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Long-term effects of early maternal deprivation on goat social behaviour

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ABSTRACT

Early maternal deprivation has been shown to disrupt goat kids' social behaviour and stress-coping strategy, and has long-term effects in other species like cattle. We studied the long-term effects of early maternal deprivation on 18-month-old goats. Seventeen goats were raised together with their dams (**DR** kids) and other lactating goats and kids, while 18 goats were separated from their dams three days after birth and artificially reared together (**AR** kids). Kids of both treatments were weaned around two to three months of age, grouped and raised together until this study 15 months later. Affiliative, playful, and agonistic behaviour was recorded by focal sampling in the home pen, when the focal goat had rejoined the herd after being physically isolated for 3 minutes, and after the focal goat was restrained and manipulated for 3 minutes. Behavioural observations were also conducted after the goats were introduced in groups of four in a herd of 77 unknown, lactating multiparous goats. Avoidance distance tests were performed in the home pen to assess the human-animal relationship. Salivary cortisol was measured before and after physical isolation, and faecal glucocorticoid metabolites were measured before and 24 hours after introduction in the lactating herd. In the home pen, AR goats were involved in less head nudging than DR goats, but other social behaviours or their behavioural and physiological response to the various stressful situations were not affected by their rearing treatment. Upon introduction in a DR lactating herd, most of the agonistic interactions observed were initiated by multiparous goats towards the introduced AR and DR goats alike. AR goats received more threats from the multiparous goats than DR goats, but were involved in less clashing than DR goats. AR goats showed less avoidance of familiar and unfamiliar humans than DR goats. Overall, previously AR and DR goats showed only a few differences in affiliative and agonistic behaviours in their home pen or after being exposed to different stressors 15 months later. Nonetheless, after being introduced into a multiparous goat herd, AR goats were still threatened more often than DR goats, and DR goats clashed more than AR goats, suggesting some persistent differences in their social abilities observed at an early age before and after weaning. As predicted, AR goats remained less fearful of humans than DR goats.

Implications

Maternal deprivation and artificial rearing affect goat kids' development but remain common practices in dairy farming. Goats previously reared without their mother as kids were still less fearful of humans 18 months later, bleated more during social isolation, but also received more threats and clashed less when introduced in the lactating herd. Nevertheless, other behavioural and physiological responses to isolation or restraint were unaffected by the rearing treatment. Rearing kids artificially appear to have some long-term effects on the measures collected in this study as goats 1.5 years later, but kids of both treatments were mixed together after weaning, which may have weakened differences due to social learning.

Introduction

A goat dam is not only her kid's primary source of food, warmth, and security (Nowak et al., 2000; Poindron, 2005; Newberry and Swanson, 2008) but also the kid's primary affiliative relationship and role model (Collias, 1956). Modern dairy farming typically disturbs this bond by separating the kid from its dam in the first days after birth and rearing it with same-age peers (von Korn et al., 2013). Such maternal deprivation was found to affect the kid's development and behaviour. A goat kid reared only with same-

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age peers is more behaviourally active in stressful situations, less neophobic towards social and non-social stimuli, less fearful of humans than a kid reared with its mother, but also initiates less aggressive interactions towards unknown kids after weaning (Hersher, 1969; Lyons and Price, 1987; Lyons et al., 1988a; 1988b; Toinon et al., 2021; 2022a). Yet, little is yet known about the long-term effects of early maternal deprivation in goats. Maternal deprivation can also have long-term effects on other ruminants' sociability and social competence such as cattle (Wagner et al., 2012), which are especially important for lactating ruminants who typically undergo introduction in a herd composed of unknown individuals or regrouping between each lactation cycle, which can disturb social stability (Patt et al., 2012 and 2013). Mother-reared heifers are more reactive and more motivated to re-establish social contact during an isolation test and show more submissive behaviour when introduced into a lactating herd than heifers separated from their dam after birth and reared with peers (Wagner et al., 2012; 2015). A previous study from our group showed that mother-reared goats maintained closer proximity to familiar peers after being introduced in a herd of unknown, older adult individuals than goats reared with same-age peers (Szabò et al., 2013). This propensity to stay closer to known peers could reflect greater abilities to utilise social support, the process by which a companion can enhance an animal's stress-coping abilities (Rault, 2012).

This study compared the behavioural and physiological responses during farm-relevant stressful situations of 18-month-old goats reared with their mothers in a herd of goats and kids vs. goats reared only with peers of a similar age during the first two months of life. We expected dam-reared goats to display more affiliative and agonistic behaviours (i.e. predicting a richer behavioural repertoire) in their home pen, after different stressors, or after introduction in a herd of unknown individuals compared to goats that had been reared only with same-aged peers. We also expected goats reared with their dams to be more fearful of humans than goats reared with same-age peers. Preliminary results have been published in an abstract form (Toinon et al., 2022b).

Material and methods

Animals and housing

The study was performed at an organic dairy goat farm in Austria and focused on 18-month-old Saanen goats reared in different social environments during their early life. Twenty females were raised together with their dam as kids (**DR** kids) in a herd composed of other lactating goats and kids, while 20 females were separated from their dam three days after birth and artificially reared together with other kids (**AR** kids), visually separated from the milking herd (for more details on the rearing treatments, see Toinon et al., 2021). All kids were disbudded and therefore did not have horns. We used mothers that were themselves dam-reared for the dam-reared kids as this farm typically uses a dam-rearing system, and because we consider being reared by its mother the biological norm for an altricial animal like the domestic goat. All animals were mixed together at three months of age and stayed in the same pen. Three DR and two AR animals died between weaning and the time of this study, but two DR animals were added that were not focal animals in the previous studies (Toinon et al., 2021; 2022a) but that were reared together with the other DR animals.

Seven DR and five AR animals were successfully mated by the buck at 9 months of age, gave birth and were subsequently integrated in a lactating herd of 76 individuals as primiparous focal goats five months before the beginning of the trial. The remaining 12 DR and 13 AR nulliparous focal goats stayed in their pen, to which 42 younger dam-reared nulliparous goats were added two months before the beginning of the trial. All 67 nulliparous goats were marked using long-lasting hair-dye the day before the beginning of the trial, and each focal primiparous goat was marked using hair-dye spray that was re-applied when the marking started fading. The lactating goats were milked twice a day, from 0430 h to 0600 h and from 1630 h to 1800 h. Concentrate was distributed to the animals three times a day and fresh hay every 36 hours. All animals were housed on deep litter straw in rectangular pens (36.0 m × 4.4 m) delimited by opaque walls on three sides and by a raised feeding table on the fourth long side.

Behavioural observations in the home pen

Behavioural observations were performed for 14 consecutive days by one trained observer blind to the treatment using "Animal behaviour pro 1.4.4" (Newton-Fisher, 2012) while the goats were in their home pen. Lying in contact behaviour (Table 1) for all nulliparous goats and the 12 focal primiparous goats were observed through scan sampling sessions. These sessions were performed between 0800 h and 1630 h with a half an hour interval, resulting in 18 scans per day, recording the identities of the goats lying in physical contact with each other. The other social behaviours of the nulliparous DR and AR goats were observed for 10 minutes twice a day through continuous sampling, once in the morning, once in the afternoon, every second day using an ethogram (Table 1). Observations were divided into four periods of 50 minutes between 0800 h and 1200 h, and four periods between 1220 h and 1630 h. Each goat was allocated to one period of observation in the morning and one period in the afternoon, with 3:50–5:10 hours between observation periods. This allocation alternated in a predetermined order every second day so that each goat was observed during all periods over four days of observation. Each period included three or four focal observations, and the exact time when one goat was observed within the period was randomised. The focal goat partner's identity was recorded for each affiliative, social play and agonistic behaviour (Table 1). In addition, the role of the focal goat was recorded as initiator or receiver for behaviours that have clear directions: rubbing another individual, allogrooming, stepping-on, and agonistic interaction.

Behavioural tests

The nulliparous goats went through two consecutive stressful tests interspersed by behavioural observations. The testing apparatus was built in the goats' home pen and included an isolation pen (1.2 m × 1.5 m) separated from the home pen by opaque walls on three sides and a 4.6 m empty corridor on the fourth side (Fig. 1). See-through doors separated the isolation pen from the empty corridor and the empty corridor from the home pen, and could be opened by an experimenter standing on the feeding table, outside the pen.

Testing procedure

The tests were conducted over three consecutive days, between 0800 h and 1630 h. At least half an hour before being tested, each goat was examined for lameness using a 5-point gait scoring system (Deeming et al., 2018) and restrained to be fitted with a heart rate monitoring device (Polar S810 heart rate monitor) and sampled for saliva using a Salimetrics Children's Swab (SalivaBio, Carlsbad, USA). Each focal goat was caught by the same unfamiliar experimenter, led through the empty corridor to the isolation pen, and left alone in the isolation pen for 120 seconds, while the number of its vocalisation was recorded. Then, the door separating

Table 1
Behaviours of the goats recorded during focal observations and scan sampling sessions (source: Szabò et al., 2013).

Category	Behaviour	Definition
Affiliative behaviours	Rubbing[1,2,3]	The initiator scrapes its head, horns or neck towards the passive receiver's head, horns, neck or body, without causing the recipient withdrawal
	Allogrooming[1,2,3]	The initiator uses its tongue, lips or teeth to scrape the head or body, except vulva and anus, of the recipient without causing its withdrawal
	Standing in contact[1,3]	The focal goat stands static while in physical contact with another (standing or lying) static goat. If the leg is the only body part in contact with the other goat, 5 cm^2 of leg has to touch the other goat.
	Lying in contact[1,3,4]	The ventral surface of a goat is in contact with the floor, and a part of her body is in contact with another lying goat. If the leg is the only body part in contact with the other goat, 5 cm^2 of leg has to touch the other goat, otherwise, the goat is considered as resting.
	Stepping-on[1,2,3]	The initiator is standing while having at least one leg in contact with the back of the receiver's body, without causing its withdrawal
Social play behaviour	Play fighting[1,2,3]	Two individuals simulate a fight without causing the withdrawal of one of the individuals, making contact with their foreheads or clashing their foreheads with little strength, eventually pushing each other without strength or circling each other, often interspersed with affiliative behaviour.
Agonistic behaviours	Avoiding[1,4]	The focal goat retreats when another one approaches. In case of feeding, she leaves her feeding space without any visible agonistic behaviour demonstrated by the approaching goat. The avoidance movement itself can be either slow or fast.
	Threatening[1,2,4]	The initiator presents her horns or forehead, indicates biting, or moves her head or body quickly towards the receiver.
	Head nudging[1,2,4]	Interactions of little intensity between two goats feeding or standing close together, such as sideways head movements towards neighbouring goats.
	Pushing[1,2,4]	The initiator pushes the receiver away from the feeding barrier using her shoulder or neck/head.
	Butting[1,2,4]	The initiator hits with her forehead/horn base, but without upward movement, any part of the receiver's body except the head.
	Head kicking[1,2,4]	The initiator performs a quick upward movement with her head and hits the receiver with the tip of her horns or forehead, but all extremities of the receiver stay on the ground.
	Lifting[1,2,4]	The initiator performs a quick upward movement with her head and hits the receiver with the tip of her horns or forehead, and at least one extremity of the receiving goat loses contact with the ground.
	Clashing[1,4]	Both goats face each other, eventually rear onto their hind legs, and strike forward, making contact with their foreheads.
	Biting[1,2,4]	The initiator bites the receiver on any part of its body, except vulva or anus.
Non-social behaviour	Resting alone[3]	The ventral surface of the focal goat is in contact with the floor but no part of her body is in contact with another lying goat.
	Other non-social[3]	The focal goat is involved in active non-social behaviours such as walking, drinking, eating or standing without physical contact with another goat.

[1] Behaviour for which the partner's ID was recorded.
[2] Behaviour for which the identities of the donor and receiver were recorded.
[3] State.
[4] Event.

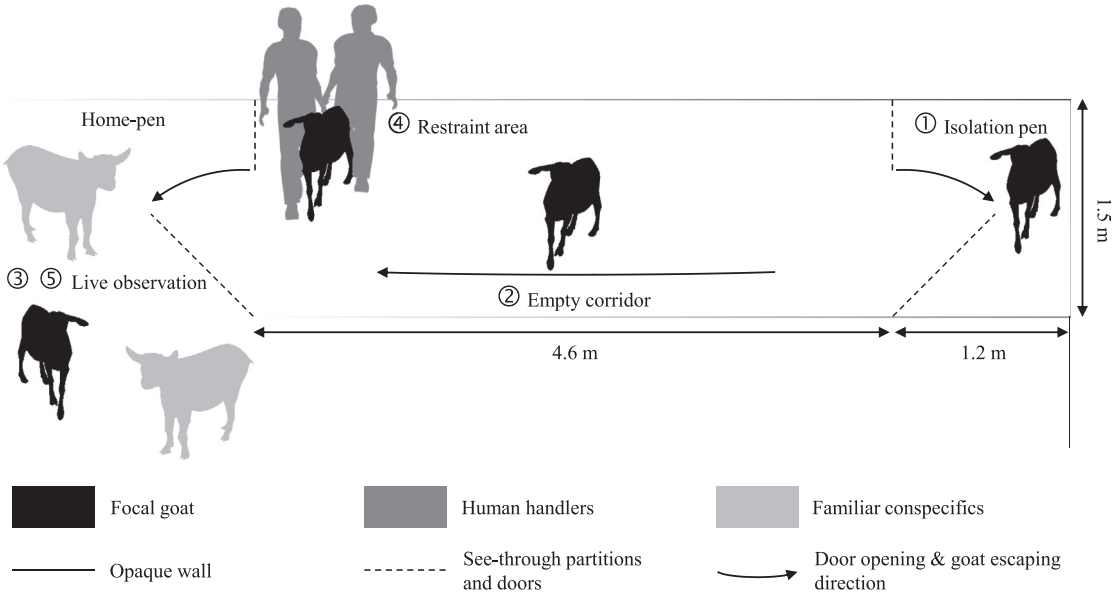

Fig. 1. Schematic representation of the setup created for the social reinstatement and social support tests. ① The goat stayed in the isolation pen for 2 minutes. ② The doors opened, allowing the goat to leave the isolation pen, cross the empty corridor and enter the home pen. ③ The social behaviour of the goat was recorded for 10 minutes. ④ The goat was brought to the restraint area and manipulated for 3 minutes. ⑤ The social behaviour of the goat was recorded for 10 minutes.

the isolation pen from the empty corridor was opened, allowing the goat to exit the isolation pen and cross the empty corridor back to its home pen. When the goat has crossed two third of the empty corridor, the door separating it from the home pen was opened, and the goat was able to rejoin its group in the home pen. The behaviour of the goat in the empty corridor was video-recorded

to measure the latency to cross the empty corridor. After the goat re-entered the home pen, its behaviour (Table 1) was recorded for 10 minutes by continuous focal sampling.

After the end of the 10-minute observation session, the goat was brought to the restraint area for one unfamiliar person to collect a saliva sample and perform a rectal faeces collection while being restrained by another unfamiliar person for 3 minutes. The goat was restrained manually facing the home pen and could see its conspecifics through the see-through partition wall (Fig. 1). After the restraint period, the goat was brought back to its group in the home pen and its behaviour (Table 1) was recorded for another 10 minutes. Finally, the heart rate monitoring device was removed from the goat.

Salivary cortisol concentration

Saliva samples were collected while fitting the heart rate monitor on the focal goat to measure baseline salivary (free) cortisol concentration, and during the restraint period to quantify the effect of the isolation period on salivary cortisol concentration. Saliva samples were collected, stored and analysed using previously reported methods in our laboratory (Toinon et al., 2021). Samples with sufficient quantity, for eight DR and 11 AR goats, were analysed using the Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit from Salimetrics® (USA, Item No. 1-3002). All the samples were processed on the same plate, with an intra-assay CV $\leq$ 9.5%.

Human-animal relationship tests

Two trained experimenters, one familiar and one unfamiliar to the goats, performed avoidance distance tests on each focal goat on the same day following the method used by Toinon et al. (2021) in the goats' home pens, and were both blind to treatments. The familiar experimenter had blonde hair and exclusively cared for the AR goats during their first weeks of life, and the unfamiliar experimenter had grey hair and never saw the animals before testing them. Both experimenters were similar height, female, and wore green overalls and rubber boots. The experimenter stood 2 m in front of the individual goat until it was standing and gazing in their direction. Then, the experimenter moved one step per second towards the goat. At the first avoidance reaction from the goat, the experimenter visually estimated the distance between their hand and the goat's muzzle with a resolution of 10 cm, which represented the avoidance distance for that goat. In case the experimenter was able to establish physical contact with that goat but it withdrew right after being touched, the avoidance distance was counted as 0.01 m. If the experimenter was able to stroke the goat on its head, the avoidance distance was counted as 0 m. If the experimenter could not approach the goat at the starting distance of 2 m without the animal withdrawing, the avoidance distance was counted as 2 m. The test was restarted if any disturbance occurred, such as the goat stopped gazing at the experimenter, or if another goat crossed the path of the experimenter or interacted with the tested goat.

Introduction to a new herd trial

Nine groups of four focal goats were formed and introduced one at a time into the multiparous lactating goat herd for 33 hours, to mimic the introduction of replacement goats to the lactating herd commonly done on dairy farms. The 77 multiparous goats were all dam reared and lactating for more than eight months, but had diverse age and horn status. For each introduced group, the four goats composing it were retrieved from their herd, sampled for faeces, and brought in the multiparous herd's pen at 0730 h on day 1. They stayed in the multiparous herd, were sampled for faeces again at 0730 h on day 2, and were brought back to their home pen at 1630 h on day 2.

Group formation

Each group of four goats was balanced for rearing treatment (i.e. two AR and two DR goats) and included only one highly affiliated dyad among the six possible dyadic relationships between those four goats. The highly affiliated dyad either consisted of two DR goats or two AR goats, and this was balanced across groups. The simple-ratio-index (SRI) of each dyad was computed based on the records of lying in contact behaviour collected during the scan sampling observations done prior and used as a proxy for the strength of the affiliative bond of one dyad (Farine and Whitehead, 2015), with $SRI_{AB} = x/(x + yAB + yA + yB)$ the index of the dyad composed of the goats A and B, and:

x: the number of scan samples where A and B were observed lying in contact together.
yAB: the number of scan samples where both goats were recorded lying in contact with other goats but not together.
yA: the number of scan samples where goat A was observed lying in contact with another peer while goat B was not lying in contact with anyone.
yB: the number of scan samples goat B was observed lying in contact with another peer while goat A was not lying in contact with any peer.

Behavioural observation

Behavioural observations were conducted on each individual of the group of four introduced goats by two trained observers using Animal behaviour pro 1.4.4 (Newton-Fisher, 2012). Inter-observer reliability testing was performed on videos until reaching at least 80% agreement among raters on each video prior to starting the observations. Each introduced goat was observed for its social behaviour through 32 continuous focal sampling sessions of 10 minutes following the method detailed previously. The observation sessions were equally distributed over the two days spent in the multiparous herd, with half of the observations conducted between 0750 h and 1200 h and half of the observations conducted between 1230 h and 1640 h. The observations of each goat were also balanced between observers.

Faecal glucocorticoid metabolites

Faecal samples were collected right before introducing the group in the multiparous herd to assess baseline faecal glucocorticoid metabolite (**FCM**) concentration, and 24 h later to quantify the short-term effect of introduction into the multiparous goats' herd on FCM concentration reflecting their adrenocortical response to this event. About one gram of faeces was taken from the rectum, immediately put on ice and stored at −20 °C once all goats were sampled. Samples were later thawed, extracted by centrifuging 0.5 g of faeces with 5 mL of 80% methanol (Palme et al., 2013), and FCMs were determined using a group-specific 11-oxoaetiocholanolone enzyme immunoassay (Möstl et al., 2002), which has been validated for use in goats (Kleinsasser et al., 2010). Intra- and interassay CVs were below 10 and 15%, respectively.

Statistical methods

Statistical analyses were performed using RStudio (version 1.2.5033; RStudio Team 2019). Each behaviour (Table 1) observed in the home pen and during the behavioural tests was considered a distinct response variable and was analysed using a generalised linear mixed-effects model. Whether the different behaviours (Table 1) occurred or not during each observation session was

analysed using binomial regressions, as the different behaviours did not occur often enough to analyse their duration or frequency. Each model included the fixed effects of treatment (DR, AR), stressor type (no stressor type, isolation, restraint), the interaction between treatment and stressor type, type of partner (DR, AR, non-focal; if relevant for this behaviour), role (initiator, receiver; if relevant for this behaviour), and the z-transformed time of the day and testing day as fixed effects. Goat's identity and dam (as two third of the focal goats were twins; Toinon et al., 2021) were included as random effects, and the random slopes for type of partner, stressor type, day and time within goat's identity and dam were included. Bleats during the isolation test were analysed using a negative binomial model including the fixed factors treatment and time of the day, and the random factors goat's identity, dam, and testing day. Latency to cross the empty corridor was analysed using a linear mixed model including the fixed factors treatment, time of the day, and gait score, and the random factors goat's identity, dam, and testing day. Salivary cortisol concentration was analysed using a linear mixed model including the fixed factors treatment, time point (before or after isolation), the interaction between treatment and time point, and time of the day, and the random factors goat's identity, dam, and testing day. The human avoidance distance test results were beta-transformed to fit in the open interval (0,1) before being analysed using a Beta regression model including the fixed factors treatment, experimenter (familiar or unfamiliar human), and the interaction between treatment and experimenter, and the random factors goat's identity and dam.

Each behaviour observed in the multiparous goat herd (Table 1) was considered a distinct response variable and was analysed using a generalised linear mixed-effects model. Whether the different behaviours (Table 1) occurred or not during each observation session were analysed using binomial regressions, as the different behaviours did not occur often enough to analyse their duration or frequency. Each model included the fixed effects treatment (DR, AR), the strength of the highest affiliative bond with another introduced goat, the interaction between treatment and affiliative bond strength, type of partner (introduced, resident; if relevant for this behaviour), role (initiator, receiver; if relevant for this behaviour), the z-transformed time since introduction, and observer as fixed effects. Goat's ID, dam and group were included as random effects, and the random slopes for type of partner, time, observer and role within goat's identity and dam, treatment, strength of the bond, type of partner, time, role, and observer within group were included. FCM concentrations were analysed using a linear mixed model including the fixed factors treatment, time point (before or after introduction), the interaction between treatment and time point, and the strength of the highest affiliative bond with another introduced goat, and the random factors goat's identity, dam, and group and the random slopes for treatment, day, and affiliative bond within group. For each response variable, the effect of treatment was tested by conducting a full-null model comparison using a likelihood ratio test, with the null model only differing from the full model by lacking the treatment factor.

Results

Behaviour in the home pen

A total of 617 social interactions were recorded, including 173 head nudging, 101 standing in contact, 51 stepping-on, 44 lying in contact, 44 threatening, 44 pushing, 29 head kicking, 28 butting, 27 biting, 21 rubbing, 19 allogrooming, 17 avoiding, 11 play fighting, and 8 clashing, but no lifting. Head nudging occurred less in AR goats than in DR goats (χ^2 = 3.7, df = 1, P = 0.05, Table 2), tended to occur more after isolation than after restraint or no stressor type (χ^2 = 5.0, df = 2, P = 0.08, Table 3), and occurred more between one focal goat and one younger non-focal goat than between two focal goats (χ^2 = 18.8, df = 2, P < 0.001, Table 3). Lying in contact occurred more after isolation or no stressor type than after restraint (χ^2 = 6.3, df = 2, P = 0.04, Table 3), and occurred more between two DR goats and two AR goats than between a DR goat and an AR goat or between a focal goat and a non-focal goat (χ^2 = 28.3, df = 2, P < 0.001, Table 3). Head kicking occurred more after no stressor type than after isolation or restraint (χ^2 = 7.4, df = 2, P = 0.02, Table 3), but treatment, the interaction between treatment and stressor type, and type of partner were not significant. Standing in contact occurred more between a focal goat and a DR goat than between a focal goat and an AR goat or a non-focal goat (χ^2 = 9.6, df = 2, P = 0.01, Table 3), but treatment, stressor type, and the interaction between treatment and stressor type were not significant. Threatening, pushing, and butting occurred more between one AR or DR goat and one non-focal goat than between two focal goats (threatening: χ^2 = 12.1, df = 2, P = 0.04; pushing: χ^2 = 6.8, df = 2, P = 0.04; butting: χ^2 = 16.7, df = 2, P < 0.001; Table 3), and biting occurred more towards AR goats, and then non-focal goats, than towards DR goats (χ^2 = 12.4, df = 2, P = 0.002; Table 3), but treatment, stressor type, and the interaction between treatment and stressor type were not significant. Play-fighting increased across days (χ^2 = 158.7, df = 1, P = 0.02), and standing in contact decreased across days (χ^2 = 4.5, df = 1, P = 0.03). Standing in contact decreased over the course of a day (χ^2 = 10.2, df = 1, P = 0.001). Resting alone, stepping-on, and avoiding did not significantly differ according to any of the factors.

Table 2
Estimated percentage of observation of the goats in the home pen where at least one occurrence of the behaviour was recorded ± SE and *P*-value of the factor treatment. AR: artificially reared goats, DR: dam-reared goats.

	Treatment		
Behaviour (%)	AR	DR	*P*-value
Rubbing*	–	–	–
Allogrooming*	–	–	–
Standing in contact	0.0 ± 4.7	0.1 ± 1.5	0.47
Lying in contact	16.7[a] ± 0.5	0.0[b] ± 125	<0.001
Stepping-on	3.5 ± 0.4	3.3 ± 0.4	0.91
Play fighting*	–	–	–
Avoiding	1.3 ± 0.8	0.3 ± 1.3	1.00
Threatening	0.7 ± 1.4	0.5 ± 0.5	0.55
Head nudging	6.7[a] ± 0.3	11.3[b] ± 0.4	0.05
Pushing	1.6 ± 0.7	2.3 ± 0.4	0.49
Butting	0.4 ± 1.1	0.4 ± 0.4	1.00
Head kicking	0.5 ± 1.0	0.8 ± 0.5	0.57
Biting	1.1 ± 0.8	1.1 ± 0.5	0.80
Resting alone	17.7 ± 0.5	17.0 ± 0.5	1.00

* Considered too low occurrence to allow robust statistical analyses.

Behavioural and human-animal relationship tests

The avoidance distance of the human experimenter of AR goats was lower than that of DR goats (χ^2 = 39.4, df = 2, P < 0.001; Fig. 2), but experimenter familiarity and the interaction between treatment and experimenter familiarity were not significant.

Bleating during the isolation period was more frequent in AR goats than in DR goats (AR: 30.2 ± 0.2, DR: 17.4 ± 0.3, χ^2 = 4.0, df = 1, P = 0.05), but time of the day was not significant. The salivary cortisol concentration was higher 10 minutes after the isolation period than before isolation (before: 0.026 ± 0.01 µg/dL, after: 0.118 ± 0.01 µg/dL, χ^2 = 5.2, df = 1, P = 0.02), but treatment, the interaction between treatment and time point, and time of the day were not significant. The latency to cross the empty corridor

Table 3
Estimated percentage of observation of the goats in the home pen where at least one occurrence of the behaviour was recorded ± SE and *P*-value of the factors stressor type and type of partner. AR: artificially reared goats, DR: dam-reared goats, NF: Non-focal nulliparous goats.

		Stressor type				Type of partner			
Behaviour (%)	Treatment	No stressor	Isolation	Restraint	*P*-value	Partner AR	Partner DR	Partner NF	*P*-value
Rubbing*		–	–	–	–	–	–	–	–
Allogrooming*		–	–	–	–	–	–	–	–
Standing in contact		4.1 ± 4.7	13.1 ± 1.0	6.6 ± 8.9	0.39	4.1^{a} ± 4.7	90.4^{b} ± 4.3	0.3^{a} ± 5.2	0.01
Lying in contact	AR	0.0^{a} ± 0.5	0.0^{a} ± 1.2	0.0^{b} ± 26.1	0.04	16.7^{a} ± 0.5	0.0^{b} ± 574	0.0^{b} ± 415	<0.001
	DR					0.0^{a} ± 125	12.5^{b} ± 138	0.0^{a} ± 132	
Stepping-on		15.6 ± 0.4	18.8 ± 0.8	7.3 ± 1.1	0.56	15.6 ± 0.4	16.2 ± 0.5	25.8 ± 0.4	0.29
Play fighting*		–	–	–	–	–	–	–	–
Avoiding		5.4 ± 0.8	40.9 ± 1.5	37.3 ± 1.5	0.54	5.4 ± 0.8	0.1 ± 2.6	0.0 ± 504	0.20
Threatening		6.5 ± 1.4	7.6 ± 0.8	5.1 ± 1.1	0.89	6.5^{a} ± 1.4	16.1^{a} ± 1.5	41.9^{b} ± 1.4	0.002
Head nudging		26.2 ± 0.3	42.8 ± 0.4	25.7 ± 0.6	0.08	26.2^{a} ± 0.3	17.4^{a} ± 0.4	43.3^{b} ± 0.3	<0.001
Pushing		13.3 ± 0.7	1.2 ± 2.0	2.3 ± 1.7	0.18	13.3^{a} ± 0.7	11.7^{a} ± 0.7	30.6^{b} ± 0.7	0.04
Butting		5.9 ± 1.1	4.3 ± 1.0	0.7 ± 2.1	0.27	5.9^{a} ± 1.1	3.8^{a} ± 1.7	35.5^{b} ± 1.0	<0.001
Head kicking		8.4^{a} ± 1.0	1.1^{b} ± 1.7	0.0^{b} ± 3.5	0.02	8.4 ± 1.0	6.9 ± 1.2	22.8 ± 1.0	0.36
Biting		9.7 ± 0.8	2.6 ± 1.6	0.0 ± 4.2	0.18	9.7^{a} ± 0.8	0.1^{b} ± 4.5	2.3^{c} ± 0.7	0.002
Resting alone		31.2 ± 0.5	11.2 ± 1.1	31.7 ± 0.9	0.29	-	-	-	-

* Considered too low occurrence to allow robust statistical analyses.

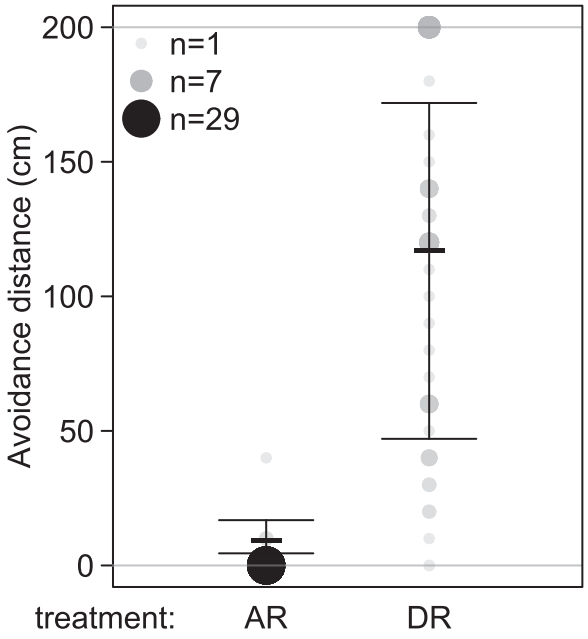

Fig. 2. Avoidance distance by the goats of the human: estimated means and confidence interval. AR: artificially reared goats. DR: dam-reared goats.

did not significantly differ according to treatment, gait score, or time of the day (7.9 ± 1.2 s).

Behaviour after the introduction into the multiparous goat herd

A total of 8 079 social interactions were recorded, including 2 043 head nudging, 1 960 threatening, 1 045 avoiding, 976 standing in contact, 496 lying in contact, 372 head kicking, 266 butting, 236 biting, 139 play-fighting, 132 rubbing, 107 lifting, 105 pushing, 90 allogrooming, 89 stepping-on, and 23 clashing. Threatening occurred more in AR than in DR goats (χ^2 = 6.8, df = 1, *P* = 0.01, Table 4), occurred more between one introduced goat and one resident goat than between two introduced goats (χ^2 = 34.5, df = 1, *P* < 0.001, Table 5), with the introduced goats being the receivers in most of these interactions (χ^2 = 109.9, df = 1, *P* < 0.001, Table 5), and sooner after introduction in the herd (χ^2 = 6.5, df = 1, *P* < 0.01, Table 5), but the strength of the affiliative bond was not significant.

Table 4
Estimated percentage of observation in the multiparous goat pen where at least one occurrence of the behaviour was recorded ± SE and *P*-value of the factor treatment. AR: artificially reared goats, DR: dam-reared goats.

	Treatment		
Behaviour (%)	AR	DR	*P*-value
Rubbing	0.4 ± 0.5	0.4 ± 0.3	0.87
Allogrooming	0.2 ± 0.8	0.3 ± 0.4	0.92
Standing in contact	25.3 ± 0.2	25.9 ± 0.1	0.76
Lying in contact	0.7 ± 0.5	0.7 ± 0.2	0.49
Stepping-on	0.0 ± 3.1	0.0 ± 0.5	0.92
Play fighting	2.0 ± 0.3	2.1 ± 0.3	0.55
Avoiding	42.0 ± 0.2	47.1 ± 0.1	0.30
Threatening	1.4^{a} ± 0.4	1.3^{b} ± 0.1	0.01
Head nudging	9.2 ± 0.2	8.7 ± 0.1	0.37
Pushing	1.2 ± 0.4	1.2 ± 0.2	0.74
Butting	0.8 ± 0.6	0.5 ± 0.3	0.1
Head kicking	0.3 ± 0.8	4.5 ± 0.2	0.59
Lifting	0.1 ± 1.7	0.0 ± 0.3	0.99
Clashing	0.0^{a} ± 2.2	0.3^{b} ± 1.7	0.02
Biting	0.9 ± 0.4	1.2 ± 0.2	0.42
Resting alone	16.4 ± 0.2	18.6 ± 0.3	0.28

Clashing occurred more in DR goats than in AR goats (χ^2 = 5.3, df = 1, *P* = 0.02, Table 4), and type of partner, the strength of the bond, the interaction between treatment and bond, and time since introduction were not significant. Avoiding occurred the most in introduced goats because of a resident goat (χ^2 = 26.5, df = 1, *P* < 0.001, Table 5), decreased over time after introduction (χ^2 = 6.4, df = 1, *P* = 0.01, Table 5), but treatment, strength of the bond, and the interaction between treatment and strength of the bond were not significant. Lifting and biting occurred more between one introduced goat and one resident goat than between two introduced goats (lifting: χ^2 = 14.6, df = 1, *P* < 0.001; biting: χ^2 = 9.9, df = 1, *P* = 0.002; Table 5), with the introduced goats being the receivers in most of these interactions (lifting: χ^2 = 17.1, df = 1, *P* < 0.001; biting: χ^2 = 18.7, df = 1, *P* < 0.001; Table 5), increased across time since introduction (lifting: χ^2 = 4.0, df = 1, *P* = 0.04; biting: χ^2 = 5.3, df = 1, *P* = 0.02; Table 5), but treatment, strength of the bond, and the interaction between treatment and the strength of the bond were not significant. Rubbing occurred more between two introduced goats than between one introduced goat and one resident goat (χ^2 = 7.8, df = 1, *P* = 0.005, Table 5), decreased over time since introduction (χ^2 = 6.4, df = 1, *P* = 0.01, Table 5), but treatment, strength of the bond, and the interaction between treatment and the strength of the bond were not significant. Lying in contact occurred more between two introduced goats than

Table 5
Estimated percentage of observation in the multiparous goat pen where at least one occurrence of the behaviour was recorded ± SE and *P*-value of the factors type of partner, strength of the bond, and time since introduction.

	Type of partner			Strength of the bond			Time since introduction		
Behaviour (%)	Introduced	Resident	*P*-value	Weak bond	Strong bond	*P*-value	Beginning	End	*P*-value
Rubbing	26.8^{a} ± 0.5	6.1^{b} ± 0.5	0.005	1.1 ± 0.2	6.7 ± 0.2	0.60	9.1^{a} ± 0.1	4.3^{b} ± 0.1	0.01
Allogrooming	29.8^{a} ± 0.7	4.8^{b} ± 0.8	0.001	0.6 ± 0.3	5.9 ± 0.3	0.44	4.9 ± 0.2	4.8 ± 0.2	0.92
Standing in contact	76.3^{a} ± 0.1	37.2^{b} ± 0.2	<0.001	37.0 ± 0.1	37.3 ± 0.1	0.95	49.1 ± 0.1	32.5 ± 0.1	0.13
Lying in contact	89.8^{a} ± 0.5	7.6^{b} ± 0.5	<0.001	0.0 ± 0.1	8.0 ± 0.1	0.76	5.6^{a} ± 0.1	10.1^{b} ± 0.1	0.03
Stepping-on	78.0^{a} ± 3.0	0.7^{b} ± 3.1	<0.001	0.0 ± 0.5	0.7 ± 0.5	0.88	0.8 ± 0.2	0.7 ± 0.2	0.43
Play fighting	2.0^{a} ± 0.4	2.1^{b} ± 0.3	0.003	3.4 ± 0.2	11.0 ± 0.2	0.39	17.6 ± 0.1	10.0 ± 0.1	0.09
Avoiding	0.8^{a} ± 0.5	48.6^{b} ± 0.2	<0.001	46.5 ± 0.1	50.6 ± 0.1	0.45	70.8^{a} ± 0.1	42.2^{b} ± 0.1	0.01
Threatening	0.5^{a} ± 0.2	10.6^{b} ± 0.4	<0.001	10.6 ± 0.1	10.6 ± 0.1	0.99	10.7^{a} ± 0.1	10.5^{b} ± 0.1	0.01
Head nudging	14.7^{a} ± 0.2	2.4^{b} ± 0.2	0.004	21.1 ± 0.1	26.2 ± 0.1	0.18	21.9 ± 0.1	24.7 ± 0.1	0.44
Pushing	23.6^{a} ± 0.3	10.0^{b} ± 0.4	0.003	3.8 ± 0.4	6.4 ± 0.4	0.10	9.2 ± 0.2	10.6 ± 0.2	0.69
Butting	0.9^{a} ± 0.4	6.5^{b} ± 0.6	<0.001	5.7 ± 0.3	7.6 ± 0.3	0.55	6.8 ± 0.1	6.3 ± 0.1	0.78
Head kicking	0.3^{a} ± 0.6	4.7^{b} ± 0.8	<0.001	4.3 ± 0.2	5.2 ± 0.2	0.54	4.4 ± 0.1	5.1 ± 0.1	0.62
Lifting	0.1^{a} ± 0.9	2.1^{b} ± 1.7	<0.001	36.4 ± 0.3	2.2 ± 0.3	0.79	1.5^{a} ± 0.2	2.9^{b} ± 0.2	0.04
Clashing	9.9 ± 1.4	5.0 ± 2.2	0.57	2.5 ± 0.6	10.8 ± 0.6	0.13	7.2 ± 0.5	3.6 ± 0.5	0.44
Biting	2.9^{a} ± 0.2	10.1^{b} ± 0.4	0.002	29.0 ± 0.2	10.3 ± 0.2	0.49	7.2^{a} ± 0.1	13.2^{b} ± 0.1	0.02
Resting alone	–	–	–	39.6 ± 0.2	25.9 ± 0.2	0.07	35.1 ± 0.2	31.1 ± 0.2	0.49

between one introduced goat and one resident goat (χ^2 = 26.1, df = 1, P < 0.001, Table 5), increased across time since introduction (χ^2 = 4.5, df = 1, P = 0.03, Table 5), but treatment, strength of the bond, and the interaction between treatment and the strength of the bond were not significant. Play fighting occurred more in resident goats than introduced goats (χ^2 = 9.0, df = 1, P = 0.003, Table 5), tended to decrease over time since introduction (χ^2 = 2.9, df = 1, P = 0.09, Table 5), but treatment, strength of the bond, the interaction between treatment and the strength of the bond were not significant. Head nudging, butting and head kicking occurred more between one introduced goat and one resident goat than between two introduced goats (head nudging: χ^2 = 8.0, df = 1, P = 0.004; butting: χ^2 = 13.4, df = 1, P < 0.001; head kicking: χ^2 = 12.7, df = 1, P < 0.001; Table 5), with the introduced goats being the receivers in most of these interactions (head nudging: χ^2 = 21.5, df = 1, P < 0.001; butting: χ^2 = 19.3, df = 1, P < 0.001; head kicking: χ^2 = 15.5, df = 1, P < 0.001; Table 5), but treatment, strength of the bond, the interaction between treatment and the strength of the bond, and time since introduction were not significant. Standing in contact, stepping-on, allogrooming, and pushing occurred more between two introduced goats than between one introduced goat and one resident goat (standing in contact χ^2 = 25.8, df = 1, P < 0.001; stepping-on: χ^2 = 15.0, df = 1, P < 0.001; allogrooming: χ^2 = 10.9, df = 1, P < 0.001; pushing: χ^2 = 8.8, df = 1, P = 0.003; Table 5), but treatment, strength of the bond, the interaction between treatment and the strength of the bond, and time since introduction were not significant. Resting alone tended to occur less with stronger affiliative bond with another introduced goat (χ^2 = 3.4, df = 1, P = 0.06, Table 5), but treatment, the interaction between treatment and strength of the bond, and time since introduction were not significant. FCM concentration was higher after 24 h in the multiparous goat herd (before introduction: 295.1 ± 55.8 ng/g; after introduction: 590.2 ± 84.0 ng/g; χ^2 = 7.6, df = 1, P = 0.01), but treatment, the interaction between treatment and day, and the strength of the bond were not significant. Allogrooming, standing in contact, pushing, butting, and biting varied between the two observers (allogrooming: χ^2 = 7.8, df = 1, P = 0.005; standing in contact: χ^2 = 9.8, df = 1, P = 0.002; pushing: χ^2 = 9.4, df = 1, P = 0.002; butting: χ^2 = 10.0, df = 1, P = 0.002; biting: χ^2 = 11.6, df = 1, P < 0.001).

Discussion

AR goats let the human experimenter approach them much closer than DR goats during the avoidance distance tests, and this difference was not affected by the familiarity of the experimenter. Therefore, the human-animal relationship difference found towards a caretaker when the animals were four months old (Toinon et al., 2021) remained 14 months later, and AR goats generalised their response to an unknown human (Jones, 1994; Hemsworth et al., 1996; Waiblinger et al., 2006).

AR goats also bleated more than DR goats during the isolation period, similarly to their vocalisation response during a similar behavioural test as one-month-old kids (Toinon et al., 2021), suggesting that the higher behavioural reactivity of AR goats during social isolation is a stress-coping difference that remain in the long-term. Such effect of rearing social environment on goats' personality has been previously shown in a shorter-term study in goat kids, although the behaviour of the DR kids changed after they were separated from their dams at 14 weeks of age as they tended to react more, and more like AR kids at 22 and 30 weeks of age (Lyons et al., 1988a).

Another long-lasting social behaviour difference between treatments was in the higher involvement of DR compared to AR goats in agonistic interactions. After the focal goats had been weaned and mixed with kids of the opposite treatment at two to three months of age, AR kids received more agonistic interactions from DR kids than the opposite (Toinon et al., 2022a). In this study, sixteen months later, AR goats were still threatened more often than DR goats and DR goats clashed more than AR goats after being introduced into the multiparous goat herd. These behavioural differences indicate some long-lasting effects of early social and maternal deprivation on social strategy or social competence, as shown in cattle (Le Neindre, 1989; Wagner et al., 2012). DR goats seem to be better able to avoid receiving aggression during their introduction into an adult herd. However, the increase in FCMs did not differ between treatments. Therefore, although rearing treatment differed in their social skills, it did not translate to detectable differences in the level of stress experienced after being introduced to the multiparous goat herd. Whether one strategy is better in terms of access to resources and welfare state warrants research (Miranda-de la Lama and Mattiello, 2010).

Interestingly, AR and DR goats preferentially laid down in contact with a goat from their own treatment, despite being housed together with the other treatment for the last 14 months, suggesting either long-lasting affiliative relationships, as shown in cattle (Bouissou and Andrieu, 1978), or preference for one own's kind. However, the social behaviours in the home pen remain relatively unaffected by the stressful experiences the goats went through, except for less head kicking after restraint by the humans. The stressors used might have been too mild or irrelevant for the goats

to need to physically interact with each other while the disappearance of the stressor and the mere proximity of their peers may have buffered their stress response (Cohen and Wills, 1985). The use of different stressors such as novelty or human encounters without restraint might be more appropriate stimuli to study the mobilisation of social support in goats (Cohen and Wills, 1985; Lyons et al., 1988b; Rault, 2012). Moreover, the method of observation and the low number of focal sampling sessions might have not allowed detecting potential subtle differences between treatments. Indeed, focal sampling recorded too few bouts of rarely occuring social behaviours to allow analysing their duration or frequency.

The methodology that we used to carry out this study on a commercial goat dairy farm does have some limitations to keep in mind. Since AR and DR kids were mixed together after weaning, social learning or social transmission may have reduced differences between treatments (Rørvang et al., 2018). As group-living animals, AR kids might have learned to show aggression towards DR kids and potentially dominate some of them by goal emulation, local enhancement or social facilitation (Nicol, 1995; Lefebvre et al., 1996; Rørvang et al., 2018). DR and in a lesser extent AR goats preferentially showed agonistic interactions towards younger nulliparous goats, probably reflecting dominance relationships as the lower body mass of the younger goats is associated with a lower rank in the herd (Barroso et al., 2000; Miranda-de la Lama and Mattiello, 2010). Whether AR kids would have developed their agonistic behavioural repertoire without being mixed with DR kids is to be further studied, as well as the potential long-term implications of lacking such social skills.

In conclusion, previously AR and DR goats showed only few differences in affiliative and agonistic behaviours in their home pen or after being exposed to different stressors 15 months later. Nonetheless, after being introduced into a multiparous goat herd, AR goats were still threatened more often than DR goats, and DR goats clashed more than AR goats, suggesting some persistent differences in their social abilities observed at an early age before and after weaning (Toinon et al., 2021; 2022a). As predicted, AR goats remained less fearful of humans than DR goats.

Ethics approval

All procedures were approved by the institutional ethics and animal welfare committee of the University of Veterinary Medicine, Vienna, following the guidelines for Good Scientific Practices and the national legislation (project numbers ETK-043/03/2020 and ETK-135/09/2020).

Data and model availability statement

None of the data were deposited in an official repository. Information can be made available from the authors upon request.

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Declaration of interest

None.

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