2003]. Reactivity in animals includes both behavioural (motor activity, vocalizations, defecations, etc.) and physiological (heart rate, blood pressure, hormones, respiration rate, etc.) indicators – McCall *et al.* [2006]. Elevated heart rate occurs during exposition of horses to novel objects or under social isolation [Jezierski and Górecka 2000, Sapuła *et al*. 2006, Sołtys 2006, Sapuła 2008]. Fearfulness tests have been used for temperament evaluation in horses [Momozawa *et al*. 2003, McCall *et al*. 2006, Lansade *et al*. 2008], cattle [Welp *et al*. 2004] and sheep [Murphy *et al*. 1998]. Fearfulness has been defined as a tendency to react with fear which can be seen as short term reaction to stimulations provoking aversion [Jones 1987], as a propensity to experience fear or anxiety [Wohlt *et al*. 1994] or as a predisposition to react in a similar way to various fear-eliciting events [Lansade *et al*. 2008]. It can be inferred from the behavioural states expressed by the individual in potentially frightening situations [Lansade *et al*. 2008]. According to Forkman *et al*. [2007], fear-related reactions are characterized by

physiological and behavioural reactions preparing the animal to deal with the danger. In the present study, fearfulness test [Budzyński 1984] was used to assess the level of mares' fearfulness. Earlier findings of Houpt [2002], Grandinson [2005] and Dwyer [2008] on dam's care showed that maternal vocalizations and motor activity are useful as indicators of proper maternal behaviour. The present study involves the recording of behavioural parameters such as vocalizations number, defecation frequency and locomotor activity when the dam was separated from the foal to evaluate the intensity of maternal behaviour.

Budzyński *et al*. [1995] reported a relationship between fearfulness and mares' reproductive traits. Fearless mares showed a significantly higher fertility and foaling rate and were used for breeding as the stud dams for longer period compared to fearful mares. By studying mares' behaviour more knowledge on factors affecting maternal ability crucial to survival of the young can be gathered. The aim of this study was to test the hypothesis that mares' fearfulness is a potential factor differentiating the level of maternal behaviour. Evaluated was which indicator(s) is (are) the most useful to assess maternal behaviour.

Material and methods

Animals

The study included 20 purebred Arab mares. A number of experimental animals was limited by the need to select mares with similar traits (age, number of reared foals and number of days that passed from parturition) – Table 1. The mares with their foals were housed in boxes and fed with oat grain twice daily with free access to hay and water. The dams with their offspring were allowed to graze for 7 hours (8:00-12:00 and 14:00-17:00) daily. Since first weeks of offspring's life the pairs mare-foal were submitted to short-term separation events in order to take mares for mating or to follow the other farm procedures.

Table 1. Description of experimental mares (means±SD)

Variable	Fearless mares (16-18 points) n=8	Medium-fearful mares (13-15 points) n=7	Fearful mares (9-12 points) n=5
Age (years)	6.88±1.81	8.29±3.55	8.00±1.87
Number of reared foals	2.63±1.69	3.57±2.70	3.80±1.79
Number of days from parturition	111.38±43.52	115.43±39.47	102.20±47.71

Experimental

In part one of the study (in autumn – October), fearfulness in pregnant mares was assessed (on the mean of day 176.70±39.06 of pregnancy) by their behavioural reactivity and heart rate (HR) in response to rotating black-and-white squares. In part

two (in spring – June), maternal behaviour of mares having foals (mean age of foals was 110.50±40.14 days) was evaluated by mare-foal separation tests.

Fearfulness level was assessed with fearfulness test [Budzyński 1984] conducted at the arena next to the stable. In the present work, fearfulness was considered as a behavioural and physiological reactivity expressed by specified manifestations of locomotor behaviour (behavioural patterns according to 10-point scale described below) and HR changes during potentially fear-eliciting situation when a horse was exposed to the moving novel object. The tested mare was confronted with a moving novel visual stimulus through walking by a stableman along a path between two rotating (40 rotations/min) black-and-white squares (1x1 m). The visual devices located 5 m apart were hidden by vertical boards to prevent seeing them by a horse before testing procedure. Horse behaviour was assessed by one observer using 10-point scale [Budzyński 1984] according to decreasing reactivity of animal as follows:

1-2 points = fearful horse, jumping and/or moving back, willing to escape and not passing by the rotating squares despite encouragement;
3-4 points = skittish horse, jumping and/or moving back, passing after several trials of encouragement;
5-6 points = shy horse, stopping and/or moving back, passing with hesitation;
7-8 points = watchful horse, slowing and/or walking sideways and/or looking at rotating squares, passing with interest towards the objects;
9-10 points = fearless horse, no change in pace, no interest towards the objects, passing without hesitation.

Every mare was scored twice (p1 – first point scoring the response to moving novel stimulus, p2 – second point scoring the response to moving novel stimulus). First time the mare was led next to the devices generating visual stimuli hidden by the boards and after reversing by a stableman, the mare again was led next to the devices generating the stimuli, this time not hidden by the boards. The first scored mare's passing by the rotating squares was directly followed by the second one. As a total score the sum of two scorings (points p1+p2) was considered. Based on the total score the mares were divided into three following groups related to the fearfulness intensity:

1 – fearless (16-18 points, n=8) passing easily, with a little interest or without response towards rotating squares,
2 – medium-fearful (13-15 points, n=7) passing with some hesitation, and
3 – fearful (9-12 points, n=5) passing after encouragement.

Like in the other *herbivora* endangered by predators, the visual system of the horse is its crucial surviving trait [Saslow 2002].

Level of maternal behaviour was assessed by separation test. The test was conducted once in every mare-foal pair and arranged in two sessions each lasting 3 min. **Session 1** involved dam's separation from the foal (with visual, auditory and olfactory contact between them) – a mare led out of the box (1m from box's door), foal staying alone in the box, and **session 2** involving dam's separation from the foal

(no contact between them) – a mare led out of the stable (for distance 15-20 m), a foal staying alone in the box. The following behavioural indicators: mare's vocalization (neighs) and defecation frequency, locomotor response (on 1-3 points scale, according to decreasing reactivity shown by the dam, where 1 point – excitable, continual and vigorous movement of head and legs; 2 points – medium excitment, occasionally vigorous movement of head and legs; 3 points – very quite, motionless behaviour), were recorded by direct observation during two test sessions.

Prior to both tests (fearfulness test and mare-foal separation test, session 1 and 2), the HR monitor and receiver (Polar 810i, OY ELECTRO, Finland) were attached to the mare in the box. For all the mares, HR was continuously recorded in 5 sec intervals during both tests.

All examined variables had no normal distribution and were subjected to non-parametric analyses. Wilcoxon pair-matched test, U Mann-Whitney test and Kruskall-Wallis test were applied (Stanisz 2005) and Spearman correlations were estimated (STATISTICA 6.0). Correlation coefficients were considered as significant when $P<0.05$ and tending to be significant when $P<0.1$. This is in accordance with the studies on horses by Visser *et al*. [2001], Lansade *et al*. [2008], and Lansade and Simon [2010] where significance-tending correlations ($P<0.1$) were considered to identify trends of some behavioural traits.

Results and discussion

The present results support the hypothesis on the relationship between the mares' fearfulness and intensity of maternal behaviour as evaluated by mare-foal separation test. It was shown that fearless dams when separated from their foals vocalized more and showed higher heart rate, compared to fearful dams.

The results of fearfulness test are shown in Table 2. The HR of fearless occurred lower compared to fearful mares (mean rank 6.88 *vs*. 15.30, $P<0.05$). The HR was negatively correlated with total score of behaviour in fearfulness test (R_s = -0.55, $P<0.02$) which validated categorization of horses as per fearfulness.

Table 2. Means (±SD) and mean ranks (bolded) of behavioural and HR responses to visual stimulus in mares during fearfulness test as related to fearfulness level

Indicator	Fearless mares (16-18 points) n=8		Medium-fearful mares (13-15 points) n=7		Fearful mares (9-12 points) n=5	
p1 (points)	8.50±0.53	**16.25**^{AB}	7.00±0.58	**9.21**^{AC}	5.00±0.77	**3.10**^{BC}
p2 (points)	8.38±0.52	**16.50**^{AB}	7.00±0.00	**8.50**^{Aa}	5.80±0.84	**3.70**^{Ba}
p1 + p2 (points)	16.88±0.83	**16.50**^{AB}	14.00±0.58	**9.00**^{AC}	10.80±1.30	**3.00**^{BC}
HR (beats/min)	114.25±27.57	**6.88**^{b}	133.86±32.50	**11.21**	173.40±42.25	**15.30**^{b}

^{AC}Means within rows bearing the same superscript letter are significantly different at: small letters – $P<0.05$, capitals – $P<0.01$.

Vocalization is a major feature of animal reactivity and many calls are incorporated into responses concerned with threat and alarm [Fraser 1985]. The frequency of vocalizations is indicative of distress and poor welfare that was shown on cattle [Budzynska and Weary 2008, Jasper *et al*. 2008], horses [Houpt 2002, Moons *et al*. 2005] and pigs [Weary *et al*. 1997]. Vocalization also plays an important part in the maintenance of communication between bonded individuals. Dam's vocal signals are significant components of maternal behaviour, especially related to responsiveness and attentiveness towards the offspring [Fraser 1985, Grandinson 2005]. In the present study, during session 1, there were no significant differences in vocal activity of mares in relation to their fearfulness (Tab. 3). During session 2, fearless mares vocalized more compared to those classified as fearful (calls' mean rank 13.00 *vs*. 6.10 during 3 min separation, $P<0.05$). Mean number of vocalizations/min during full separation from foals was also significantly higher in fearless than in fearful mares (Tab. 3). A positive trend ($R_s = 0.34$, $P<0.1$) was shown between total score in fearfulness test and vocal activity in mare-foal separation test: the more fearless mares were, the more they tended to vocalize. Moreover, when fully separated from their foals (session 2), fearless and medium-fearful dams showed significantly higher vocalization rate (during particular minutes of separation as well as per 3 min and vocalization no./min) than during session 1, in which dams were still in visual, auditory and olfactory contact with their young (Tab. 3). Thus, it can be stated that vocalization frequency in dams during foals separation is a good indicator of maternal behaviour significantly related to their fearfulness.

The obtained results are in accordance with relations found in sheep where calm ewes showed better maternal ability by higher bleating frequency towards their lambs [Murphy *et al*. 1998]. Selection for desirable temperament traits is becoming increasingly important, particularly for high mothering ablility in pigs [Grandinson 2005]. Positive relationship between fearlessness and maternal ability has been reported in pigs by Janczak *et al.* [2003] who measured fear-related behaviour using voluntary human approach test. Gilts showing low levels of fear had a better maternal ability when they later became dams. Lower total mortality in their litters during the first three weeks post-farrowing was observed as well as higher responsiveness when crushing a piglet. Le Neindre *et al.* [2002] found a relation between response to humans and maternal behaviour of Limousine cows. Heifers reacting positively to human handling spent more time licking their calves after birth.

Defecation – the other behavioural manifestation measurable during the mare-foal separation test – was too rarely expressed to find any significant difference between fearfulness groups. However, concerning locomotor activity (Tab. 4), fearless and medium-fearful mares showed lower point score (higher locomotor response) during total separation from foals (in session 2) than in session 1 of the test. Moreover, a trend ($R_s = - 0.39$, $P<0.1$) between locomotor activity and vocalizations was shown: the more restless mares were, the more they tended to vocalize. Thus, defecation rate occurred not useful enough to assess maternal behaviour in this study, whereas

locomotor activity seems to be better indicator of maternal behaviour, significantly related to mares' fearfulness. The pony mares showed an increase in locomotor activity and vocalization rate when separated from their foals [Houpt 2002]. The relationship between vocalizing and locomotion activity has also been observed in sows [Marchant *et al*. 2001] and growing pigs [Fraser 1974].

Table 3. Means (±SD) and mean ranks (values M-W according to the Mann-Whitney test, values W according to the Wilcoxon test) of vocalizations number in mares during separation test as related to their fearfulness level

Vocaliz. Number	Fearless mares (n=8)			Medium-fearful mares (n=7)			Fearful mares (n=5)		
	mean±SD	M-W	W	mean ±SD	M-W	W	mean ±SD	M-W	W
Session 1 (contact with foal)									
minute 1	0.88±2.10	9.13	1.06^a	1.14±1.68	11.00	1.21	1.00±1.00	12.00	1.20
minute 2	1.00±1.85	10.75	1.13	0.71±1.50	10.79	1.14^e	0.20±0.45	9.70	1.20
minute 3	0.38±0.74	10.81	1.13^b	0.86±2.27	10.14	1.21	0.40±0.89	10.50	1.30
sum per 3 min	2.25±4.30	9.94	1.00^c	2.71±4.89	10.64	1.00^f	1.60±1.82	11.20	1.20
vocaliz. no./min	0.75±1.43	9.94	1.00^d	0.90±1.63	10.64	1.00^g	0.53±0.61	11.20	1.20
Session 2 (no contact with foal)									
minute 1	4.00±2.14	13.06	1.94^a	2.86±3.18	9.79	1.79	1.60±1.52	7.40	1.80
minute 2	3.38±2.00	12.06	1.88	3.29±3.09	11.21	1.86^e	1.40±1.67	7.00	1.80
minute 3	3.25±2.55	12.19	1.88^b	2.71±2.69	11.14	1.79	1.00±1.22	6.90	1.70
sum per 3 min	10.63±5.32	13.00^a	2.00^c	8.86±8.34	10.79	2.00^f	4.00±3.74	6.10^a	1.80
vocaliz. no./min	3.54±1.77	13.00^b	2.00^d	2.95±2.78	10.79	2.00^g	1.33±1.25	6.10^b	1.80

$^{a-b}$Within rows M-W values bearing the same superscript letters are significantly different at P<0.05.
$^{a-g}$Within each W column values bearing the same superscript letters are significantly different at P<0.05.

Table 4. Means (±SD) and mean ranks (bolded) of motor activity in mares during separation test as related to their fearfulness level

Motor activity	Fearless mares n=8	Medium-fearful mares n=7	Fearful mares n=5
Session 1 (contact with foal)			
points/3min	3.00±0.00 **1.88***	3.00±0.00 **1.93***	3.00±0.00 **1.80**
Session 2 (no contact with foal)			
points/3min	2.00±0.76 **1.13***	1.71±0.76 **1.07***	2.40±0.55 **1.20**

*Within columns differences between sessions are significant at P = 0.03.

When fully separated from their foals (session 2), fearless, medium-fearful and fearful dams showed significantly higher HR than during session 1, in which they were still in visual, auditory and olfactory contact with their young (Tab. 5). During fearfulness test, mares classified as fearless on the basis of their behaviour, showed the lowest HR level (Tab. 2). In contrast to this, during separation test (Tab. 5), and especially in session 2, HR was higher in fearless than in fearful dams (mean rank 15.40 *vs.* 7.23 in the second minute, P<0.05). Moreover, HR in this test was positively

Table 5. Means (±SD) and mean ranks (values M-W according to the Mann-Whitney test, values W according to the Wilcoxon test) of heart rate (beats/min) in mares during separation test as related to their fearfulness level

Heart rate	Fearless mares (n=8)			Medium-fearful mares (n=7)			Fearful mares (n=5)		
	mean ±SD	M-W	W	mean ±SD	M-W	W	mean ±SD	M-W	W
Session 1 (contact with foal)									
minute 1	80.49±16.06	10.05	1.00^{a}	78.73 ±16.02	10.29	1.14	78.85±19.51	10.80	1.00^{h}
minute 2	68.43±14.22	8.63	1.00^{b}	80.82±23.10	12.57	1.00^{e}	75.48±26.93	10.60	1.20
minute 3	61.11±14.02	10.63	1.00^{c}	65.58±25.67	10.14	1.00^{f}	66.65±20.31	10.80	1.00^{i}
sum per 3 min	69.40±13.05	9.63	1.00^{d}	75.04±20.64	11.14	1.00^{g}	73.66±19.37	11.00	1.00^{j}
Session 2 (no contact with foal)									
minute 1	118.60±16.63	11.25	2.00^{a}	108.70±33.37	10.86	1.86	110.30±14.59	8.80	2.00^{h}
minute 2	138.00±22.66	15.40^{a}	2.00^{b}	135.50±29.95	10.85	2.00^{e}	116.00±20.32	7.23^{a}	1.80
minute 3	130.10±27.76	15.30^{b}	2.00^{c}	128.00±28.60	10.73	2.00^{f}	102.40±15.23	7.13^{b}	2.00^{i}
sum per 3 min	128.51±19.88	12.00	2.00^{d}	124.08±29.08	11.57	2.00^{g}	109.56±14.81	6.60	2.00^{j}

$^{a-b}$Within rows M-W values bearing the same superscript letters differ significantly at P<0.05.
$^{a-j}$Within each W column values bearing the same superscript letters are significantly different at P<0.05.

correlated with mares' vocal activity (R_s = 0.68, P<0.01). Elevated activation of sympathetic system expressed by higher HR and by increased vocal activity in fearless mares may be explained by good maternal attentiveness and responsiveness towards the foal. Pajor *et al*. [1999] reported that sows showing strong maternal qualities made more calls, indicating a higher level of distress induced by isolation them from their offspring. In another study on pigs [Marchant *et al.* 2001], the positive correlation between vocalizing frequency and HR was identified.

In conclusion, it can be stated that there is a significant relationship between mares' fearfulness level assessed by fearfulness test and their maternal behaviour evaluated by mare-foal separation test. Thus, mares selected for low fearfulness may generally display improved maternal ability that is vitally important in successful breeding.

This study shows that the level of maternal behaviour in mares can be assessed most successfully by measuring the vocalization frequency and heart rate. The results of earlier study by Budzyński *et al*. [1995] reporting the favourable reproductive traits (high fertility and foaling rate) occurring in fearless mares as well as the present findings show that fearfulness is an important factor that can have a large impact on maternal as well as breeding success. Finally, selecting horses for low fearfulness ensures appropriate maternal behaviour of the brood mares.

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