

1 Genetics for maternal reactivity

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3 **Maternal reactivity of ewes at lambing is genetically linked to their behavioral**
 4 **reactivity in an arena test**

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ABSTRACT

In sheep, the bond between the dam and her lambs is established during the first hours of a lamb's life. Genetic variability for behavioral reactivity of ewes assessed in an arena test performed 24 h after lambing has already been reported. However, there is no evidence that this reactivity represents the ewe's maternal reactivity at lambing in outdoor conditions. The objective of this study was to investigate whether or not the behavioral reactivity of ewes in the arena test is genetically related to their maternal reactivity measured at lambing. A total of 935 Romane ewes were studied. The maternal reactivity of ewes at the outdoor lambing site was recorded in response to a human approach and to the handling of the lambs. Their behavioral reactivity was also recorded 24 h post-lambing in the arena test that involved a separation from the litter and a human presence. Flight distance, aggressive reaction, time to restore contact with the litter at the lambing site and maternal behavior scores were moderately heritable (0.18 to 0.34), and vocalizations were slightly heritable (0.16). All of these behaviors were genetically correlated with the behavioral reactivity in the arena test. The highest genetic correlations (from 0.60 to 0.90) were found for maternal behavioral scores, flight distance and high-pitched bleats. In conclusion, behavioral reactivity in the arena test can be used to assess early maternal reactivity in standardized conditions. Such phenotyping could be used for genetic improvement of maternal behavior in sheep.

Keywords: genetic correlations, behavioral reactivity, lambing, arena test

INTRODUCTION

The behavior of both ewes and lambs at lambing and their interaction are important for the survival of their offspring, especially in extensive farming conditions (Dwyer, 2014; Nowak, Porter, Levy, Orgeur, & Schaal, 2000). Moreover, genetic improvement of lamb survival at birth is not efficient due to its very low heritability (Brien et al., 2014). Inappropriate behavior and low offspring survival could hinder the development of extensive farming systems, whereas agroecology promotes grazing systems for ruminants in order to limit competition between animal and human food (Phocas et al., 2016). Developing genetic selection of maternal behaviors could therefore be advantageous to improve offspring survival and growth, and to reduce labor and stress, as suggested by Mignon-Grasteau et al. (2005).

The development and the strength of the bond between ewes and their lambs are affected by several factors, including maternal experience, temperament, nutrition during pregnancy, breed and, to some extent, by the behavior of lambs (Dwyer, 2008a, 2008b). Individual differences in the maternal behavior of ewes result in the formation of bonds of varying degrees of strength with their lambs. In extensive conditions, perturbations can occur at the onset of maternal behavior and increase variations in maternal bonding with lambs. Early measurement of maternal behavior is therefore relevant. A scoring system was developed for use at lambing sites by O'Connor et al. (1985) to assess the ability of ewes to care for lambs. Nevertheless, the heritability of such a maternal score in sheep is generally low (Brown et al., 2015; Everett-Hincks & Cullen, 2009; Lambe, Conington, Bishop, Waterhouse, & Simm, 2001; Plush, Hebart, Brien, & Hynd, 2011). Maternal behavior in beef cows assessed through a scoring method was also lowly heritable (Michenet, Saintilan, Venot, & Phocas, 2016). For ewes that lamb outdoors in extensive farming systems, low

heritability scores for maternal behavior could be partly due to the large number of variation factors such as the social and grazing environments. In addition, assessment of maternal behavior at lambing in extensive conditions is limited by the high number of ewes that lamb during the night (i.e., almost 40% in our study) and also requests to the shepherd to spent more time waiting for lambing. To avoid such biases and limitations, investigation of the genetic component of maternal behavior has been recently done by exposing ewes reared in extensive conditions to a challenging standardized indoor situation. Moderate to high heritabilities for the behavioral reactivity of ewes in an arena test 24 h post-lambing have been reported (Hazard et al., 2020). Since selective bonding between ewes and lambs is constructed during the first six to 24 h after lambing (Keller et al., 2003), maternal reactivity has to be assessed after such a period of consolidation. However, the genetic relationships between maternal reactivity at lambing and behavioral reactivity post-lambing in the arena test remain unknown.

We addressed whether the measurement of behavioral reactivity in an arena test 24 h post-lambing can be used to estimate maternal reactivity at the lambing site 2 h post-lambing. We hypothesized that such a behavioral reactivity is genetically linked to early maternal reactivity.

MATERIALS AND METHODS

Ethical compliances

The experiments described here fully comply with applicable legislation on research involving animal subjects in accordance with the European Union Council directive (2010/63/UE). The investigators who carried out the experiments were certified by the relevant French governmental authority. All experimental procedures

were performed according to the guidelines for the care and use of experimental animals and approved by the local ethics committee (approval number SSA_2018_011).

Animals and management

A total of 570 primiparous Romane ewes (single lambing: 126; twin lambing: 295; triple and more lambing: 149) and 736 multiparous Romane ewes (single lambing: 85; twin lambing: 303; triple and more lambing: 348) were phenotyped. A total of 305 ewes were phenotyped twice or more. The ewes originated from 82 sires. The experiment was conducted at INRAE's La Fage Experimental Farm (Roquefort sur Soulzon, France) over a period of 11 years, and an average of 120 ewes were tested each year.

Ewes were reared exclusively outdoors under the harsh conditions of the Causses du Larzac plateau. Reproductive ewes were maintained in a single flock (250 reproductive females) reared on 280 ha of rangelands divided into paddocks (average surface per paddock: 15 ha; 150 m² per ewe at lambing, and up to 450 m² per ewe after weaning). The farming system, management and environmental characteristics were previously described by Gonzalez et al. (2014). Lambing takes place in the spring and outdoors. All lambs were identified at birth using electronic ear tags and weaned at 85 ± 4 days of age.

General experimental design and behavioral measurements

Maternal reactivity was assessed at the lambing site approximately 2 h after lambing, only on ewes that lambbed during daylight when the shepherd approached the lambing ewes to catch lambs for weighing and identification. For more clarity in the text, the

term, “maternal reactivity”, is used to describe all behaviors performed by ewes toward their litter right after lambing in response to the shepherd’s approach and the catching of lambs. Thus, in this study, maternal reactivity was characterized for ewes kept outside at their lambing site.

Measurement of maternal reactivity at the lambing site (LS) consisted of two successive phases: (1) when the shepherd approached the lambs; and (2) the capture and displacement of the lambs by the shepherd. In the first phase (LS1), the shepherd stood approximately 15 m away from the lambing spot and approached the ewes and the lambs at a regular speed (1 m/s). In the second phase (LS2), the shepherd caught all the lambs at the same time and moved away from the lambing spot in the same direction as that of the approach, stopping at the starting point where he placed the lambs back on the ground and then moved 15 m away to allow the ewe to restore contact with her lambs. This second phase of the test was not applied to ewes that flee at the approach of the shepherd and do not return within 60 s after the end of LS1.

A scoring system, close to those defined by O’Connor et al. (1985), was developed for each of the two phases to evaluate maternal reactivity. In LS1, a maternal behavior score (LS1-MBS) was recorded on a 5-point scale as follows: 1 - ewe flees and doesn’t return to the lambs within 60 s; 2 - ewe retreats (i.e., at least 2-3 m) but comes back to her lambs within 60 s; 3 - ewe retreats with at least one lamb and comes back; 4 - ewe retreats and returns repeatedly; 5 - ewe stays close to the lambing spot. In LS2, a second maternal behavior score (LS2-MBS) was recorded on a 4-point scale as follows: 1 - ewe flees; 2 - ewe stays close to the lambing spot, 3 - ewe follows but from a distance (i.e., 1 to 2 m), 4 - ewe follows, staying close to the shepherd (i.e., less than 1 m). The ewe’s flight distance from the lambing spot (LS1-FLIGHT) was recorded in LS1 as follows: more than 6 m, 2 to 6 m, and 0 to 2 m,

respectively, for classes 1, 2 and 3. Maternal aggressiveness (LS1-THREAT) towards the shepherd, expressed as striking its leg on the soil, was recorded in LS1 as a binary trait: class 1 = no and class 2 = yes. The time needed by the ewe to restore contact with her litter (LS2-CONTACT) was recorded in LS2 as follows: no contact within maximum duration of the test (i.e., 90 s), after human leaves; within 30 s; within 3 s, respectively, for class 1, 2, 3 and 4. The number of high- and low-pitched bleats (LS1/2-HBLEAT, LS1/2-LBLEAT) was recorded in LS1 and LS2 as categorical variables as follows: 0, 1 to 3, 4 to 6, and more than 6 bleats, respectively, for classes 1, 2, 3 and 4.

The ewes with their lambs were then introduced into the lactating female group while waiting for the next behavioral test. Indeed, the same experimental ewes were also individually exposed to an arena test (AT) 24 h post-lambing, as previously described by Hazard et al. (2020). Behaviors recorded in the three phases of the test (AT1/2/3) included high-pitched bleats (HBLEAT), low-pitched bleats (LBLEAT), locomotor activity (i.e., number of virtual zones crossed, LOCOM), time spent in vigilance postures (VIGIL), and the ewe's proximity to the litter and/or the human during phases 1 and 3 (PROX). For more clarity in the text, the term, "behavioral reactivity", was used to describe behaviors performed by ewes toward their litter in the AT. This behavioral reactivity in the AT corresponded successively to the ewe's (1) attraction to her litter, (2) reactivity to social separation from her litter, and (3) reactivity to a conflict between social attraction to her litter and avoidance of a motionless human. Thus, in the present study, behavioral reactivity characterized ewes moved indoors and submitted to a standardized behavioral test.

Statistical analysis

Descriptive statistics

Analyses of variance taking the repeated measures into account were performed to assess differences between ewes at different ages at the time of their first lambing, parity, number of lambs born and reared, and the year of measurement. The age at first lambing effect took ewes lambing for the first time at 1 or 2 years of age (classes 1 and 2, respectively) into account. The parity effect took first, second and third or more lambing (classes 1, 2, and 3, respectively) into account. The litter size effect was classified according to the number of lambs born and suckled (i.e., class 1: ewes lambing and suckling singletons; class 2: ewes lambing twins and suckling one; class 3: ewes lambing and suckling twins; and class 4: ewes lambing and suckling more than two lambs). Finally, the year effect took the 11 years of data collection into account. The GENMOD procedure of SAS® software was applied to the categorical variables to test the fixed effects and first-order interactions and to determine factors of variations for behaviors to be included in subsequent genetic analyses.

Genetic analysis

The (co)variance components for categorical behaviors were estimated by MCMC and Gibbs sampling methods using a threshold model in TM software (Legarra, Varona, & Lopez de Maturana, 2008). All analyses assumed a repeatability linear model with behavior measured across productive cycles considered to be the same trait with a constant variance. Random effects included a direct additive genetic effect of the animal (i.e., ewe) and a permanent environmental effect of the animal. The following animal mixed model was fitted:

$$y = Xb + Za + Wc + e \text{ [I]}$$

where y is the vector of observations corresponding to the trait(s) in the analysis; b is the vector of appropriate fixed effects (age at first lambing, parity of the ewe, litter

size and year of measurement); a is the vector of random genetic effects and c is the vector of permanent environmental effects; e is the vector of residual effects; X , Z and W are incidence matrices linking fixed effects, random animal genetic effects and random permanent environmental effects to the trait, respectively; a , c and e were assumed to be normally distributed with means equal to zero and (co)variances $A\sigma_a^2$, $I_c\sigma_c^2$, $I_e\sigma_e^2$ for a , c and e , respectively; A is the additive relationship matrix based on the pedigree; I are identity matrices of appropriate size, where σ_a^2 is the additive genetic variance, σ_c^2 is the variance due to the permanent environmental effect, and σ_e^2 is the residual variance.

Univariate analyses were performed to estimate variances for each trait. Multivariate analyses were performed to estimate genetic and phenotypic correlations between traits using the same model as the one used in univariate analyses. Variance estimates in the multi-trait analyses were very similar to those from single-trait analyses. Genetic and phenotypic correlations between quantitative traits and categorical traits were performed using TM software.

Three parameters were defined on the basis of the variance components: (1) heritability or proportion of total phenotypic variance attributed to the additive genetic effect, $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$; (2) proportion of total phenotypic variance attributed to the permanent environmental effect, $c^2 = \sigma_c^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$; and (3) proportion of total phenotypic variance attributed to the residual effect, $e^2 = \sigma_e^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$. In addition, repeatability (R) was defined as the sum of h^2 and c^2 .

RESULTS

Behavioral responses of the ewe at the lambing site

Descriptive statistics of behaviors are summarized in Table 1. The maternal behavior score in LS1 indicated that 59.3% of ewes stayed close to the lambing spot, and 38.1% of ewes retreated in different ways and came back. Only 2.6% of ewes fled and did not return. Eighty-five percent of ewes stayed between 0 and 2 m from the lambing spot in LS1. Ninety percent of ewes did not threaten the shepherd when he approached. The maternal behavior score in LS2 indicated that 77.2% of ewes followed their lambs, either staying close to the shepherd (62.5%) or keeping a distance from the shepherd (14.7%), while 10.1% stayed close to the lambing spot and 12.7% fled. In LS2, 71.2% of ewes restored contact with their litter within 3 s after moving their lambs, and 20% of ewes within 30 s. Nearly 95% of ewes did not make high-pitched bleats in LS1, while 42.1% of ewes made at least one high-pitched bleat in LS2. Fifty-nine percent of ewes made at least one low-pitched bleat in LS1, while 84.4% made at least one low-pitched bleat in LS2, with 49.9% of ewes making more than three low-pitched bleats.

Biological sources of variations in ewe behavior at the lambing site

The number of HBLEATs in LS2 significantly decreased with parity and age at the first lambing and increased with litter size (Table 2). The number of LBLEATs significantly increased in LS2 as parity increased. However, no significant parity, age at first lambing or litter size effects were observed on the number of high and LBLEATs in the LS1 test. Flight distance of ewes in LS1 and time to restore contact with their litter in LS2 increased as parity increased. Flight distance of ewes also decreased as litter size increased. Few variations of aggressive reactions were observed with parity, age at first lambing or litter size. The maternal behavior score in

LS1 increased as parity or litter size increased. The maternal behavior score in LS2 increased with the increase in parity.

Genetic parameters of ewe behavior at the lambing site

Heritabilities were moderate for maternal behavior scores, flight distance, aggressive behavior, and time to restore contact with litter (0.18 ± 0.05 to 0.34 ± 0.11) (Table 3).

Low heritabilities were measured for vocalization behaviors in LS1 and LS2 (0.12 ± 0.02 to 0.16 ± 0.04). Permanent environmental effects were low to moderate for almost all of the traits except aggressive behavior, which had a strong effect.

Repeatabilities ranged from 0.27 to 0.57, and reached 0.84 for aggressive behavior.

Genetic and phenotypic correlations for maternal reactivity traits measured on LS are presented in Table 4. Genetic correlations between LS2-HBLEAT and LS1-

HBLEAT, LS2-HBLEAT and LS1-LBLEAT, LS2-HBLEAT and LS1-MBS were moderate and positive, whereas they were high and negative between LS2-HBLEAT

and LS2-LBLEAT. High positive genetic correlations were found between LS1-FLIGHT and LS1-HBLEAT, LS1-FLIGHT and LS2-LBLEAT, LS1-FLIGHT and

LS2-CONTACT, LS1-FLIGHT and LS1-MBS, and LS1-FLIGHT and LS2-MBS. A high negative genetic correlation was observed between LS1-THREAT and LS1-

LBLEAT. Genetic correlations between LS2-LBLEAT and LS2-CONTACT, and

LS2-LBLEAT and LS2-MBS were positive and moderate. High positive genetic correlations were found between LS2-CONTACT and LS1-MBS, and LS2-

CONTACT and LS2-MBS, while a moderate negative genetic correlation was found between LS2-CONTACT and LS1-HBLEAT. A high and positive genetic correlation

was found between LS1-MBS and LS2-MBS. In general, for all maternal reactivity

traits at the LS, phenotypic correlations were of similar signs and lower than genetic correlations.

Genetic correlations between ewe behaviors at the lambing site and their reactions in the arena test

Genetic correlations between maternal reactivity traits measured at the LS and behavioral reactivity traits measured in the AT are presented in Table 5. High and positive genetic correlations were found between AT1-HBLEAT and HBLEAT at the LS. No significant or low correlations were found between HBLEAT in the AT and LBLEAT at the LS. No significant correlations were found between LBLEAT in the AT and vocalization traits (i.e., HBLEAT or LBLEAT) at the LS. High positive genetic correlations were found between AT2-LOCOM and LS1-HBLEAT, and AT3-LOCOM and LS1-HBLEAT, whereas a high negative genetic correlation was found between AT1-LOCOM and LS2-HBLEAT. A moderate negative genetic correlation was found between AT1-PROX and LS1-HBLEAT. Vigilance behavior in AT1 and AT2 were positively correlated (moderate to high genetic correlations) with HBLEAT and LBLEAT at LS1. Flight distance in LS1 was positively correlated (moderate to high genetic correlations) with AT1-HBLEAT, AT2-HBLEAT, AT3-LBLEAT, AT1-LOCOM, AT3-PROX and AT2-VIGIL, whereas a high negative genetic correlation was found between LS1-FLIGHT and AT1-LBLEAT. Aggressive behavior in LS1 was positively correlated (low to moderate genetic correlations) with AT2-HBLEAT, AT1-LOCOM and AT3-VIGIL, and negatively correlated (moderate to high genetic correlations) with AT1-LBLEAT, AT3-LOCOM and AT1-PROX. A low positive genetic correlation was found between LS2-CONTACT and AT3-PROX, and moderate to high negative genetic correlations were found between LS2-CONTACT

and AT1-PROX, and LS2-CONTACT and AT3-VIGIL. Both maternal behavior scores at the LS were positively correlated with HBLEAT in the AT, and genetic correlations were higher in LS1 than in LS2. A low positive genetic correlation was found between LS2-MBS and AT2-LBLEAT. Moderate positive genetic correlations were found between AT1-LOCOM and both maternal behavior scores at the LS. Proximity scores in the AT were positively correlated with both maternal behavior scores at the LS (moderate to high genetic correlations). The maternal behavior score in LS2 was highly and negatively correlated with vigilance behavior in AT1 and AT3, while a low positive genetic correlation was found between LS1-MBS and AT2-VIGIL.

DISCUSSION

Phenotypic variability of maternal reactivity and sources of variation

Phenotypic variability was observed for various behavioral reactions of the ewes recorded in this study at lambing. An increase in the variability was observed for maternal reactivity at the lambing spot in response to the handling of lambs by a shepherd compared to the approach by a shepherd, as seen by the variability in vocalizations and the maternal behavior score. The ewe's perception of the approaching shepherd may be different from the perception of the handling of lambs by the shepherd. The present results also highlighted the fact that low-pitched bleats were more frequent than high-pitched bleats at the lambing spot several hours after lambing. These results were consistent with a previous study showing that sheep preferentially use the low-pitched bleat as a specific lambing vocalization made almost exclusively to strengthen bonding with the lamb (Dwyer et al., 1998).

Various biological and physiological factors have been widely reported to influence maternal behavior toward the litter at lambing in sheep (Dwyer, 2008a; Kendrick, Lévy, & Keverne, 1991; Poindron, Lévy, & Krehbiel, 1988; Simitzis, Galani, Vasiliou, Koutsouli, & Bizelis, 2016). For instance, an increase in parity and/or litter size was reported in the literature to increase specific maternal behaviors (i.e., grooming, licking, etc.). As expected, maternal reactivity traits measured at lambing spots in our conditions increased with increases in parity and litter size, supporting the hypothesis that maternal attachment to the litter was greater in ewes with maternal experience (i.e., greater bond between ewe and lamb), as reported in the literature (Everett-Hincks, Lopez-Villalobos, Blair, & Stafford, 2005; Hernandez et al., 2009; Lambe et al., 2001; O'Connor et al., 1985).

Genetic determinism of maternal reactivity

Genetic variability within breeds is poorly documented for maternal reactivity in sheep, whereas genetic variations between breeds are well documented (for a review, see (Dwyer, 2008a);(von Borstel, Moors, Schichowski, & Gauly, 2011). Heritabilities for vocalizations were lower at the lambing spot than those previously reported in the arena test (Boissy et al., 2005; Hazard et al., 2016; Hazard et al., 2020; Wolf, McBride, Lewis, Davies, & Haresign, 2008). This could be explained by the more simplified and controlled environment of the arena test compared to the open environment at the lambing spot. Heritabilities for maternal behavior scores in the approaching phase and the handling phase at the lambing spot were equivalent or slightly higher than the heritability values previously reported in sheep for a single maternal behavioral score (Brown et al., 2015; Everett-Hincks & Cullen, 2009; Lambe et al., 2001; Plush et al., 2011). Heritabilities for flight and threat behaviors of

ewes in response to an approaching shepherd at the lambing spot, as well as the delay needed by the ewe to restore contact with her litter after the handling of lambs, have not yet been described.

Genetic relationships

Each behavior measured at the lambing site 2 h post-lambing was genetically linked with at least one or several behavioral reactions measured in the arena test 24 h post-lambing. These results suggested that bonding between ewes and lambs, previously described to be complete at 6 to 24 h post-lambing (Keller et al., 2003), was expressed early in our extensive conditions (i.e., 2 h after lambing). This was consistent with delays previously reported for the achievement of the construction of the social link between ewes and their lambs that occurred soon after lambing, up to a maximum of 24 h for primiparous ewes (Keller et al., 2003). Interestingly, high-pitched bleats and proximity scores with the litter in arena tests were highly genetically linked with maternal reactivity at the lambing spot (i.e., maternal behavior scores and/or flight distance). The only genetic link found for vocalizations between both conditions concerned high bleats expressed by ewes that still saw their litter in the arena test, and high bleats expressed at the lambing spot. We hypothesized that the vocal reactions of ewes to maintain contact with their litter may differ between the open field of the lambing spot and the restricted area of the arena test, probably due to the more stressful perception of the visual separation from the litter or the presence of a motionless human in the arena test. Genetic correlations also indicated that maternal reactivity observed at the lambing spot were strongly and favorably genetically linked with locomotion of ewes that can see their litter without a human presence and time spent close to their litter in the presence of a shepherd in the arena test. However,

locomotion of ewes visually separated from their litter or in the presence of a motionless human, as well as vigilance behavior in the arena test, were mainly genetically linked with a high-pitched bleat response to the shepherd's approach at the lambing spot. This suggested that these behaviors might be more representative of a response to a perturbation than to the expression of the maternal attachment to the litter. In addition, negative genetic relationships between the maternal behavior score after the handling of lambs at the lambing spot and vigilance in the arena test suggested that ewes that exhibited a high capacity to follow caught lambs are expected to express low levels of vigilance in the arena test. Finally, aggressiveness toward the approaching shepherd was favorably genetically linked to several traits of behavioral reactivity in the arena test, and ewes that show high levels of proximity and low bleats as well as a low level of locomotion, in particular, are expected to be less aggressive towards the shepherd.

At the lambing spot, both maternal behavior scores were highly genetically linked, suggesting that both criteria were rather similar traits and that bonding between ewes and lambs was expected to be similarly expressed during the approach of the shepherd and during the handling of lambs. Flight distance of the ewe from the lambing spot during the approach of the shepherd and time spent by the ewe to restore contact with the litter were also strong indicators of such ewe-lamb bonding, as suggested by high genetic correlations with maternal behavior scores. On the other hand, the ewe-lamb bond evaluated through maternal behavior scores was not clearly genetically related to vocalizations. Similarly, aggressive reactions toward an approaching human appeared to be genetically independent of most of the ewes' behaviors expressed toward the litter, except for low-pitched bleats. Aggressiveness at lambing could be explained by a greater ewe-lamb bonding to protect the neonate, as described in cow

(Boissy, Nowak, Orgeur, & Veissier, 2001; Phocas et al., 2006). Thus, we hypothesized that the greater ewe-lamb bond in aggressive ewes did not require a higher number of low-pitched bleats to strengthen the bond between the ewe and her lambs, as mentioned above. Reciprocally, ewes that expressed a greater bond through high levels of low-pitched bleats were not expected to exhibit higher levels of aggressiveness. Aggressiveness is undesirable for facilitating labor and improving welfare. Here, the absence of a genetic relationship with other maternal reactivity traits was favorable for genetic improvement of the ewe-lamb bond without increasing aggressiveness.

In conclusion, maternal reactivity assessed at the lambing site was heritable. Moderate to high genetic correlations found between maternal reactivity at the lambing site and behavioral reactivity in arena tests 24 h post-lambing suggested that ewe-lamb bonding was expressed very early in our extensive conditions. Thus, maternal reactivity of ewes, which is difficult to study at lambing, can be assessed through their behavioral reactivity towards their litter measured under standardized conditions, the arena test, performed 24 h later. Genetic improvement of maternal behavior could involve phenotyping of the ewe-lamb bond through a quick and simplified standardized behavioral test performed 1 day post-lambing, which provides higher heritable traits.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

LITERATURE CITED

- Boissy, A., Bouix, J., Orgeur, P., Poindron, P., Bibe, B., & Le Neindre, P. (2005). Genetic analysis of emotional reactivity in sheep: effects of the genotypes of the lambs and of their dams. *Genetics Selection Evolution*, 37, 381-401. doi:10.1051/gse:2005007
- Boissy, A., Nowak, R., Orgeur, P., & Veissier, I. (2001). Social relationships in domestic ruminants: constraints and means for the integration of the animal into its environment. *Inra Productions Animales*, 14, 79-90.
- Brown, D. J., Fogarty, N. M., Iker, C. L., Ferguson, D. M., Blache, D., & Gaunt, G. M. (2015). Genetic evaluation of maternal behaviour and temperament in Australian sheep. *Animal Production Science*, 56, 767-774. doi:10.1071/an14945
- Dwyer, C. M. (2008a). Genetic and physiological determinants of maternal behavior and lamb survival: Implications for low-input sheep management. *Journal of Animal Science*, 86, E246-E258. doi:10.2527/jas.2007-0404
- Dwyer, C. M. (2008b). Individual Variation in the Expression of Maternal Behaviour: A Review of the Neuroendocrine Mechanisms in the Sheep. *Journal of Neuroendocrinology*, 20, 526-534. doi:10.1111/j.1365-2826.2008.01657.x
- Dwyer, C. M. (2014). Maternal behaviour and lamb survival: from neuroendocrinology to practical application. *animal*, 8, 102-112. doi:10.1017/S1751731113001614
- Dwyer, C. M., McLean, K. A., Deans, L. A., Chirnside, J., Calvert, S. K., & Lawrence, A. B. (1998). Vocalisations between mother and young in sheep: effects of breed and maternal experience. *Applied Animal Behaviour Science*, 58, 105-119. doi:10.1016/s0168-1591(97)00113-5

- 457 Everett-Hincks, J. M., & Cullen, N. G. (2009). Genetic parameters for ewe rearing
458 performance. *J. Anim Sci.*, 87, 2753-2758. doi:10.2527/jas.2008-0858
- 459 Everett-Hincks, J. M., Lopez-Villalobos, N., Blair, H. T., & Stafford, K. J. (2005).
460 The effect of ewe maternal behaviour score on lamb and litter survival.
461 *Livestock Production Science*, 93, 51-61.
462 doi:<https://doi.org/10.1016/j.livprodsci.2004.11.006>
- 463 Gonzalez-Garcia, E., de Figueiredo, V. G., Foulquie, D., Jousserand, E., Autran, P.,
464 Camous, S., . . . Jouven, M. (2014). Circannual body reserve dynamics and
465 metabolic profile changes in Romane ewes grazing on rangelands. *Domestic*
466 *Animal Endocrinology*, 46, 37-48. doi:10.1016/j.domaniend.2013.10.002
- 467 Hazard, D., Bouix, J., Chassier, M., Delval, E., Foulquie, D., Fossier, T., . . . Boissy,
468 A. (2016). Genotype by environment interactions for behavioral reactivity in
469 sheep. *Journal of Animal Science*, 94, 1459-1471. doi:10.2527/jas2015-0277
- 470 Hazard, D., Macé, T., Kempeneers, A., Delval, E., Foulquie, D., Bouix, J., & Boissy,
471 A. (2020). Genetic parameters estimates for ewes' behavioural reactivity
472 towards their litter after lambing. *Journal of Animal Breeding and Genetics*,
473 *n/a*. doi:10.1111/jbg.12474
- 474 Hernandez, C. E., Harding, J. E., Oliver, M. H., Bloomfield, F. H., Held, S. D. E., &
475 Matthews, L. R. (2009). Effects of litter size, sex and periconceptional ewe
476 nutrition on side preference and cognitive flexibility in the offspring.
477 *Behavioural Brain Research*, 204, 82-87.
478 doi:<https://doi.org/10.1016/j.bbr.2009.05.019>
- 479 Keller, M., Meurisse, M., Poindron, P., Nowak, R., Ferreira, G., Shayit, M., & Levy,
480 F. (2003). Maternal experience influences the establishment of visual/auditory,
481 but not olfactory recognition of the newborn lamb by ewes at parturition.
482 *Developmental Psychobiology*, 43, 167-176. doi:10.1002/dev.10130
- 483 Kendrick, K. M., Lévy, F., & Keverne, E. B. (1991). Importance of vaginocervical
484 stimulation for the formation of maternal bonding in primiparous and
485 multiparous parturient ewes. *Physiology & Behavior*, 50, 595-600.
486 doi:[https://doi.org/10.1016/0031-9384\(91\)90551-X](https://doi.org/10.1016/0031-9384(91)90551-X)
- 487 Lambe, N. R., Conington, J., Bishop, S. C., Waterhouse, A., & Simm, G. (2001). A
488 genetic analysis of maternal behaviour score in Scottish Blackface sheep.
489 *Animal Science*, 72, 415-425.
- 490 Legarra, A., Varona, L., & Lopez de Maturana, E. (2008). TM Threshold model.
491 GenoToul Bioinformatics, Toulouse, France.
- 492 Michenet, A., Saintilan, R., Venot, E., & Phocas, F. (2016). Insights into the genetic
493 variation of maternal behavior and suckling performance of continental beef
494 cows. *Genetics Selection Evolution*, 48, 45. doi:10.1186/s12711-016-0223-z
- 495 Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, J.-M., Fisher, A. D., Hinch, G. N.,
496 . . . Beaumont, C. (2005). Genetics of adaptation and domestication in
497 livestock. *Livestock Production Science*, 93, 3-14.
498 doi:10.1016/j.livprodsci.2004.11.001
- 499 Nowak, R., Porter, R. H., Levy, F., Orgeur, P., & Schaal, B. (2000). Role of mother-
500 young interactions in the survival of offspring in domestic mammals. *Reviews*
501 *of Reproduction*, 5, 153-163. doi:10.1530/revreprod/5.3.153
- 502 O'Connor, C. E., Jay, N. P., Nicol, A. M., & Beatson, P. R. (1985). Ewe maternal
503 behaviour score and lamb survival. *Proceedings of the New Zealand Society of*
504 *Animal Production*, 45 159-162.
- 505 Phocas, F., Belloc, C., Bidanel, J., Delaby, L., Dourmad, J. Y., Dumont, B., . . .
506 Brochard, M. (2016). Review: Towards the agroecological management of

- 507 ruminants, pigs and poultry through the development of sustainable breeding
508 programmes: I-selection goals and criteria. *animal*, 10, 1749-1759.
509 doi:10.1017/s1751731116000926
- 510 Phocas, F., Boivin, X., Sapa, J., Trillat, G., Boissy, A., & Neindre, P. (2006). Genetic
511 correlations between temperament and breeding traits in Limousin heifers.
512 *Animal Science*, 82. doi:10.1017/asc200696
- 513 Plush, K. J., Hebart, M. L., Brien, F. D., & Hynd, P. I. (2011). The genetics of
514 temperament in Merino sheep and relationships with lamb survival. *Applied*
515 *Animal Behaviour Science*, 134, 130-135. doi:10.1016/j.applanim.2011.07.009
- 516 Poindron, P., Lévy, F., & Krehbiel, D. (1988). Genital, olfactory, and endocrine
517 interactions in the development of maternal behaviour in the parturient ewe.
518 *Psychoneuroendocrinology*, 13, 99-125. doi:[https://doi.org/10.1016/0306-](https://doi.org/10.1016/0306-4530(88)90009-1)
519 [4530\(88\)90009-1](https://doi.org/10.1016/0306-4530(88)90009-1)
- 520 Simitzis, P., Galani, K., Vasiliou, P., Koutsouli, P., & Bizelis, I. (2016). Effect of
521 breed and litter size on the display of maternal perinatal and offspring
522 postnatal behavior in dairy sheep. *Journal of Veterinary Behavior: Clinical*
523 *Applications and Research*, 13, 10-18.
524 doi:<https://doi.org/10.1016/j.jveb.2016.02.008>
- 525 von Borstel, U. K., Moors, E., Schichowski, C., & Gauly, M. (2011). Breed
526 differences in maternal behaviour in relation to lamb (*Ovis orientalis aries*)
527 productivity. *Livestock Science*, 137, 42-48. doi:10.1016/j.livsci.2010.09.028
- 528 Wolf, B. T., McBride, S. D., Lewis, R. M., Davies, M. H., & Haresign, W. (2008).
529 Estimates of the genetic parameters and repeatability of behavioural traits of
530 sheep in an arena test. *Applied Animal Behaviour Science*, 112, 68-80.
531 doi:10.1016/j.applanim.2007.07.011

533

Table 1 – Distribution of maternal reactivity traits of ewes at the lambing site in response to an approaching shepherd (LS1) and then to moving lambs by the shepherd (LS2).

Variables ¹	Number of records	Class ³				
		1	2	3	4	5
LS1-HBLEAT	960	94.4 ²	4.9	0.6	0.1	
LS2-HBLEAT	940	57.9	26.5	11.0	4.6	
LS1-LBLEAT	960	40.8	48.2	9.3	1.7	
LS2-LBLEAT	940	15.6	34.5	27.0	22.9	
LS1-FLIGHT	1306	2.8	12.2	85.0		
LS1-THREAT	1306	90.0	10.0			
LS2-CONTACT	1274	2.8	5.8	20.2	71.2	
LS1-MBS	1306	2.6	5.9	11.0	21.2	59.3
LS2-MBS	1274	12.7	10.1	14.7	62.5	

¹ LS1: approach of the shepherd to the lambing site; LS2: handling and moving of lamb(s) by the shepherd from the lambing site; HBLEAT: high bleats; LBLEAT: low bleats; FLIGHT: flight distance; THREAT: aggressive reaction; MBS: maternal behavior score; CONTACT: time to restore contact with litter.

² For each behavior, results are expressed as the percentage of records in each class.

³ HBLEAT/LBLEAT: class 1 = 0, class 2 = 1 to 3, class 3 = 4 to 6, class 4 = more than 6 high-pitched bleats; FLIGHT: class 1 = more than 6 m, class 2 = 2 to 6 m, class 3 = 0 to 2 m; THREAT: class 1 = no, class 2 = yes; CONTACT: class 1 = no contact within the duration of the test (i.e., 90 s), class 2 = after human leaves, class 3 = within 30 s, class 4 = within 3 s; LS1-MBS: class 1 = ewe flees and doesn't return, class 2 = ewe retreats and comes back, class 3 = ewe retreats with lamb and comes back, class 4 = ewe retreats and returns repeatedly, class 5 = ewe stays close to the lambing spot; LS2-MBS: class 1 = ewe flees, class 2 = ewe stays close to the lambing spot, class 3 = ewe follows but remains at a distance, class 4 = ewe follows, staying close to the shepherd.

Table 2 – Least squares means for maternal reactivity traits of ewes recorded at the lambing site for each level of parity, litter size and age at first lambing.

Variables ¹	Parity				Litter size				First Lambing		
	p-val	1	2	3	p-val	1	2	3	p-val	1	2
<i>n records (%)</i>		44	37	19		17	45	38		49	51
LS1-HBLEAT	NS	1.09	1.04	1.06	NS	1.06	1.06	1.07	NS	1.07	1.05
LS2-HBLEAT	***	1.84	1.44	1.35	**	1.44	1.52	1.67	*	1.60	1.49
LS1-LBLEAT	NS	1.72	1.79	1.57	NS	1.68	1.64	1.76	NS	1.7	1.7
LS2-LBLEAT	***	2.33	2.84	2.73	NS	2.68	2.61	2.60	NS	2.61	2.65
LS1-FLIGHT	***	2.74	2.87	2.87	**	2.78	2.82	2.89	NS	2.83	2.82
LS1-THREAT	NS	1.90	1.90	1.92	*	1.90	1.88	1.93	NS	1.89	1.92
LS2-CONTACT	***	3.17	3.87	3.94	NS	3.66	3.67	3.64	NS	3.64	3.68
LS1-MBS	***	4.04	4.29	4.55	*	4.16	4.31	4.40	NS	4.30	4.28
LS2-MBS	***	2.75	3.67	3.77	NS	3.42	3.39	3.38	NS	3.41	3.39

¹LS1: approach of the shepherd to the lambing site; LS2: handling and moving of lamb(s) by the shepherd from the lambing site; HBLEAT: high bleats; LBLEAT: low bleats; LOCOM: Locomotion; PROX: Proximity; VIGIL: vigilance; FLIGHT: flight distance; THREAT: aggressive reaction; MBS: maternal behavior score; CONTACT: time to restore contact with litter; P-val: p-value; *, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; NS: non-significant.

Table 3 – Estimates of heritability, repeatability and permanent and residual effects
(\pm S.E.) for maternal reactivity traits of ewes recorded at the lambing site.

Variables	n	Component			Total σ_p^2	Repeatability (R)
		Animal (h^2)	Perm (c^2)	Residual (e^2)		
LS1-HBLEAT	696	0.16 \pm 0.10	0.18 \pm 0.12	0.66 \pm 0.14	0.95 \pm 0.07	0.34 \pm 0.10
LS2-HBLEAT	686	0.14 \pm 0.06	0.13 \pm 0.08	0.73 \pm 0.07	1.45 \pm 0.12	0.27 \pm 0.08
LS1-LBLEAT	696	0.12 \pm 0.06	0.20 \pm 0.08	0.67 \pm 0.07	0.45 \pm 0.03	0.32 \pm 0.08
LS2-LBLEAT	686	0.16 \pm 0.07	0.15 \pm 0.08	0.69 \pm 0.06	0.95 \pm 0.06	0.31 \pm 0.08
LS1-FLIGHT	934	0.18 \pm 0.05	0.18 \pm 0.04	0.63 \pm 0.06	2.78 \pm 0.40	0.36 \pm 0.06
LS1-THREAT	934	0.34 \pm 0.11	0.49 \pm 0.11	0.17 \pm 0.05	0.08 \pm 0.01	0.84 \pm 0.11
LS2-CONTACT	918	0.31 \pm 0.09	0.26 \pm 0.04	0.43 \pm 0.08	4.32 \pm 0.60	0.57 \pm 0.10
LS1-MBS	934	0.23 \pm 0.06	0.14 \pm 0.07	0.63 \pm 0.07	4.98 \pm 0.33	0.38 \pm 0.07
LS2-MBS	918	0.19 \pm 0.05	0.10 \pm 0.05	0.70 \pm 0.06	6.49 \pm 1.08	0.30 \pm 0.05

LS1: approach of the shepherd to the lambing site; LS2: handling and moving of lamb(s) by the shepherd from the lambing site; HBLEAT: high bleats; LBLEAT: low bleats; LOCOM: Locomotion; PROX: Proximity; VIGIL: vigilance; FLIGHT: flight distance; THREAT: aggressive reaction; MBS: maternal behavior score; CONTACT: time to restore contact with litter; h^2 , c^2 , e^2 = proportion of total phenotypic variance attributed to additive genetic, permanent and residual effects, respectively; Total σ_p^2 = total phenotypic variance; R = $h^2 + c^2$; n: number of animals.

Table 4 – Genetic (above the diagonal) and phenotypic (below the diagonal) correlations (\pm S.E.) for maternal reactivity traits of ewes recorded at the lambing site.

	LS1- HBLEAT	LS2- HBLEAT	LS1- LBLEAT	LS2- LBLEAT	LS1- FLIGHT	LS1- THREAT	LS2- CONTACT	LS1- MBS	LS2- MBS
LS1-HBLEAT		0.58 (0.29)	NS	NS	0.63 (0.30)	NS	-0.58 (0.15)	NS	NS
LS2-HBLEAT	0.21 (0.07)		0.43 (0.19)	-0.64 (0.14)	NS	NS	NS	0.51 (0.13)	NS
LS1-LBLEAT	NS	NS		NS	NS	-0.65 (0.13)	NS	NS	NS
LS2-LBLEAT	-0.31 (0.09)	-0.42 (0.05)	NS		0.63 (0.19)	NS	0.40 (0.13)	NS	0.57 (0.29)
LS1-FLIGHT	NS	-0.36 (0.08)	-0.20 (0.08)	NS		NS	0.86 (0.15)	0.85 (0.13)	0.89 (0.12)
LS1-THREAT	NS	NS	NS	NS	NS		NS	NS	NS
LS2-CONTACT	-0.57 (0.13)	-0.27 (0.09)	NS	0.35 (0.10)	0.29 (0.09)	NS		0.78 (0.21)	0.95 (0.06)
LS1-MBS	NS	-0.21 (0.06)	-0.14 (0.08)	-0.19 (0.06)	0.70 (0.33)	NS	0.20 (0.10)		0.82 (0.13)
LS2-MBS	NS	-0.36 (0.07)	-0.12 (0.07)	0.21 (0.07)	0.32 (0.08)	-0.14 (0.12)	0.81 (0.04)	0.14 (0.07)	

LS1: approach of the shepherd to the lambing site; LS2: handling and moving of lamb(s) by the shepherd from the lambing site; HBLEAT: high bleats; LBLEAT: low bleats; FLIGHT: flight distance; THREAT: aggressive reaction; MBS: maternal behavior score; CONTACT: time to restore contact with litter. NS: non-significant;

Table 5 - Genetic correlations (\pm S.E.) between maternal reactivity traits of ewes recorded at the lambing site (LS) and behavioral reactivity traits of ewes individually exposed to the arena test (AT) 24 h after lambing.

	LS1- HBLEAT	LS2- HBLEAT	LS1- LBLEAT	LS2- LBLEAT	LS1- FLIGHT	LS1- THREAT	LS2- CONTACT	LS1- MBS	LS2- MBS
AT1-HBLEAT	0.60 (0.20)	0.60 (0.19)	NS	NS	0.90 (0.13)	NS	NS	0.60 (0.13)	NS
AT2-HBLEAT	NS	0.28 (0.13)	NS	0.33 (0.12)	0.56 (0.14)	0.34 (0.11)	NS	0.63 (0.14)	0.32 (0.14)
AT3-HBLEAT	NS	NS	NS	NS	<i>-0.40¹</i> (0.21)	NS	NS	0.70 (0.13)	0.41 (0.20)
AT1-LBLEAT	NS	NS	NS	NS	-0.63 (0.17)	-0.40 (0.15)	NS	NS	NS
AT2-LBLEAT	NS	NS	NS	NS	0.43 (0.26)	NS	NS	NS	0.32 (0.10)
AT3-LBLEAT	NS	NS	<i>-0.43</i> (0.25)	NS	0.75 (0.13)	NS	NS	0.23 (0.12)	NS
AT1-LOCOM	NS	-0.56 (0.15)	NS	NS	0.68 (0.05)	0.51 (0.24)	NS	0.41 (0.16)	0.50 (0.16)
AT2-LOCOM	0.68 (0.16)	NS	0.24 (0.14)	NS	NS	NS	NS	NS	NS
AT3-LOCOM	0.55 (0.09)	0.32 (0.10)	0.23 (0.09)	0.24 (0.14)	NS	-0.31 (0.08)	NS	NS	NS
AT1-PROX	-0.39 (0.12)	0.28 (0.18)	NS	<i>-0.32</i> (0.17)	NS	-0.85 (0.06)	-0.34 (0.17)	0.64 (0.10)	NS
AT3-PROX	NS	NS	NS	NS	0.57 (0.23)	NS	0.32 (0.13)	0.70 (0.12)	0.52 (0.18)
AT1-VIGIL	0.40 (0.17)	NS	0.46 (0.21)	NS	NS	NS	NS	NS	-0.58 (0.06)
AT2-VIGIL	0.79 (0.16)	NS	NS	NS	0.44 (0.14)	NS	NS	0.25 (0.09)	-0.15 (0.08)
AT3-VIGIL	NS	NS	NS	NS	0.40 (0.24)	0.36 (0.17)	-0.57 (0.15)	NS	-0.59 (0.12)

LS1: approach of the shepherd to the lambing site; LS2: handling and moving of lamb(s) by the shepherd from the lambing site; AT1/2/3: arena test phase 1/2/3; HBLEAT: high bleats; LBLEAT: low bleats; FLIGHT: flight distance; THREAT: aggressive reaction; MBS: maternal behavior score; CONTACT: time for contact with litter; LOCOM: Locomotion; PROX: Proximity; VIGIL: vigilance. NS: non-significant. ¹Genetic correlations in italics are non-significant but indicate a strong tendency.