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Dissertation

Mare-facilitated exposure to humans reduces fear responses in foals

Ruth Dunford Patten

Pelotas, 2020

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Mare-facilitated exposure to humans reduces fear responses in foals

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“This old man had once told me that he left school when he was twelve, whereas I had spent most of the twenty-four years in my life in study. Yet when I looked back on the last hour or so I could come to only one conclusion. I'd had more of books, but he had more of learning”. James Herriot

Abstract

PATTEN, Ruth Dunford. **Mare-facilitated exposure to humans influences fear responses in foals.** 2020. 76p. Dissertation (Master's degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2020.

Generally without siblings or a present paternal figure, the mare is the primary social model for the domestic foal. Recent research on early training and handling has shown a strong correlation of reduced fear responses in the foal when the mare is handled in the foals' presence, showing a promising potential for maternal social transmission of habituation in horses. The objective of the current study was to test the persistence of mare-facilitated training when implemented under two differing husbandry practices. A total of 36 mares and their respective foals were included in this study, from 3 Thoroughbred breeding farms in São Jose dos Pinhais, Brazil. Of these, 15 mares were included as the control group and 21 mares as the experimental group, which underwent daily soft brushing sessions over the first 5 days post foaling as a form of indirect positive interaction between the human and the foal. Differences between husbandry practices were also evaluated. All foals were submitted to evaluations during three different periods: day 15 (D15), day 30 (D30-D34) and at six months post-partum. An increase in behaviours associated with rest were observed in the mares who underwent brushing. All experimental foals showed a significant increase in time spent on the same side as the experimenter, an increased number of deliberate approaches to the experimenter and interactions (sniffing, licking and nibbling), and an increase in the amount of time spent within 0.5 m of the experimenter during the initial treatment. Experimental foals showed significant decrease in fear responses in the presence of the experimenter (increased approximations, interactions) during the stationary human tests, however when contact was sought, a greater effect of husbandry between groups was observed at D30-34 and at six months. The differences observed in foal behaviour related to husbandry shows that the daily repetition of handling behaviour had a more significant influence on the foal's long-term behaviour when approached by a human, than from the brushing treatment sessions which occurred during the first 5 days post-foaling. The incorporation of indirect foal training of this form was seen as a practical way to integrate learning theory with a relatively low investment (both in terms of costs and time) into Thoroughbred breeding farms in southern Brazil.

Keywords: fear; foal; early training; maternal influence; human-animal relationship

Resumo

PATTEN, Ruth Dunford. **Exposição facilitada por égua reduz as respostas ao medo em potros em relação aos humanos.** 2020. 76f. Dissertação (Mestrado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2020.

A égua é o principal modelo social para o potro doméstico, considerando a ausências de irmãos ou de uma figura paterna. Estudos recentes em equinos tem demonstrado uma forte correlação entre reduzidas respostas ao medo associada ao manejo precoce da mãe na presença dos potros. Assim, o objetivo do presente estudo foi avaliar a persistência da aproximação e interação positiva com éguas quando implementado sob duas práticas diferentes de manejo. Foram utilizadas 36 éguas e seus respetivos potros em três criatórios de equinos Puro-sangue inglês na região de São José dos Pinhais – PR. Do total, 15 foram consideradas Grupo Controle e 21 éguas do Grupo Experimental, as quais foram submetidas a sessões diárias de escovação nos primeiros 5 dias após o parto como forma de interação positiva indireta humano-potro. Foi também avaliado o efeito da diferença de manejo entre os criatórios. Todos os potros foram submetidos a avaliações subsequentes em três momentos: 15 dias (D15); 30 dias (D30-D34) e aos seis meses de idade. Observou-se um incremento dos comportamentos relacionados ao descanso nas éguas submetidas ao escovação. Todos os potros mostraram um aumento significativo na proporção do tempo no mesmo lado que o pesquisador, um número aumentado de interações (cheirar, lamber e mordiscar) e tempo que o potro permaneceu próximo ao experimentador (≤ 0,5 m) durante o tratamento inicial nos dias 1-5. Potros do Grupo Experimental mostraram uma diminuição significativa nas respostas de medo na presença do experimentador (aumento de aproximações, interações) durante os testes em humanos estacionários, porém quando o contato humano-potro foi estimulado, um maior efeito do manejo entre os criatórios foi observado na avaliação de 30 dias e seis meses de idade. As diferenças observadas no comportamento do potro relacionadas à criação mostram que a repetição diária do manejo teve uma influência mais significativa no comportamento a longo prazo do potro do que nas sessões de tratamento de escovação abordadas por humanos nos primeiros 5 dias de pós-parto. Contudo a incorporação do treinamento indireto das éguas sobre potros foi considerada uma maneira prática de integrar a teoria da aprendizagem com um investimento relativamente baixo, tanto em termos de custos quanto de tempo, em criatórios de equinos puro-sangue inglês no sul do Brasil.

Palavras-chave: medo; potro; treinamento inicial; influência maternal; relacionamento homem-cavalo

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Abbreviations List

D	Days
ha	Hectare
m	Meter
sec	Second

Symbols List

<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to

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1 Introduction

In horses, as in all prey animals, fear has an important function (HALL et al., 2018), and it is potentially for this reason that fear continues to persist in the domestic horse (*Equus caballus*). Fearful animals are known to be more reactive, slower to learn new tasks (WOLFF; HAUSBERGER, 1996) and more likely to cause accidents than calmer animals (BOIVIN et al., 2003; KEELING et al., 2009; KING; WILLS; RANDLE; 2019; WAIBLINGER et al., 2006). Costs associated with fearful and reactive horses account for severe financial losses, as well as premature losses of animals and termination of careers due to animal injuries, as well as the associated endangerment to their handlers (HALL et al., 2018; LANSADE et al., 2007; MCLEAN; CHRISTENSEN, 2017; WATSON; MCDONNELL, 2018). Given the innate propensity towards fear-related behaviours (onset of fear in foals from 2 hours post-partum: WARING, 2003), it is therefore crucial that approaches to equine training and foal training in specific, incorporate methodologies that can alter the development of these reactions, in a way that will last beyond the early period of life. For this reason, studies that explore early training measures in foals that can decrease such behaviours is warranted.

Learning theory refers to an area of research which seeks to identify the principles that explain how animals learn (MCGREEVY et al., 2018). Although the concept of learning theory has gained attention in recent years (DOHERTY; MCGREEVY; PEARSON, 2017; WARREN-SMITH; MCGREEVY, 2008; WENTWORTH-STANLEY, 2008), the set of principles it represents have been utilized in domestic animal training for years, being fundamentally based on a knowledge of behaviour (DOHERTY; MCGREEVY; PEARSON, 2017). Even with the rise in academic interest, the application of such equine behavioural knowledge in a practical way remains under-emphasized (DOHERTY; MCGREEVY; PEARSON, 2017). The current goal therefore, is to implement what we know about the acquisition of behaviour (though modes of learning), with known training techniques, in a way that is profitable both to man and animal. By applying methodologies of learning theory into training, we are not attempting to introduce a novel manner of learning on an animal during this initial phase, but rather *we are learning* how to train in a manner that is innately familiar to the animal, and theoretically more successful.

Studies which have used the mare's influence over the foal to reduce fear can help explain the plasticity of innate fear reactions, and as such, additional study is warranted to investigate this area further (RØRVANG et al., 2018).

The general aim of this study was to evaluate the persistence of mare-facilitated foal training when implemented under active farm conditions, with the specific goal of investigating the relationship of such training with differing husbandry systems.

We hypothesize that foals of mares that received a positive experience (soft brushing) in the foal's presence but without direct contact will have (i) lower levels of known fear responses (flight, immobility), and increased amounts of behaviours associated with decreased fear (approaches and interactions with human, increased time spent within 0.5 m of human), and (ii) quicker social learning of a novel task, in comparison with the control foals whose dams received no experimental treatment.

2 Literature Review

The period occupying the foal's life prior to weaning involves a time frame of intense learning on the part of the foal. Dam influences, husbandry influences, environmental influences, foal health, and many more factors all contribute in shaping the foal during this initial phase. While many studies have sought to investigate the relationship between timings, procedures and frequencies of directly handling the foal, few have investigated the role of the mare in indirect training of the foal and even less when implemented under varying farm settings without the use of restraint. In contrast to direct foal handling, indirect early foal training is an important area of research to investigate, since it does not risk compromising the establishment of the mare-foal bond (HENRY et al., 2005; WARING, 2003). Indirect foal training is also unique in that it does not interfere in the natural ontogeny of behaviours in the foal, but incorporates learning theory into the training by permitting the foal to learn about humans (to associate humans with non-fear responses) through the mare, as with all other stimuli during the early period. The absence of restraint, integral to indirect training, allows for perhaps a truer representation of the foal's willingness to interact, as the observed behaviours are voluntary. Such experiments have been requested in the literature (LIGOUT; BOUISSOU; BOIVIN, 2008) in order to further explore both the sensitive early period of foals, and the effects of their non-constrained handling.

In horses, the critical training period in foals can either refer to the sensitive time frame occurring in the first few days of life (HENRY et al., 2005; LANSADE et al., 2005; MAL; MCCALL, 1996; MILLER, 1991; SØNDERGAARD; JAGO, 2010; WARING, 2003; WILLIAMS et al., 2002), or to various later periods occurring at some time prior to (HENRY et al., 2007; JEZIERSKI et al., 1999) or around the time of weaning (HEIRD et al., 1986; LANSADE et al., 2004; LIGOUT; BOUISSOU; BOIVIN, 2008), varying also in the frequencies and durations of the handling (SØNDERGAARD; JAGO, 2010). Nonetheless, the early period is ideal for training as it is characterized as being a time of rapid brain development wherein neural pathways are being refined (GUDSNUK; CHAMPAGNE, 2011). Research has shown that there is a certain window of opportunity for learning during this early period which can have considerable long-term effects (GUDSNUK; CHAMPAGNE, 2011; HENRY et al., 2009; WARING, 2003), making it an optimal time to shape social and performance skills (PEREIRA-

FIGEIREDO et al., 2017). The specific time frame to use therefore appears to depend more on the training method used and its schedule of reinforcement.

Early foal training can take on a number of forms, and depending on the goal, can incorporate either direct (imprinting or a derivative, gentling, teat training, handling), or indirect training of the neonate (using conspecifics or parental models) to influence behaviour. Direct early foal handling has been used as a method of desensitizing the foal and improving human-foal bonds (SIMPSON, 2002; MILLER, 1991), reducing reactive, fear-based responses (LIGOUT; BOUISSOU; BOIVIN, 2008; WARING, 2003), resulting in better and more uninhibited learning and training in the foal as a result (HEIRD et al., 1986; SIMPSON, 2002). The issue with direct training of the foal during the neonate period however, is two-fold: its implementation is costly (both in terms of time investment and in the physical effort in restraining), and its effectiveness as a whole in the literature has proven inconsistent. Depending on the specifications of the study, it appears that direct early foal handling can obtain behavioural effects in the foal which can either be positive or "desirable" (increased foal approaches: SIMPSON, 2002; decreased defensive behaviours when handling feet: SPIER at al., 2004), negative or "undesirable" (HAUSBERGER et al., 2007; human contact avoidance: HENRY et al., 2006; insecure maternal attachment: HENRY et al., 2009), have only short-term effects (LANSADE et al., 2005), long-term effects (SCHMIDEK et al., 2018; SIMPSON, 2002), or have no observed beneficial effect at all (MAL et al., 1994; WILLIAMS et al., 2002). Considering the high time investment per animal during this phase, and the associated costs when implemented on a large scale (as is the custom in horse breeding schemes), the feasibility of such treatment remains controversial. A third and perhaps even greater issue of direct neonatal training of the foal, is the quite unintentional overlooking of the natural process of behaviour formation that exists between the mare and foal, and the great potential therein. As mentioned in Henry et al. (2009) "It may be more fruitful to benefit from a well-established mother–young bond than to try to be 'part of the bonding'." With this in mind, the use of training which incorporates components of learning theory during the early phase can lead to significant benefits.

Social transmission is an umbrella term which refers to social facilitation, stimulus enhancement and local enhancement (RØRVANG et al., 2018), and is the natural phenomenon which commonly occurs when transferring information between conspecifics, whereby the behaviour of one animal influences the motivation of the observer animal to enact a similar behaviour (RØRVANG et al., 2018). Such learning is not true learning, in the sense that a novel behaviour is not being reproduced, rather it is a stimulus to use behaviours already within the animal's own behavioural repertoire. Social transmission of behaviour largely has to do with individualistic benefit, with observer animals normally being influenced by conspecifics of relative dominance and familiarity (JONES et al., 2014; VEISSIER et al., 1998), or even age (MCGREEVY et al., 2018). Social facilitation is the mechanism by which herd animals are stimulated to graze and rest in synchrony (LIGOUT, 2010; NICOL, 1995), and there is evidence of transmission of information between individuals in frightening situations (MCGREEVY et al., 2018).

Early indirect foal training is an area of study which to date has had considerably less academic interest. Indirect early training is based on the principles of learning theory, whereby training is enabled via the modeling behaviour of a conspecific. Studies have shown that conspecific training, or "horizontal social facilitation" (CHRISTENSEN, 2016) with habituated conspecifics can aid in artificial nursing (in sheep: VEISSIER; STEFANOVA, 1993) and in habituation to a fear-eliciting stimulus (in calves: BOISSY; LE NEINDRE, 1990). In horses, behaviour modelling with conspecifics has seen to have an effect in improving social behaviours (BOURJADE et al., 2008), decreasing stress at weaning (HENRY et al., 2012) and decreasing fear responses in fear-stimulating circumstances (CHRISTENSEN et al., 2008; RØRVANG; AHRENDT; CHRISTENSEN, 2015; RØRVANG; CHRISTENSEN, 2018). Such influence can be of great value in that, if taught at a sufficient frequency and during an appropriate age window, can have long term effects (GUDSNUK; CHAMPAGNE, 2011).

Based on their constant association (CROWELL-DAVIS, 2005) and the comfort or security (WARING, 2003) she provides, the maternal bond is an extremely strong influencer on the behavioural acquisition of the offspring (CHAMPAGNE, 2011; MEANEY, 2001). Mothers can influence the behaviours of their offspring in a number of ways, including in terms of food choice (in moose: EDWARDS, 1976; in sheep: LYNCH et al. 1983, VEISSIER et al., 1998; in precocious fowl: WAUTERS, et al., 2002; in herbivores: PROVENZA; PFISTER; CHENEY, 1992; in horses: BOLZAN et al., 2019; MARINIER; ALEXANDER, 1995), mate preferences (FABRICIUS, 1991; KENDRICK et al., 2001; WARING, 2003), and in reducing fear-related responses in the offspring (in goats: RUIZ-MIRANDA; CALLARD, 1992; in precocious fowl: BERTIN;

RICHARD-YRIS, 2005; PERRÉ et al., 2002; in horses: CHRISTENSEN et al., 2008; CHRISTENSEN, 2016). Generally without siblings or a present paternal figure, the mare is the primary social model for the domestic foal (HENRY et al., 2005; HENRY et al., 2007; MATEO, 2014; RØRVANG et al., 2018), and as such recent early training has sought to test the influence of the mare over the behaviour of the foal, known as “vertical social facilitation” (CHRISTENSEN, 2016). In the study by Henry et al. (2005), the concept of using mares as role models for foal behaviour was tested in a group of mixed breed horses in France during the early foal period (first 5 days post-partum). They found a strong correlation of reduced fear responses in the foal (reduced flight, increased approximations to the experimenter, increases in foal trainability) with early training of the mare in the foals’ presence, with durability lasting up until 1 year, showing a promising potential for maternal social transmission of habituation (MCGREEVY at al., 2018). A benefit of such training is that it is of relatively low time investment (15 minutes per day for 5 days) and also has less emphasis on the experience of the trainer, being easily incorporated into active farm management, as the essence of the training is facilitated through the mare.

In the current study, Thoroughbred foals were chosen as it has been shown that early training can greatly impact the future success of foals within the racing industry (DOHERTY; MCGREEVY; PEARSON, 2017). Thoroughbreds as a breed are known for being tenser and more excitable than other saddle breeds (WILK et al., 2016), making them formidable athletes as racehorses, but can cause this breed to be overlooked by buyers procuring horses for recreational purposes (MCBRIDE; MILLS, 2012). Thoroughbred temperaments can also be associated with being sociable, inquisitive (WILK et al., 2016) and intractable (MCBRIDE; MILLS, 2012), however, it is these “reactionary” characteristics, together with acquired habits from the racing industry, that give the breed a reputation of being dangerous post-racing career (WILK, et al., 2016). Other studies have shown that thoroughbreds tended to have higher heart rates and defecation when facing new objects than Anglo-Arabians (MOMOZAWA et al. 2003), and scored higher than other breeds in terms of dominance, anxiety and excitably (WILK et al., 2016). The use of Thoroughbreds in this study is therefore an interesting component to further explore the influence of the mare-foal bond and the differences of breed and emotional state. Considering the potential for injury, damage and costs associated with fearful or reactive horses, and the wastage of such horses (DOHERTY; MCGREEVY; PEARSON, 2017), strategies which can reduce the reactivity of horses at a young age are of great value, and studies investigating their implementation in various farm settings are warranted. Reduced fear in the life of an equine has implications not only in terms of the animal and in its familiar routine, but also in the animals future handling and management challenges with veterinarians in a clinical setting, and in its performance in its athletic career (SØNDERGAARD; HALEKOH, 2003). Vision of the welfare of the horse as a whole in such a way reveals the need to invest into strategies that can reduce the horses' reactivity, in order to decrease the challenges over the horses' lifetime (DOHERTY; MCGREEVY; PEARSON, 2017).

3 Articles

3.1 Article 1

Mare facilitated exposure to humans influences fear response in foals - influence of husbandry

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MARE FACILITATED EXPOSURE TO HUMANS INFLUENCES FOAL FEAR RESPONSE- INFLUENCE OF HUSBANDRY

Ruth Dunford Patten, Carlos Eduardo Wayne Nogueira, Henrique dos Reis Noronha, Hortencia Campos Mazzo, Bruna da Rosa Curcio

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Abstract

Generally, without siblings or a paternal figure, the mare is the primary social model for the domestic foal. Recent research on early training and handling has shown a strong correlation of reduced fear responses in the foal when the mare is handled in the foals' presence, showing a promising potential for maternal social transmission in horses. The objective of the current study was to test the persistence of mare-facilitated training when implemented under two different husbandry practices. A total of 36 mares and their respective foals were included in this study, originating from 3 Thoroughbred breeding farms in São Jose dos Pinhais, Brazil. 15 mare-foal pairs were included as the control group and 21 mare-foal pairs as the experimental group. Experimental mares underwent daily soft brushing sessions over the first 5 days post foaling, as a form of indirect positive interaction between human and foal. Effect of treatment was evaluated using ethogram-based evaluations, spatial information and relevant behavioural tests with the foal in the absence of restraint at days 1-5 (D1-5), day 15 (D15) and at days 30-34 (D30-34) post-partum. Mare behaviours were also recorded at the earlier evaluations. Differences between treatment groups and husbandry practices were also evaluated. In comparison with the control group, experimental mares showed an increase in behaviours associated with rest in the presence of the human experimenter. All foals from the experimental group showed a significant increase in time spent on the same side as the experimenter, as well as an increased number of direct approaches, positive contact interactions (sniffing, licking and nibbling), and an increase in the amount of time spent within 0.5 m of the experimenter during the initial treatment. Experimental foals showed a significant decrease in fear responses in the presence of the experimenter (increased approximations, interactions) during the stationary human tests at D15 and D30, however, when contact was sought, a greater effect of husbandry practices between groups was observed at D30-34. The differences observed in foal behaviour related to husbandry showed that daily repetitions of behaviour (handling) had a more significant influence on the foal's long-term behaviour when approached by a human, than from the brushing treatment sessions.

The incorporation of indirect foal training of this form was seen as a practical way to integrate learning theory with a relatively low investment (both in terms of costs and time) into Thoroughbred breeding farms in southern Brazil.

Keywords: fear; foal; early training; maternal influence; human-animal relationship, behaviour

1. INTRODUCTION

In horses, as in all prey animals, fear has an important function, and its potentially for this reason that fear continues to persist in the domestic horse (*Equus caballus*). Fearful animals are known to be more reactive, slower to learn new tasks (Wolff & Hausberger, 1996), and more likely to cause accidents than calmer animals (Boivin et al, 2003; Keeling et al., 2009; Waiblinger et al., 2006). Costs associated with fearful and reactive horses account for severe financial losses, as well as premature losses of animals and termination of careers due to animal injuries, as well as the associated endangerment to their handlers (Hall et al., 2018; Lansade et al., 2007; McLean; Christensen, 2017; Watson & McDonnell, 2018). Given the innate propensity towards fear-related behaviours (onset of fear in foals from 2 hours post-partum: Waring, 2003), it is crucial that approaches to equine training incorporate methodologies that can alter the development of these reactions, in a way that will endure beyond the early period of life.

Early foal training can take on a number of forms, and, depending on the goal, can incorporate either direct (imprinting or a derivative, gentling, teat training, handling), or indirect training of the neonate (using conspecifics or parental models). In recent years, direct early foal handling has gained much use as a method of desensitizing the foal and improving human-foal bonds (Simpson, 2002; Miller, 1991), reducing reactive, fear-based responses (Ligout et al., 2008; Waring, 2003), resulting in better and more uninhibited learning and training in the foal as a result (Heird et al., 1986; Simpson, 2002). The issue with direct training of the foal during the neonate period however, is two-fold: its implementation requires a relatively high time investment and the use of constraints, and its effectiveness as a whole in literature has proven inconsistent. Considering the high time investment per animal during this phase, and the associated costs when implemented on a large scale (as is the custom in horse breeding setups), the feasibility of such treatment remains controversial. A third and perhaps even greater issue of direct neonatal training of the foal, is the unintentional overlooking of the natural process of behaviour formation that exists between the mare and foal and its potential. As mentioned in Henry et al. (2009) "It may be more fruitful to benefit from a well-established mother–young bond than to try to be 'part of the bonding'."

Indirect early training is based on the principles of learning theory, whereby training is enabled via the modeling behaviour of a conspecific. Studies have shown that conspecific training, or "horizontal social facilitation" (Christensen, 2016) with habituated conspecifics can have an effect in improving social behaviours (Bourjade et al., 2008), decreasing stress at weaning (Henry et al., 2012) and decreasing fear responses in fear-stimulating circumstances (Christensen et al., 2008; Rørvang et al., 2015; Rørvang & Christensen, 2018). Based on their constant association (Crowell-Davis, 2005) and the comfort or security (Waring, 2003) she provides, the maternal bond is an extremely strong influencer on the behavioural acquisition of the offspring (Champagne, 2011; Meaney, 2001), including in terms of food choice (in moose: Edwards, 1976; in sheep: Lynch et al. 1983, Veissier et al., 1998; in precocious fowl: Bertin & Richard-Yris, 2005; Wauters, et al., 2002; in herbivores in general: Provenza et al., 1992; in horses: Bolzan et al., 2019; Marinier & Alexander, 1995), mate preferences (Fabricius, 1991; Kendrick et al., 2001; Waring, 2003), and in reducing fear-related responses in the offspring (in goats: Ruiz-Miranda & Callard, 1992; in precocious fowl: Perré et al., 2002; in horses: Christensen et al., 2008; Christensen, 2016). Generally without siblings or a paternal figure present, the mare is the primary social model for the domestic foal (Henry et al., 2005; Henry et al., 2007; Mateo, 2014; Rørvang et al., 2018), and as such recent early training has sought to test the influence of the mare over the behaviour of the foal, known as "vertical social facilitation" (Christensen, 2016; Henry et al. 2005). One benefit of such training is that it has less emphasis on the experience of the trainer and is easily incorporated into active farm management, as the essence of the training is facilitated through the mare.

We tested the persistence of mare-facilitated foal training when implemented under active farm conditions in English Thoroughbreds retired from the racetrack in southern Brazil. By experimentally "treating" the mare with a positive experience (soft brushing) in the presence of the foal but without direct contact, we hypothesized that foals of these mares will have (i) lower levels of known fear responses (reduced flight, reduced distances from human), (ii) increased amounts of behaviours associated with decreased fear (approaches and interactions with human, increased time spent within 0.5m of human), and (iii) quicker social learning of a novel task, in comparison with the control foals whose dams received no experimental treatment.

2. MATERIALS AND METHODS

Thirty-nine mares and their foals (13 colts, 26 fillies) were studied during the southern spring of 2018 (August- December, 2018), at 3 different Thoroughbred breeding farms in the general area of São Jose dos Pinhais, Paraná state, southern Brazil. All Thoroughbreds used in this study were randomly divided into experimental and control groups based on their parity and parturition date. Mares on average were between 4 and 20 years old (mean age of 10.2 ± 3.6). In general, the mares gave birth in stalls, and all the foals were haltered at both sites within the first 5 days of life. There was no significant difference between the average ages of mares, showing homogeneity between farms (T test two sample: Group 1: $N = 15$; $\bar{X}$: 8.8 years, SD 2.5; Group 2: $N = 21$; $\bar{X}$: 11.2 years, SD 4.2; $P = 0.06$). Foals born during this experiment were predominantly female in both the experimental (experimental: males $N = 6$, females $N = 9$) and control groups (control: males $N = 7$, females $N = 14$).

Since horses can be kept under many different management systems (Marsbøll & Christensen, 2015), we wanted to include an aspect of differential husbandry practices to test for influences. Although all animals were cared for in a similar manner regardless of farm, notable differences did exist between sites, and as such were divided into two husbandry groups, denoted as the "field group" (Farm 1, N = 15; Control: N = 5; Experimental: 10) and the "stall group" (Farm 2, N = 15; Control: 6; Experimental: 9; Farm 3, N = 9; Control: 4; Experimental: 5), with the main differences being the daily feeding and the handling of the foals, occurring either in field or in the stall. In the field group, all mare-foal pairs were housed and fed in field, whereas at the stall group, all mare-foal pairs were housed in field but were handled and brought into the stalls twice daily for feeding. In regards to handling, mares from the field group were not routinely handled on a daily basis post-foaling, but if they were brought in for a specific care, their foals were allowed to follow their dams freely without direct human contact. This was in contrast to the stall group, where both the mares and foals were handled twice daily for feedings by use of a cord around the foal's body and halter with direct physical restraint. All animals had access to water and pasture *ad libitum* when turned out.

2.1 Experimental procedure

The present study was performed during the 2018 southern breeding season. All procedures carried out in the present study were approved by the Ethical Committee on Animal Experimentation of the Federal University of Pelotas under No. 10578.

2.2 Mare brushing

All experimental procedures were carried out following the methodology of Henry et al., 2005, and through conferring with the author (personal communication, 2018). Any differences in procedures are noted below.

The first session of brushing occurred on average within 16 hours post-partum (12-21 hours, with the exception of 1 mare who was brushed earlier). All sessions occurred after food was introduced. In Group 1, where mares and foals were maintained exclusively in the field, the experimenter caught and tied the mare along the perimeter fence in a familiar area of a roughly 1 ha field, at a sufficient distance from conspecifics to avoid stress from either isolation or close proximities. In the farms associated with Group 2, the experimenter entered into the stall, approximately 4 m x 4 m, or 5 m x 5 m, caught and tied up the mare using a wall ring. In all locations, once the mare was restrained and in contact with her foal, the experimenter would then choose a position near the mares' head and remain stationary for 1.5 min. Following this, the experimenter would then to proceed to brush the mare using a soft equine grooming brush for 13.5 minutes, being attentive to her facial expressions and body reactions to the brush (Lansade et al., 2018) to avoid unnecessary agitation. Once brushing ended, the mare would then receive a small food reward (a handful of the mares' feed), was released, and the experimenter would leave the stall or the field. At no time during these brushing sessions was contact intentionally sought by the experimenter with the foal. At the experimenter's request, no participating animals were brushed during the time frame of this study. The total treatment time per animal was 15 minutes per day for the first 5 days post-foaling equalling 75 minutes per animal.

Mares which exhibited more than a safe amount of aggression-related behaviours (advances which inhibited brushing, bites and bite threats towards the experimenter), were removed from this study since protective aggression behaviours can cause injury to the handler (Simpson, 2002). Of the thirty-six remaining mare-foal pairs, 4 mares exhibited increased protective behaviours during the first five days post partum (blocking of the foal with her body, increased locomotion, varying threats to the experimenter), were noted as being more protecive than the other dams. These mares were classified as the “protective group”, and represented 2 mares from each husbandry group.

All sessions were recorded using a digital camera (Nikon, D5000), and saved daily to an external hard drive for subsequent transcription. Instantaneous focal animal sampling (Altmann, 1974) of the mare and foal, and the spatial relationship between the experimenter and the foal were recorded every 5 sec, separating the following occurrences of behaviour: feeding and drinking, maintenance behaviours (scratching, rubbing, shaking etc.), glances, sniffing, nibbling, chewing, licking, aggressions, lying down, locomotion, eliminations, nursing, protective behaviours and approaches. All occurrence continuous sampling of vocalizations of both the mare and foal were also recorded. Frequency of time that foals spent within 0.5 m and on the same side of the experimenter were also recorded. A summary of the behaviours observed can be seen in Appendix 1, and visual examples of recorded behaviours can be found in Appendix 2.

2.3 Foal tests

All methodology for foal tests followed the procedures outlined in Henry et al. (2005), with any specifications described in the following section. All experimental and control foals were subjected to standardised tests designed to incorporate components of situations classically reported to induce fear in ungulates (Lansade et al., 2005). Tests with the foals occurred on day 15 (D15), for 5 consecutive days on days 30-34 (± 8 days). Schedule and details of each test can be found in Figure 1.

The first foal test, referred hereafter as the “reaction to a stationary human” test, occurred on D15 and again on days 30-34 (D30-34), and involved the experimenter remaining stationary for 5 minutes near the restrained mares’ head without intentionally seeking contact with the foal. The second foal test, known as the “approach-contact” test, started immediately after the reaction to a stationary human test, and involved the experimenter intentionally approaching the foal from the side and attempting to initiate contact. This test was only done on D15 and had a maximum time allowance of 60 sec, whether or not touch was achieved. Latency and durations of touch were recorded, as well as flight distances from the attempts. The third foal test, the “tolerance test”, was used to test the habituation of foals to a novel task at 1 month (during the D30-34 tests). This test began with the reaction to a stationary human test, followed by the experimenter slowly approaching the foal from the side and attempting to gently introduce and place a small saddle pad onto the foals’ back. Attempts were made only once the foal remained immobile and then the pad was gently placed on the back of the foal while the pad was continuously held by the experimenter. Success in this test was obtained when the foal remained motionless with a saddle pad placed on its back for 10 seconds, and such foals rewarded with an offering of a food to the foal (handful of the mares’ normal feed), and vocal praise. A total of 3 trials were permitted per foal per day, with a maximum time of 120 seconds per trial. All tests were performed by the same experimenter (female, blonde hair) who also performed the initial brushing treatment. At any time during this or other tests, the foal was free to move away and avoid contact, its permanence and allowance of touch therefore being its voluntary will expressed rather than imposed by force.

2.4 Statistical analyses

Data distribution was determined by the Shapiro-Wilk Normality test. Normally distributed data was further analysed using Analysis of Variance (ANOVA) and Fisher's least significant difference (LSD) procedure for pairwise comparisons of groups. For data with non-parametric distribution, Wilcoxon rank statistical test for matched pair data and Kruskal- Wallis for comparison between more than 2 groups were used. Statistical significance was set with a minimum of ≤ 0.05, and a tendency inclusion where $p = 0.06$-0.08. All statistical data analyses were performed using the commercial software Statistix 10.0 (Analytical Software, Tallahassee, FL, USA).

3. RESULTS

3.1 Results of primary interactions on days 1-5 post-partum

3.1.1 Mare results

Due to the similar behaviour of groups, experimental animals of both the field and stall groups were pooled and the following statistical evaluations reflect their combined analysis (Figure 2). All mares demonstrated behaviours associated with a reduced preoccupation over the 5 days, including a reduction in number of glances directed towards the foal (LSD: D1: $\bar{X} = 13.5$; D5: $\bar{X} = 6.3$; $p < 0.001$), increase in behaviours associated with "resting" (LSD: D1: $\bar{X} = 47.3$; D5: $\bar{X} = 64.5$; $p < 0.01$), and a reduction in vocalizations both in amount (Wilcoxon: D1: $\bar{X} = 25.41$; D5: $\bar{X} = 17.60$; $p < 0.05$) and in duration (Wilcoxon: D1: $\bar{X}= 3.71$; D5: $\bar{X}= 0.94$; $p < 0.05$). Reduced preoccupation with the experimenter was also observed from D1-D5, with decreased glances (Wilcoxon: D1: $\bar{X}= 4.74$; D5: $\bar{X}= 3.24$.; $p = 0.17$), and increases in interactive or exploratory behaviors towards the experimenter (Wilcoxon: Sniffing D1: $\bar{X}= 5.17$; D5: $\bar{X}= 7.16$; $p = 0.31$; Licking D1: $\bar{X}= 0.11$; D5: $\bar{X}= 0.16$; $p = 0.70$; Nibbling D1: $\bar{X}= 0.16$; D5: $\bar{X}= 0.34$; $p = 0.15$). Mare behaviors towards the foal in general reflected a reduction in initial care and protection (Wilcoxon: Licking: D1: $\bar{X} = 1.35$; D5: $\bar{X} = 0.19$; $p < 0.01$; Sniffing: D1: $\bar{X} = 8.26$; D5: $\bar{X} = 6.06$; $p = 0.50$; Protective behaviors: D1: $\bar{X}=0.53$; D5: $\bar{X}= 0.18$; $p = 0.89$; Nibbling: D1: $\bar{X}= 0.31$; D5: $\bar{X}= 0.52$; $p = 0.13$).

The majority of mare behaviours did not differ significantly between husbandry Groups, with the exception of increased behaviours associated with maintenance in field group mares (field group: $\bar{X} = 1.39$; stall group: $\bar{X} = 4.97$; $p < 0.01$), and higher number of vocalizations in field group mares, (LSD: field group: $\bar{X}= 3.65$; stall group: $\bar{X}= 1.57$; $p < 0.05$). Feeding statistical results were omitted since the amount of food present in each site at testing time could vary and as thus influence results.

In terms of “protective group” mares, beyond exhibiting higher frequencies of aggression towards the experimenter during D1-D5 (Wilcoxon: Protective group: $\bar{X}$= 4.11; Non-protective group: $\bar{X}$= 0.00; $p < 0.001$) and locomotion (Wilcoxon: Protective group: $\bar{X}$= 7.72; Non-protective group: $\bar{X}$= 3.52; $p < 0.01$), mares from the protective group also had differences in vocalizations, with less expressed on day 1 than non-protective mares (Wilcoxon: Protective group: $\bar{X}$= 1.93 ; Non-protective group: $\bar{X}$= 2.94; $p = 0.06$).

3.1.2 Foal results

Due to the similar behaviour of groups, experimental groups were pooled and the following statistical evaluations reflect their combined analysis as presented in Figure 3. Increases in exploratory and interactive behaviors by the foal in relation to the experimenter were observed over D1-D5 (Approaches: D1: $\bar{X}$ = 0.81; D5: $\bar{X}$ = 2.66; $p < 0.01$; Sniffing: D1: $\bar{X}$= 1.17; D5: $\bar{X}$= 8.78; $p < 0.001$; Licking: D1: $\bar{X}$= 0.00; D5: $\bar{X}$= 1.15; $p < 0.01$; Nibbling: D1: $\bar{X}$= 0.00; D5: $\bar{X}$= 0.67; $p < 0.05$; Glances D1: $\bar{X}$ = 2.32; D5: $\bar{X}$ = 3.05; $p = 0.06$; Chewing of the experimenters clothing: D1: $\bar{X}$ = 0.00; D5: $\bar{X}$ = 0.22; $p = 0.08$). Voluntary foal proximity also increased from D1 to D5 (Wilcoxon: Within 0.5m: D1: $\bar{X}$ = 10.20; D5: $\bar{X}$ = 24.51; $p < 0.001$); Same side: D1: $\bar{X}$ = 15.63; D5: $\bar{X}$ = 33.92; $p < 0.001$), as seen in Figure 4. Differences in foal behaviours towards the mare also changed during this period (Sniffing (D1: $\bar{X}$ = 16.33; D5: $\bar{X}$ = 10.23; $p < 0.05$; Nibbling: D1: $\bar{X}$ = 0.00; D5: $\bar{X}$ = 0.67; $p < 0.05$).

Evidence of husbandry group was limited to foal glances towards the mare (Wilcoxon: field group: D1: $\bar{X}$ = 0.00; D5: $\bar{X}$ = 0.14; stall group: D1: $\bar{X}$ = 0.33; D5: $\bar{X}$ = 0.25; $p < 0.05$), and maintenance-related behaviours (Wilcoxon: field group: D1: $\bar{X}$ = 3.36; D5: $\bar{X}$ = 4.77; stall group: D1: $\bar{X}$ = 1.73; D5: $\bar{X}$ = 1.80; $p < 0.05$), both increased in field group foals.

Foals from “protective” mares were observed with higher frequencies of the flehmen response during D1-D5 (Wilcoxon: Protective group: $\bar{X}$ = 0.06; Non-protective group: $\bar{X}$ = 0.01; $p < 0.05$).

3.2 Results of interactions on day 15 post-partum

3.2.1 Mare results- stationary human test

Experimental mares showed less concern during the tests on D15 than control mares (Resting behaviors: Experimental: $\bar{X} = 67.3$; Control: $\bar{X} = 46.9$; $p < 0.05$; Glances towards the experimenter: Experimental: $\bar{X} = 5.32$; Control: $\bar{X} = 8.35$; $p < 0.05$).

Separating for Husbandry group, stall group mares were more interactive with both the experimenter and the foal than field group mares (Foal sniffing: field group: $\bar{X} = 0.11$; stall group: $\bar{X} = 1.39$; $p < 0.01$; Experimenter sniffing: field group: $\bar{X} = 0.11$; stall group: $\bar{X} = 1.39$; $p < 0.05$; Glancing towards experimenter: field group: $\bar{X} = 7.00$; stall group: $\bar{X} = 5.84$; $p < 0.01$). Field mares had a greater occurrence of maintenance behaviors on D15 than stall mares (field group: $\bar{X} = 11.49$; stall group: $\bar{X} = 7.73$; $p < 0.05$).

3.2.2 Foal results- stationary human test

Experimental foals on D15 sought more voluntary contact and approximation with the experimenter than control foals (In contact: Experimental: $\bar{X} = 3.58$; Control: $\bar{X} = 0.00$; $p = 0.08$). Of the experimental foals, stall group foals tended to spend a greater amount of time than control foals of the field group (Wilcoxon: field group: $\bar{X} = 1.58$; stall group: $\bar{X} = 2.41$; $p = 0.06$). Similar to mares, field group foals also expressed more behaviours related to maintenance than foals from the stall group (Wilcoxon: field group: $\bar{X} = 7.72$; stall group: $\bar{X} = 1.84$; $p < 0.05$).

3.2.3 Foal results- approach-contact test

No differences between treatment groups were observed during the approach-contact test. In terms of husbandry group, stall group foals allowed more touching by the experimenter (Wilcoxon: field group:

$\bar{X} = 0.21$; stall group: $\bar{X} = 0.91$; $p < 0.001$) for longer durations (field group: $\bar{X} = 7.47$; stall group: $\bar{X} = 17.12$; $p < 0.01$) and were easier to touch in terms of latency to first touch during the 60 sec test (field group: $\bar{X} = 52.15$; stall group: $\bar{X} = 23.28$; $p < 0.001$) as well as the number of attempts required before first touch was permitted.

3.3 Results of interactions on days 30- 34 post-partum

3.3.1 Foal results- stationary human test

Evaluations from D30 onward include only evaluations with the foal. An increased voluntary contact and approximations by foals with the experimenter was observed from D30-D34 (Wilcoxon: In contact: D30: $\bar{X} = 0.95$; D34: $\bar{X} = 7.05$; $p < 0.05$; Same side as experimenter: D30: $\bar{X} = 34.38$; D34: $\bar{X} = 54.24$; $p < 0.01$). Of these, experimental foals had greater approximations than control foals on D34 (Wilcoxon: In contact: Experimental group: $\bar{X} = 12.28$; Control group: $\bar{X} = 0.70$; $p < 0.05$; Within 0.5 m: Experimental group: $\bar{X} = 27.40$; Control group: $\bar{X} = 5.32$; $p < 0.05$). Further, comparing D15, D30 and D34, more time was spent in contact with the experimenter in the experimental group, with a significant difference between D15, D30 and D34 in terms of proportion of time spent by experimental foals on the same side as the experimenter (D15: $\bar{X} = 40.2$; D30: $\bar{X} = 34.2$; D34: $\bar{X} = 51.7$; $p < 0.05$).

3.3.2 Foal results- tolerance test

There was no longer a significant difference of treatment group with foals during the tolerance test. In terms of husbandry group, stall group foals had greater success with saddle pad training than foals from the field group (field group: $\bar{X} = 2.55$; stall group: $\bar{X} = 8.55$; $p < 0.01$), as well as better habituation to this new task, as evidenced by an increase in success during from D30 (field group: D30: $\bar{X} = 0.45$; stall group: D30: $\bar{X} = 1.45$; $p < 0.05$) to D34 (field group: D34: $\bar{X} = 0.55$; stall group: D34: $\bar{X} = 2.66$; $p < 0.01$) (Figure 5). Stall group foals on average also had a tendency to accept the saddle pad quicker initially on D30 (field group: $\bar{X} = 315.93$; stall group: $\bar{X} = 196.39$; $p = 0.06$), increasing to a significance on the final day (D34) in comparison to field group foals (field group: D30: $\bar{X} = 325.85$; D34: $\bar{X} = 313.01$; stall group: D30: $\bar{X} = 239.50$; D34: $\bar{X} = 137.10$; $p < 0.001$). Foals from the field group were more latent in their first acceptance of the saddle pad (field group: D30-34: $\bar{X} = 1242.91$; stall group: D30-34: $\bar{X} = 429.33$; $p < 0.01$), as well as on average during this period (field group: D30-34: $\bar{X} = 315.93$; stall group: D30-34: $\bar{X} = 196.39$; $p < 0.01$) (Figure 6). Furthermore, foals from the stall group had a greater immobility when approached by the experimenter during the tolerance test, and when they were prompted to flight, travelled less distances than foals from the field group (No flight: field group 1: $\bar{X} = 3.0$; stall group 2: $\bar{X} = 27.0$; $p < 0.001$; Moderate flight: field group 1: $\bar{X} = 10.5$; stall group 2: $\bar{X} = 28.0$; $p < 0.05$; High flight: field group 1: $\bar{X} = 25.3$; stall group 2: $\bar{X} = 6.0$; $p < 0.001$). A visual example of saddle pad training at D30-34 can be found in Appendix 3, and a video example of successive trials in Appendix 4.

4. DISCUSSION

Based on the results of the current experiment, there is evidence for a sensitive period for training which can have effects on the behaviour and reactions of foals during the first month of life. The decrease in the mare's attention towards her foal, together with increased resting behaviours during the initial treatment and observed in the experimental group until two weeks post-partum, is evidence that the experience of brushing was positively appraised by the experimental mares in this study. As mares normally do not "willingly" allow direct access to their foals during the neonate phase (Waring 2003), the reduction in both protective behaviours and the quantity of mare vocalizations from D1-D5, along with an increase in positive interactions with the experimenter, are important indicators of the mare relaxing her innate protectiveness. Conversely, mares who had no initial brushing with the experimenter (control group) showed a greater concern for the experimenter's location relative to the foal even at D15, as witnessed by higher levels of glances towards the experimenter, and expressed higher care behaviors with her foal in comparison with experimental mares. Although grooming and other experiences such as stroking are largely believed to be a positive experience for the horse (McBride et al., 2004; Watson & McDonnell, 2018), these mares are all retired race horses, a career where grooming was once a common part of the associated racing preparations. As such, evidence confirming its positive reception is crucial since past experiences of grooming could have potentially influenced the level of effectiveness of the brushing experience.

All foals showed an increase in voluntary exploratory interactions and approaches with the experimenter during the initial treatment. Since this period involved no intentional interactions with the foal, such voluntary interest is indicative that the foal had reduced levels of fear towards the experimenter, reaffirming the positive appraisal of the brushing experience, considering proximity seeking has been described elsewhere as showing the formation of a "bond" (Cassidy, 1999), and behaviours associated with approaching are generally indicative of a positive appraisal of the stimuli (Hall et al., 2018; Maros et al., 2010). The opposite is also true, where avoidance of proximity, or a "passive refusal to approach" a stimulus (Hall et al., 2018) can be indicative of a threatening appraisal of a stimulus and a negative consequence (Elliot et al., 2013; Villas-Boas et al., 2016).

Although an impact of the initial training can be observed in foal behaviour up until one-month post-partum, its effect was greatly limited to the foal's voluntary interaction with a stationary human. Experimental foals spent a significantly greater amount of time in contact and in close proximity with the experimenter than control foals on D34, showing a persistence rather than novel interest in comparison with control foals, suggesting that the initial treatment experienced indirectly with experimental foals had a lasting impact in affirming a positive association with the experimenter. Since control foals were first exposed to the experimenter only on D15, it is possible that they would therefore have no motivation to interact with the experimenter, nor any example of interaction with the experimenter modelled by their mares. Comparing foal's physical contact with the experimenter on D15, D30 and D34, the highest proportion of time contact was observed in experimental foals on D34 and the lowest observed in control foals on D34, providing further evidence of the stimulatory effect for interaction that brushing might have had on experimental foals.

Once the experimenter actively sought contact with the foals in the form of the approach-contact and tolerance tests, a greater effect of husbandry group was observed in foals than the influence of treatment group. Stall group foals showed significantly more motionless behaviors (no flight) or only moderate flight distances (0.5 m $\leq$ x $\leq$ 1.5 m) when approached, in comparison to field group foals who had a significantly greater occurrence of high flight behaviors (over 1.5 m) when approached. This immobility in stall group foals when tested with a novel task (saddle pad) on days D30-34, agrees with previous research which indicates that handled foals demonstrate a better ability to control their fear responses and to successfully habituate when presented with new situations (Waring, 2003). Although foals from both husbandry groups spent the majority of their days on pasture, field group foals were not subjected to the daily handling by workers, nor did they enter into stalls daily for feeding. This difference in husbandry should therefore be considered as an arguably secondary treatment reinforced during the same time period as the brushing. Since horses can readily make an association with their actions and a food reward (Heleski et al., 2008), the results of such interactions (handling leading to feeding) could definitely have lasting effects (Sankey et al., 2010) on the behavior of the foals. Furthermore, this difference in handling has been highlighted in its impact on foal perception and subsequent reactions to handling, where in a study by Ligout et al. (2008), foals which were only passively handled (i.e. were free to avoid contact), were much less likely to remain in close proximity with a handler and allow touch than foals who underwent "forced handling". Such is similar to the effects witnessed in the current study, where foals that were "handled by force" (i.e. regardless of their compliance), for daily feedings showed greater behaviours associated with reduced fear than those who were not handled daily. This would agree with previous studies which showed that a passive human presence was not sufficient in reducing fear responses in the observer animal (Henry et al., 2005; Ligout et al., 2008), but requires propinquity over time (Waring, 2003). Additionally, foals from the field group were generally more concerned with their dams (in terms of glances D1-D5), less interactive with the experimenter (allowed less touch and required more time to be touched on D15; had a lower and slower rate of acceptance of saddle pads during D30-34 than stall group foals), and remained further away from the experimenter than foals from the stall group, further suggesting that the method of handling, and the horse's perception of such handling, can indeed impact its subsequent relationship with humans (Lansade et al., 2019). The avoidance behaviours observed in field group foals, paired with the latencies to learning the saddle pad task, suggest a persistent fearfulness in the foals of this group, since fearful animals tend to learn slower on average (Wolff & Hausberger, 1996). As no restraint (including cornering of foals in the stalls) was used, the stall structure itself can be excluded as a cause for the differences observed.

Knowingly, the differences between husbandry groups reflected the effect of the physical environment of testing, with both mares and foals from the field group having significantly more behaviors associated with maintenance behaviors (scratching, shaking, rubbing etc.) on D1-5 and D15. Notwithstanding, in-field training was an important component of this experiment as it allowed for the incorporation of this indirect technique without having to alter the current husbandry practices of the animals. That being said, components of the environment could have possibly diluted the impact of training and should be considered for future implementation. The incorporation itself of this technique was seen as a viable way to integrate learning theory into an active farm setting.

5. CONCLUSION

There is a sensitive period for training in foals which can have effects on the behaviour and reactions of foals during over the foals first month. Foal training in the absence of direct handling or restraint had a significant effect on the foal’s voluntary behaviours when in the presence of a stationary human, with experimental foals showing lower levels of known fear responses (reduced distances from human), increased amounts of behaviours associated with decreased fear (approaches and interactions, increased time spent within 0.5m of human) in this treatment group. However, when contact was intentionally sought with the foal a greater impact of husbandry group on foal behaviour was observed, with foals from the group which was handled daily showing quicker social learning and reduced flight distances in comparison with the foals of the field group.

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Table 1. Description of animals in each husbandry group.

	Group 1		Group 2		Total	
Description	Experimental	Control	Experimental	Control	Experimental	Control
No. animals	10	5	14	10	24	15
No. colts	3	1	4	5	7	6
No. fillies	7	4	10	5	17	9
No. primiparous	1	0	1	3	2	3
Total animals	15		24		39	

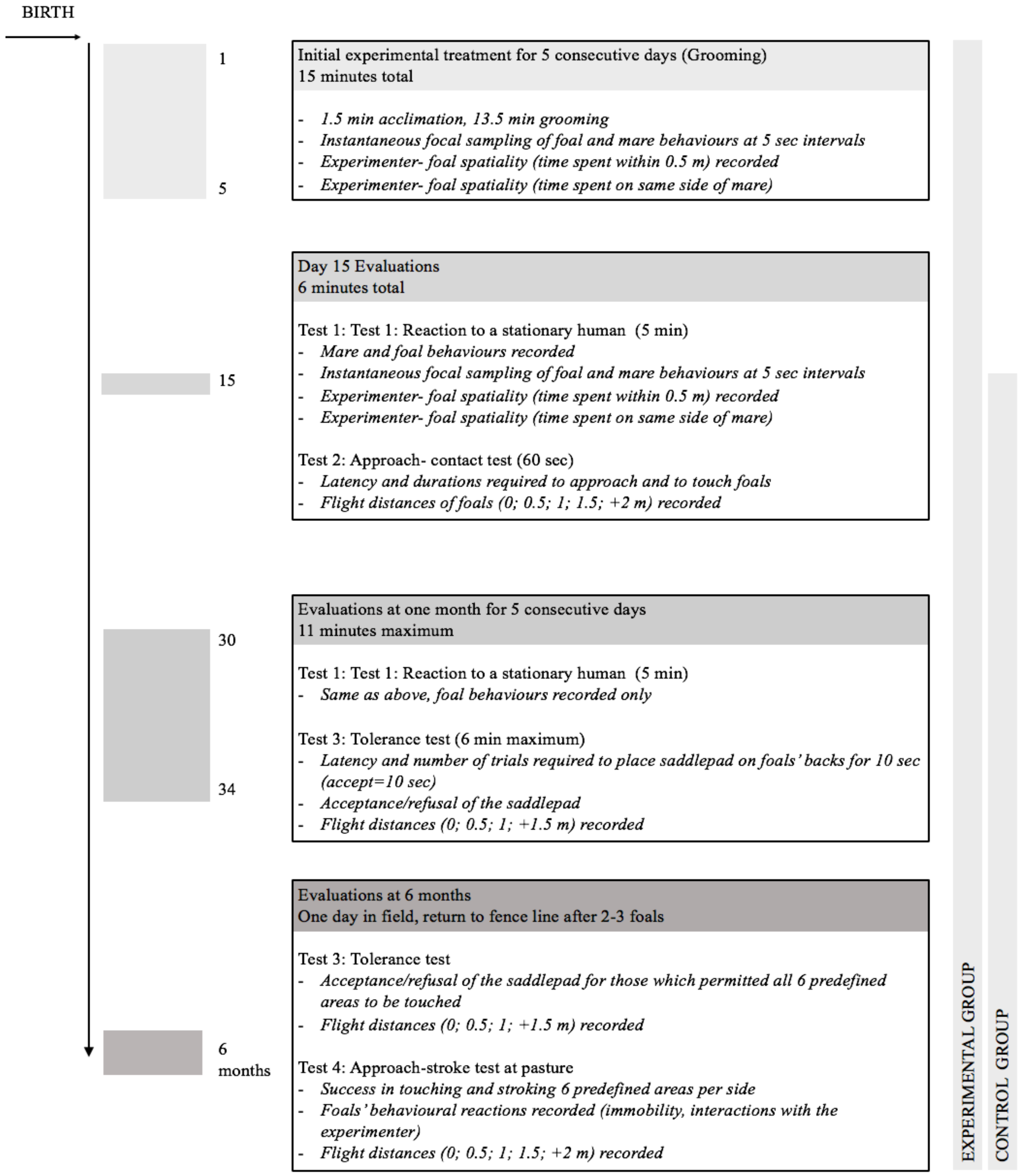


Fig. 1. Overview of foal tests and recordings.

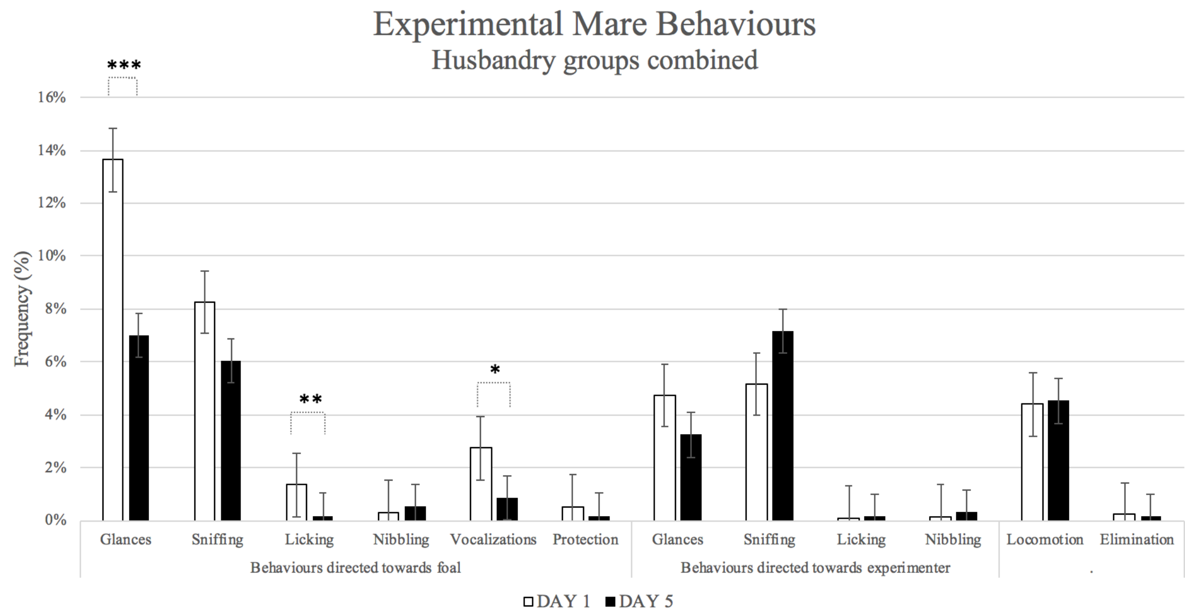


Fig. 2. Comparison of mare behaviours during the first and fifth days of the initial treatment, with husbandry Groups combined. Level of significance: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (LSD test).

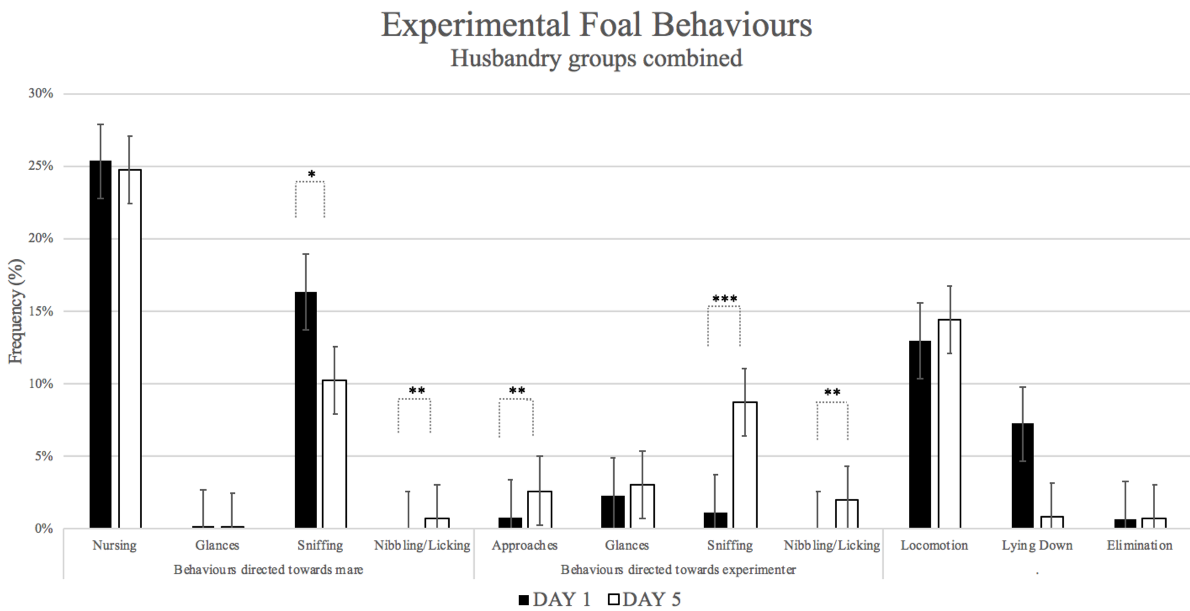


Fig. 3. Comparison of foal behaviours during the first and fifth days of the initial treatment, with husbandry Groups combined. Level of significance: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (Wilcoxon test).

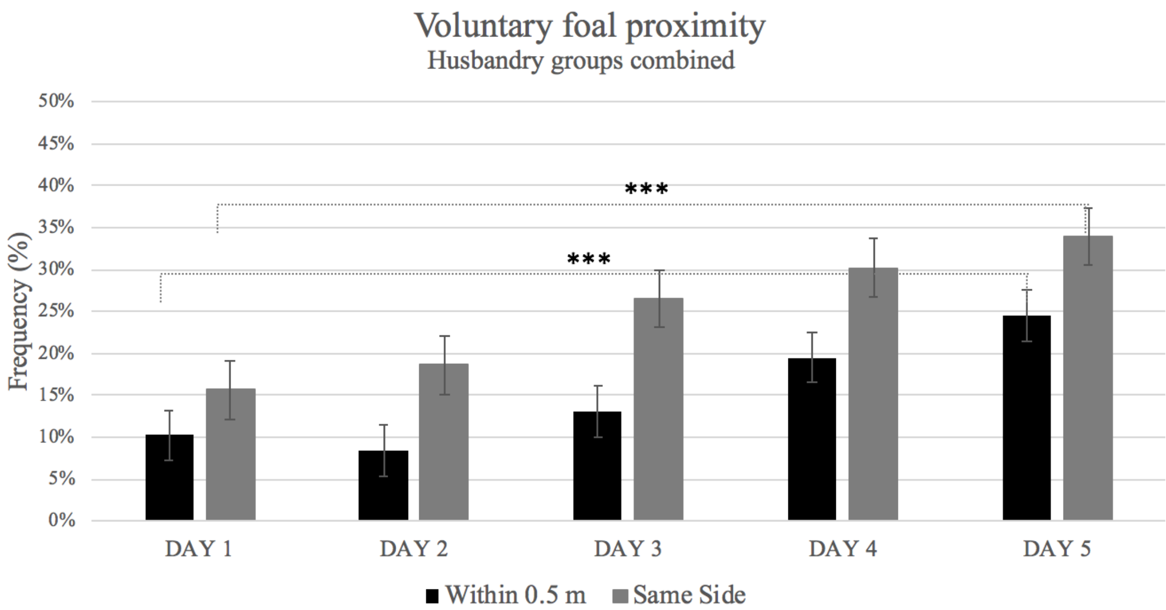


Fig. 4. Voluntary foal proximity data in relation to experimenter during the initial soft brushing sessions D1-D5, with both proportion of time where foal was within 0.5 m of the experimenter and on the same side as the experimenter, with combined husbandry group data. Level of significance: ***$p < 0.001$ (Wilcoxon test).

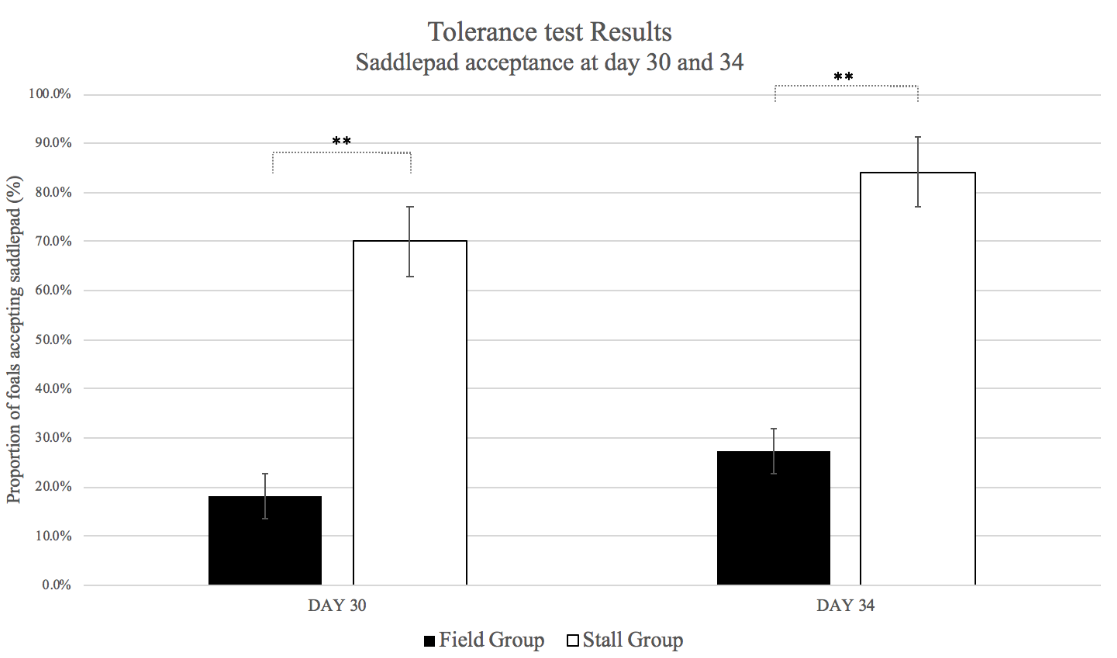


Fig. 5. Successful saddlepad acceptance during the Tolerance test, performed on foals from D30 and D34, with husbandry data separated. Level of significance: **$p < 0.01$ (Wilcoxon test).

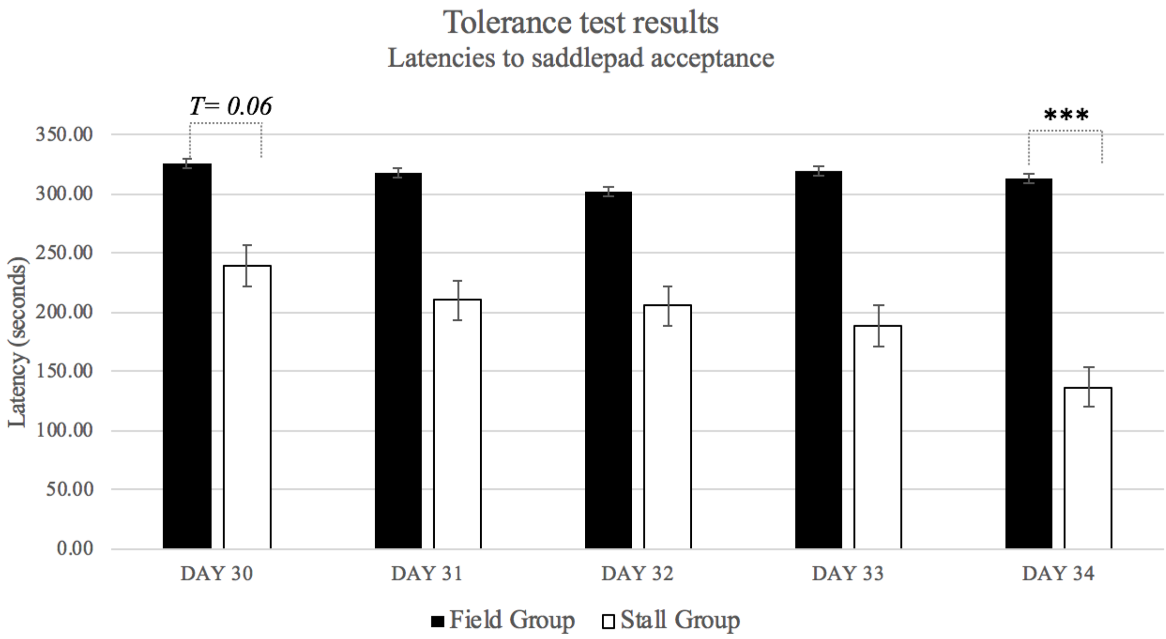


Fig. 6. Latency to accept the saddlepad on D30-D34 of the Tolerance tests with foals, with husbandry data separated. Level of significance: ***p < 0.001 (Wilcoxon test).

Appendix 1. Ethogram of recorded behaviours (adapted from Christensen, 2016; Henry et al., 2005; McDonnell, 2003; McDonnell and Poulin, 2002).

Behaviour	Description
Aggression	Aggressive threats and associated behaviours: horse briefly flattens *both* ears backwards with or without showing its teeth and turns its neck quickly (~1 sec) towards the object, accompanied with or without an approach; behaviours are accompanied by tense facial muscles
Approaches	Forward direct movement at any gait toward the experimenter in a straight or curving path, ending in a close proximity (less than 1 m)
Chewing	Part of clothes of the experimenter are taken into the mouth with a side-to-side grinding motion of upper and lower jaw
Elimination	Defecation and urination
Glances	Head and ears directed towards object with a visible gaze, usually a result of turning the head
Locomotion	Any purposeful movement of the feet of the horse to relocate or to propel motion
Lying down	Body being in contact with the ground (sternal or recumbent); includes the falling behaviour into the lying position
Maintenance	Self-scratching, rubbing, shaking
Nibbling	With jaws closed the upper lip is moved upward and downward on the object
Protection	Mare using body as a barrier between foal and experimenter, i.e. maintaining head towards the direction of the foal but not in a swift manner an in 'glance'- usually accompanies with flattened ears, with or without visible flexed neck muscles and facial veins but no active aggression
Rest	Absence of other separated activities; Rest Standing, Sleep standing
Sniffing	Head held momentarily within 10 cm of object, with or without visible contact

Appendix 2. Visual examples of behaviours recorded during testing.

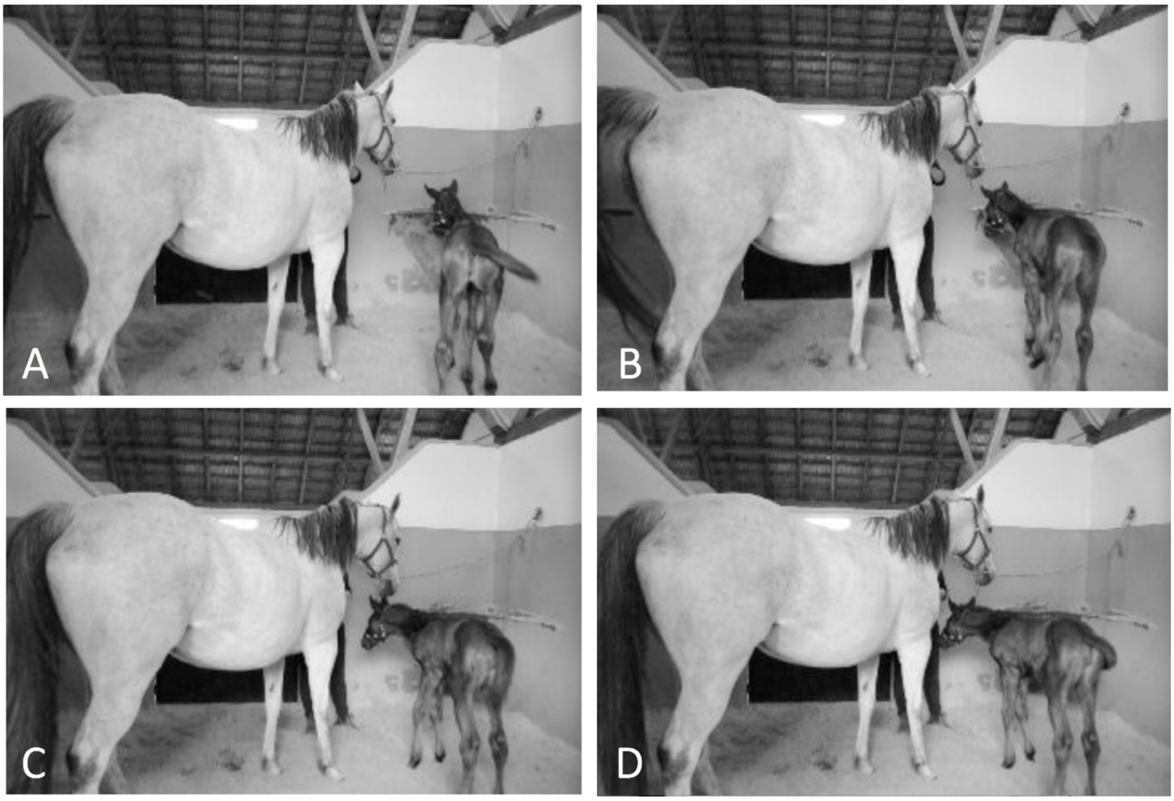


Figure 1. Visual example of the behaviours associated with approach in the foal, read from A-D. Note the intentional direct forward movement toward the experimenter ending in a close proximity (less than 1 m).

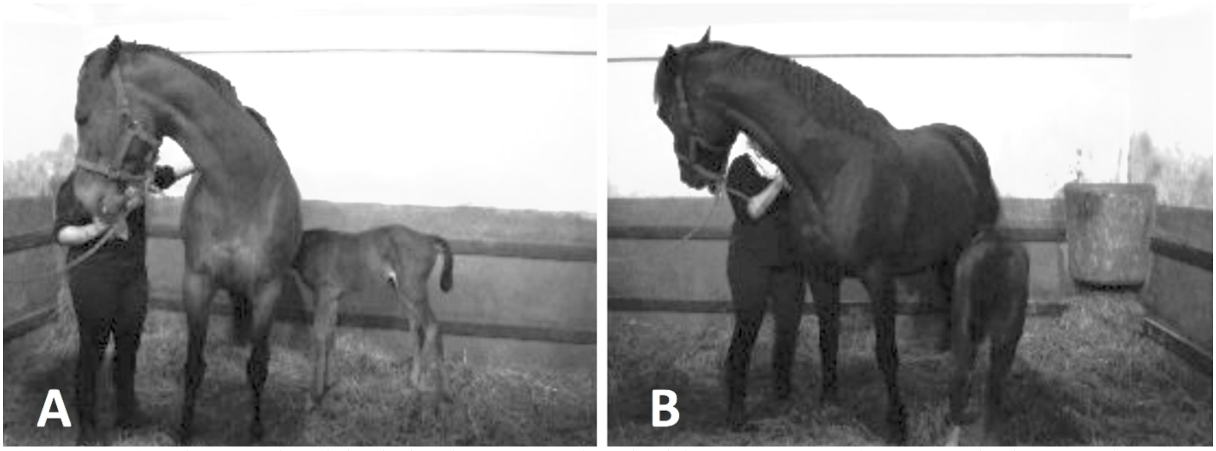


Figure 2. Visual example of the behaviours associated with mare aggression towards the experimenter, in this image a bite threat is occurring from A-B. Note the pinning of both ears, teeth shown, and tense neck and facial muscles.

Figure 3. Visual example of the behaviours associated with sniffing, both by the foal to the experimenter during brushing (Image A), and by the foal to the experimenter during the stationary human test (Image B). In Image B the mare is also demonstrating behaviours associated with sniffing towards her foal.

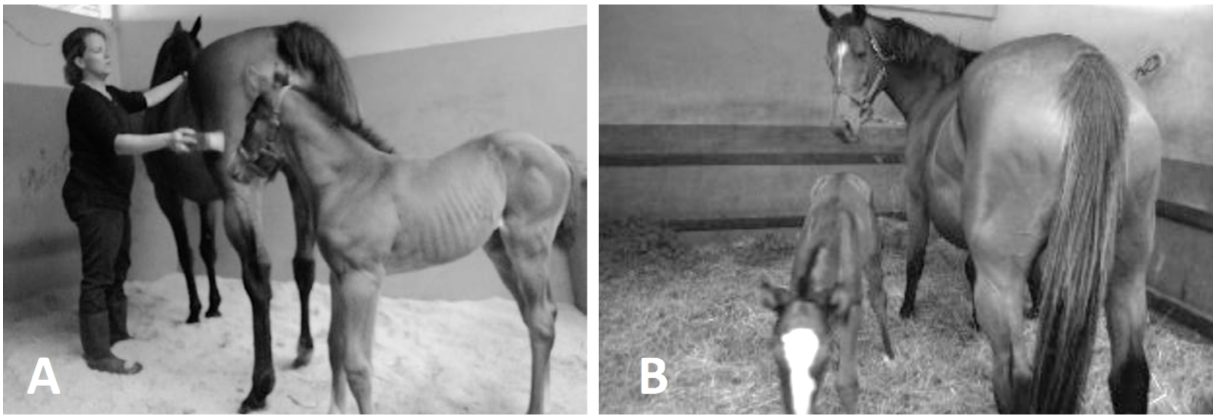


Figure 4. Visual example of the behaviours associated with glances, both demonstrated by the foal to the experimenter (Image A), and by the mare towards the foal (Image B).

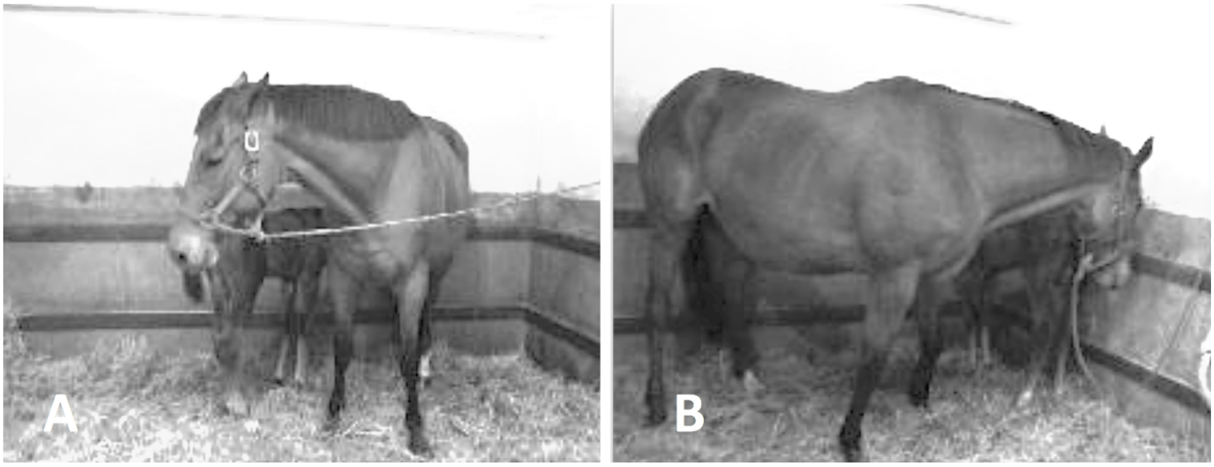


Figure 5. Visual example of the behaviours associated with mare protection, represented by the mare blocking the foal with her body (Image A), as well as cornering the foal (Image B), where tense neck and facial muscles are present but no threat is made but this position is “held”. Note the mare’s head in the direction of the foal.

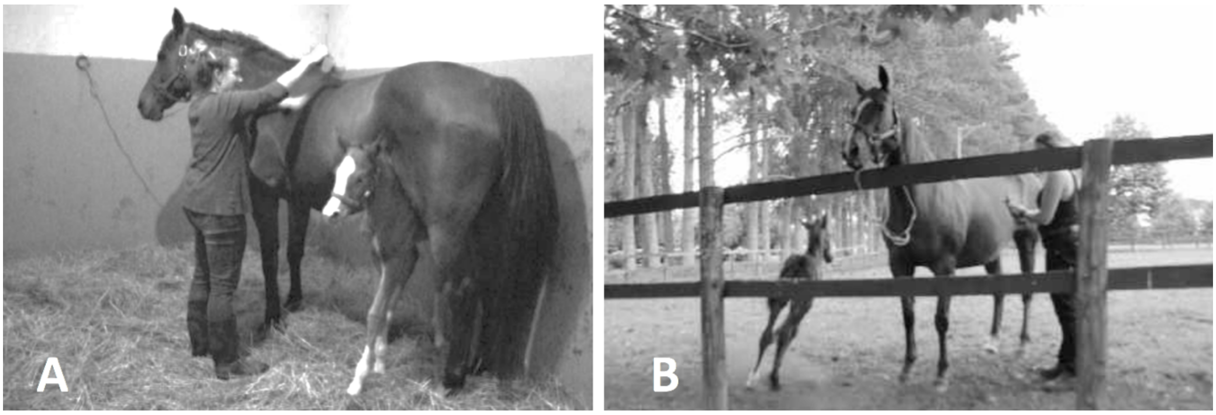


Figure 6. Visual example of the behaviours associated with locomotion in the foal, including any purposeful movement to either to relocate (Image A) or to play (Image B).

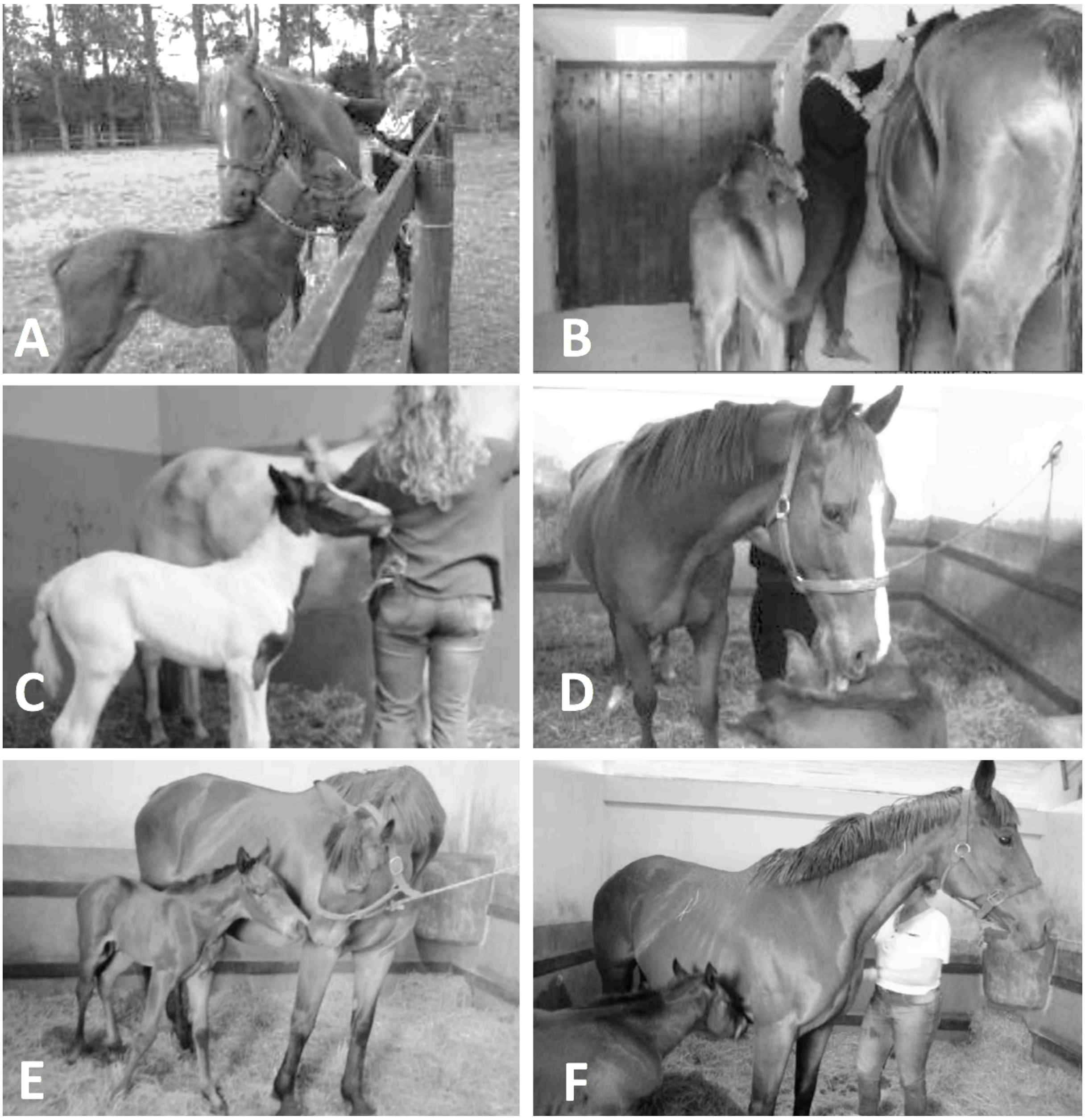


Figure 7. Visual example of various affiliative behaviours, including mare nibbling foal (Image A), foal licking experimenter (Image B), foal chewing experimenter's clothing (Image C), mare licking foal (Image D), sniffing of the mare to the foal and vice versa (Image E) and foal sniffing mare (Image F). Note that in Image F sniffing also includes the foal's head being held directly in under the mare's body but within 10 cm.

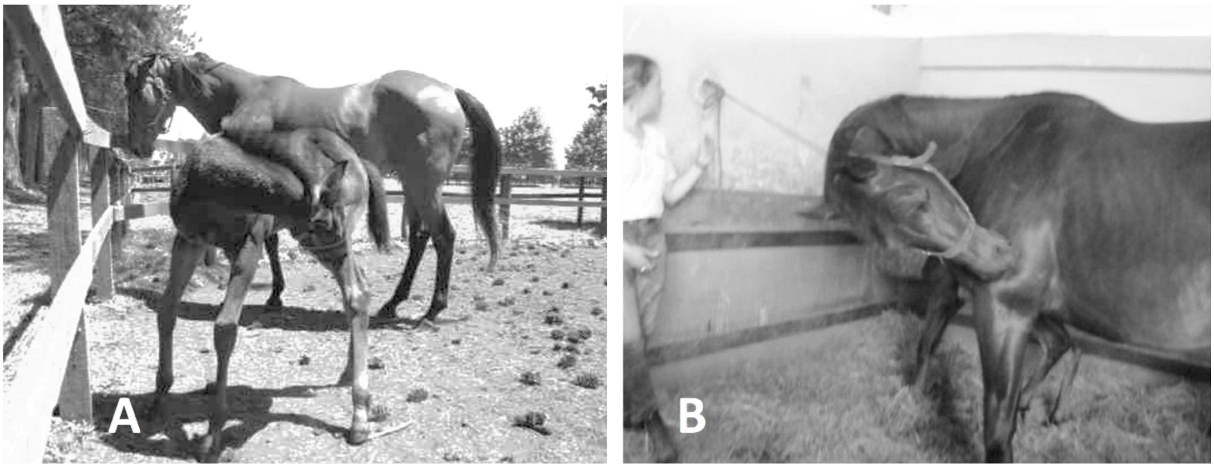


Figure 8. Visual example of the behaviours associated with maintenance, demonstrated by self-scratching in the foal (Image A) and the mare (Image B).

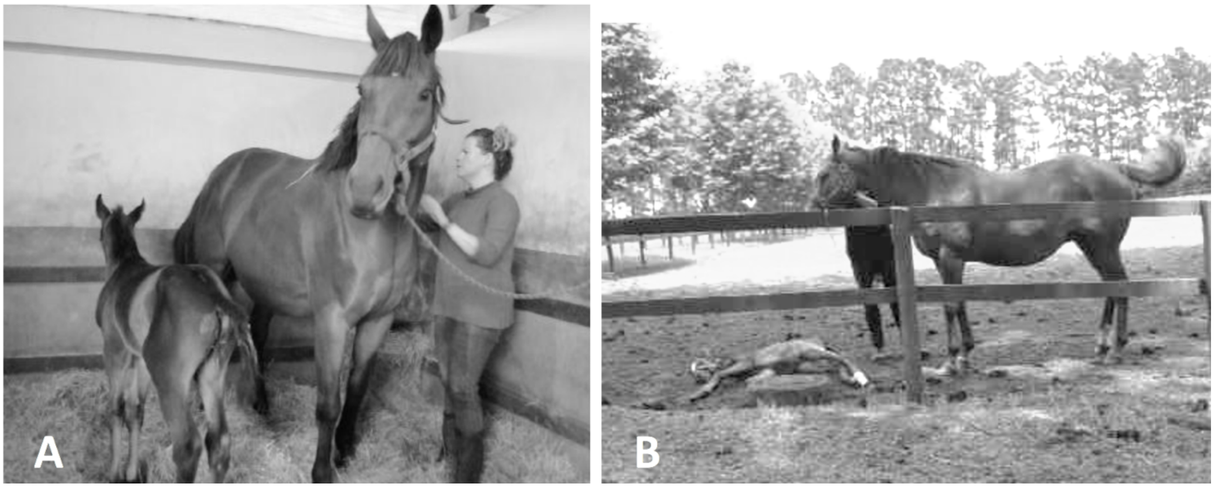
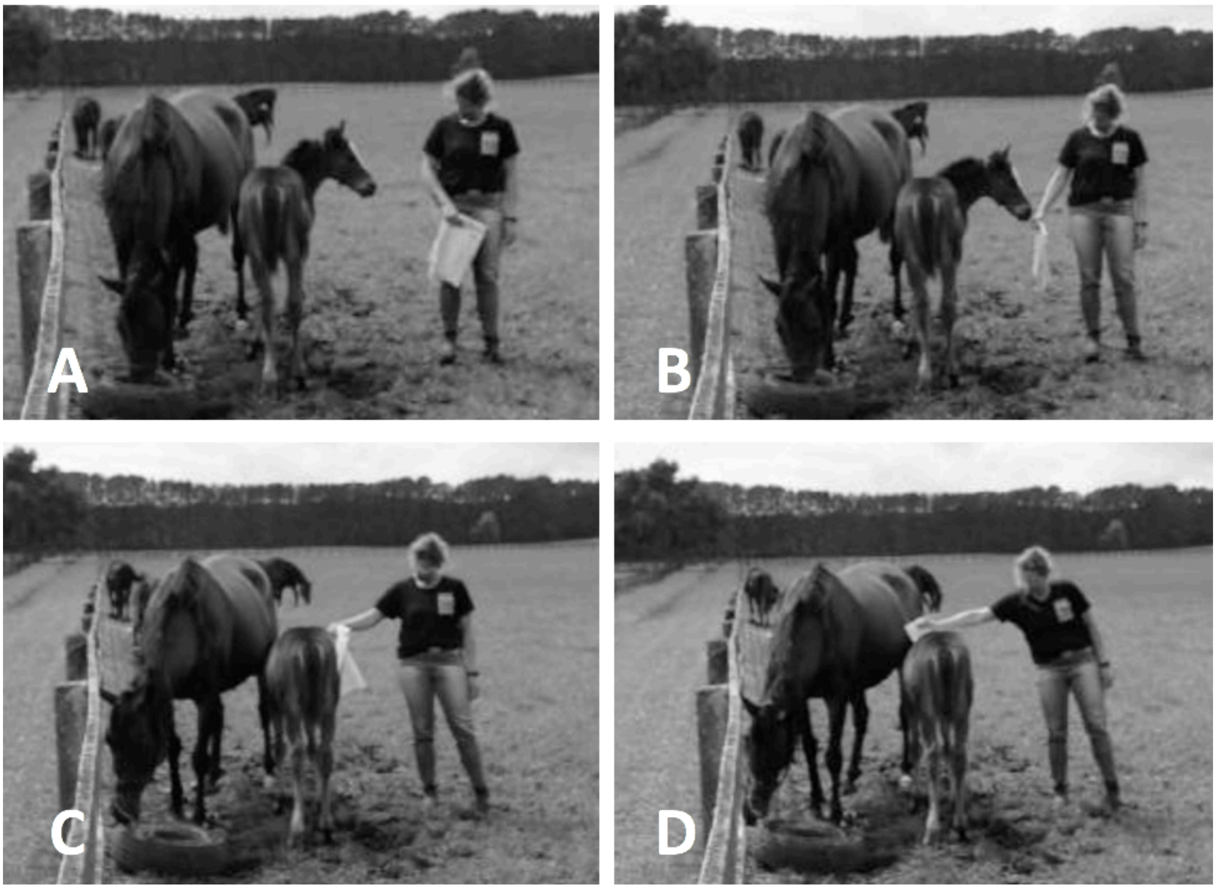


Figure 9. Visual example of the behaviours associated with resting, characterized as an absence of the other behaviours, demonstrated by both the mare and the foal (Image A), and by the mare (Image B). The foal in Image B is demonstrating behaviours associated with lying down.

Appendix 3. Visual example of the Tolerance test, performed with foals on D30-34, read from A-D.



Appendix 4. **Video example of the successive saddle pad accepts during the Tolerance test.**

4 Final Considerations

Based on the results of the current experiment, there is evidence for a sensitive period for training in foals which can have effects on the behaviour and reactions of foals during the first 6 months of life. Foal training in the absence of direct handling or restraint had a significant effect on the foal's voluntary behaviours when in the presence of a stationary human, indicative of a reduced level of fear. However, once the interaction between the human and the foal involved approaches and deliberate contact the effect of husbandry group showed a greater influence on foal behaviour. We can therefore conclude that certain known fear responses decreased in foals of the experimental group (increased interactions, reduced distances from human), while others such as flight tended to be affected more by the husbandry group than relating to treatment group. In terms of learning and habituation, a quicker acceptance of the novel task was observed in foals from Group 2, again demonstrating the effect of the husbandry group rather than the treatment group. The incorporation of this technique was seen as a viable way to integrate learning theory with a relatively low investment (both in terms of costs and time) into an active farm setting, however, the impact of husbandry procedures and environmental should be considered to enhance treatment outcomes of foals.

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Appendix

1st page:

Appendix I - Document from the Animal Ethics and Experimentation Committee

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **72/2019/CEEA/REITORIA**

PROCESSO Nº 23110.014910/2019-46

Certificado

Certificamos que a proposta intitulada **"Influência do reforço positivo em éguas no período puerperal no relacionamento égua-potro-humano"**, registrada com o n° **23110.014910/2019-46,** sob a responsabilidade de Bruna da Rosa Curcio - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **15 de agosto de 2019**.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	19/08/2019 a 01/06/2021
Espécie/linhagem/raça	Equina/Puro-sangue Inglês
N° de animais	84
Idade	42 fêmeas adultas e 42 potros
Sexo	Fêmeas e machos
Origem	Haras Springfield, Haras São Jose da Serra, Haras Santarém em São Jose dos Pinhais- Paraná.

Código para cadastro nº **CEEA 14910-2019**

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M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Documento assinado eletronicamente por **ANELIZE DE OLIVEIRA CAMPELLO FELIX**, **Médico Veterinário**, em 19/08/2019, às 10:06, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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