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## ► To cite this version:

Marie-Madeleine Mialon, Raymond Nowak, Patricia Falourd, Didier Marcon, Romain Lardy, et al.. Are early-life lambs' characteristics and behavioural reactivity related to later survival and growth performance during artificial feeding?. *Applied Animal Behaviour Science*, 2023, 262, pp.105918. 10.1016/j.applanim.2023.105918 . hal-04104440

**HAL Id: hal-04104440**

**<https://hal.inrae.fr/hal-04104440>**

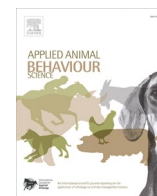
Submitted on 24 May 2023

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# Are early-life lambs' characteristics and behavioural reactivity related to later survival and growth performance during artificial feeding?

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## ARTICLE INFO

### Keywords:

Sheep  
Behavioural reactivity  
Human-animal relationship  
Meat lamb mortality  
Growth rate

## ABSTRACT

The use of prolific sheep breeds often leads farmers to rear some lambs with an automatic milk feeder to improve survival and growth. However, the success of these challenging situations could depend on lamb's early individual characteristics, and their behavioural reactivity in a stressful context. The study was performed on 567 Romane lambs. We tested the hypothesis that early-life characteristics, including behaviour and physiology, could predict survival and growth performances until weaning. In addition, on a random subsample of one third of these lambs we assessed whether behavioural reactivity to social isolation and unknown human presence was a further predictor of individual variability in growth rate. Lambs were characterised by birth weight, rectal temperature and scores for vigour, handling and sucking of first bottle milk on arrival at nursing. After three weeks of artificial rearing (i.e. halfway to weaning age), 202 randomly-chosen lambs were studied in a 2-phase test to record their reaction to social isolation in a novel arena test and presence of an unknown human. Test variables were summarised phase-wise by principal component analysis. The factor scores characterised lamb general activity in phase 1 and affinity to humans in phase 2. Data was examined using descriptive statistics, survival analysis and multivariate regression models to identify the key factors for survival and average daily gain (ADG) until weaning. Cumulative early death rate at one week was 5% and global death rate at the end of the rearing period was 11.6%. There was a higher hazard ratio for death for weak sucking at the first milk-bottle test ( $P = 0.011$ ), low rectal temperature ( $P < 0.05$ ), and dam age of 4–5 years versus 2–3 years ( $P < 0.01$ ). A low ADG until weaning was associated with a low vigour score ( $P < 0.01$ ), weak sucking ( $P < 0.05$ ) and low birth weight ( $P < 0.001$ ). Our data also supports the hypothesis of a relationship between growth and behavioural reactivity in artificially-reared lambs. A low growth rate until weaning was also associated with higher lamb affinity shown to humans ( $P < 0.001$ ) and lower activity expressed in social isolation in a new arena ( $P < 0.05$ ). Our study suggests that early-age characteristics but also reactivity to social isolation and affinity to humans could be valuable predictors of lamb survival and growth in artificial rearing. Our results possibly reveal an over-dependency on humans in some lambs in such a system.

## 1. Introduction

The concept of behavioural reactivity is reflecting individual temperament traits consistently expressed when an animal is exposed to challenging situations (e.g. sociability, boldness/shyness, response to

humans, Dodd et al., 2012, Atkinson et al., 2022). Temperament traits can be durably determined by early life factors (postnatal environment, experience with human and handling) and their expression is modulated by the environmental context. Dodd et al. (2012) also emphasized the negative relationship between behavioural reactivity, stress physiology

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on the one side and individual productivity, health and welfare on the other. The most reactive animals have decreased growth rates or milk production, poorer welfare and health, degraded meat quality (Mota-Rojas et al., 2020; Terlouw, 2015). Both Dodd et al. (2012) and Neave et al. (2020) pointed out the growing interest in this field and the need for further studies, especially in domesticated ruminants.

In meat sheep systems, productivity is strongly linked to ewe prolificacy, which has led to selection programs favouring litter size (over two lambs per ewe; Gootwine, 2020). Unfortunately, higher litter sizes create difficulties if ewes are unable to suckle all their lambs, resulting in increased mortality rates (Gootwine, 2020). To counteract this effect, artificial feeding was developed and is now a common practice in many countries (Eurosheat Network, 2023). It is used as a solution to rescue lambs likely to receive insufficient milk supply or inadequate maternal care (David et al., 2014; McCoard et al., 2021). Despite all the care provided to the lambs by farmers, artificial rearing with a milk replacer is a challenge for the lambs (Napolitano et al., 2008).

Beyond the nutritional challenge, the lambs are separated from their attachment figure, their dam, without adult models to rely on. They are exposed to potentially stressful events such as new environments and objects, noises, human presence and the constraint to get milk from an artificial feeder which requires some training ... Artificial rearing has short-term and possibly long-term negative effects on animal welfare, health and performance, with uncertain profit (Napolitano et al., 2008; Ward et al., 2017; Belanche et al., 2019). And mortality rate is still higher than in lambs reared by their mothers, up to 50% (Boivin et al., 2017).

Researchers have been trying for a long time to characterise potential ways to reduce mortality and improve welfare and performance in artificially fed lambs (Napolitano et al., 2008). The actionable factors identified run from diet quality, demonstrators to help naive lambs to suckle, care provided by the farmer, and more (Dwyer et al., 2016). These levers which are sometimes difficult to implement on-farm, only partially compensate for early maternal separation (Mialon et al., 2021). One reason for this could be a lack of knowledge about how certain individual characteristics, particularly their behavioural reactivity, are related to their chances of survival or growth rate. In lambs reared by their dams, Matheson et al. (2012) showed that neonatal traits like birth assistance, lamb vigour (recorded at 5 min of age), and sucking assistance by human are important predictors of future survival. In artificial feeding system, the acceptance for handling and the ability to suck from an artificial teat during training could potentially be other early indicators to bear in mind, as they are crucial for coping with this challenging system. Such indicators have not yet been evaluated. Behavioural reactivity in sheep can also be assessed in standardised reactivity tests that mimic husbandry challenges such as social isolation, response to novel environments or to human's presence or handling (for review, see Dodd et al., 2012). It is accepted that behavioural responses in standardised tests are moderately to highly reproducible over time. Gavojdian et al. (2015) found significant genetic correlations between behavioural reactivity evaluated at weighing at 5 months of age and preweaning and postweaning growth rate. However, in contrast to these situations in mothered lambs, there is practically no relevant information in the context of artificial rearing.

In the current study, we performed an early individual characterisation—including behavioural reactivity and physiology/fitness indicators—on meat lambs. We tested whether this characterisation can serve to predict later trajectories in terms of survival and growth performances until weaning.

## 2. Material and methods

This study was conducted at INRAE experimental facility UE 0332 (Bourges, France; <https://doi.org/10.15454/1.5483259352597417E12>) with approval from the Val-de-Loire institutional animal care and use committee (No. 19, France; agreement No. 00820.31; 08 July 2014).

The same two stockpersons took care of the lambs throughout the three years of the study and performed all the individual measurement and behavioural scoring at the beginning of artificial feeding. They were a woman and a man, about 50 years old, with a more than 15 years' experience in lambs artificial rearing in the same experimental farm. They scored 63% and 37% of the lambs respectively. Also, the 2 people who performed the reactivity test were the same throughout the study.

### 2.1. Experimental design

#### 2.1.1. Animals and rearing practices

A total of 567 lambs (52% males and 48% females) of the prolific Romane breed were reared under artificial feeding conditions and the ones that survived followed until weaning ( $n = 501$ ). They were born from 406 different dams in five consecutive lambing periods ranging from 80 to 176 individuals per period over 3 years. Sex ratio was not significantly different among periods ( $P > 0.05$ ). Lambs were born from 14% twin-bearing, 71% triplet-bearing and 15% quadruplet-bearing ewes (age:  $3.75 \pm 1.49$  years at lambing).

The standard practice on this experimental farm is based on recommendations issued by the French CIIRPO (Interregional Information and Research Centre for Sheep Production; Sagot, 2015). After giving birth, ewes and their progeny were placed in individual pens for a few hours to ensure proper colostrum intake in all the lambs. The lambs were ear-tagged at between 12 and 24 h of age, given a preventive injection of Duphacycline and Selephos and vaccinated against Ovilis Pastovax respiratory diseases at one month.

Separation from the mother took place between 12 and 24 h after birth. As usual, stockperson leave two lambs per litter with their dam, as similar in weight as possible. The other lambs were reared artificially. Sex was not considered a determining factor. If a lamb was considered as having an empty stomach (and therefore suspected to have ingested insufficient amounts of colostrum), a bottle of thawed bovine colostrum was given just after the bottle acceptance test (see procedure below).

On arrival at the nursery, after the behavioural tests, the lambs were placed in a small pen ( $1.9 \text{ m}^2$  for 8–10 lambs; 3 teats for 10 lambs) to be trained by the stockperson to suck the milk replacer from a rubber teat. The duration of time in this pen depended on the way the lamb adapted to the feeding system. It took between 2 and 4 days for most of the lambs. Once capable of feeding without human assistance, they were moved into a larger pen ( $0.5 \text{ m}^2$  per lamb; 3 teats for 25 lambs) and fed milk replacer via an automatic milk feeder until weaning according to the supplier's recommendations (Agnodor Tradition plus, Soreal Animal Nutrition, Vonnas, France). A solid diet and water were made available ad libitum from 8 days of age. Weaning which consisted of denying access to the milk feeder on a specific day for all lambs occurred at a live weight of 12–13 kg.

### 2.2. Physiology/fitness indicators on dams and lambs (Table 1)

Dam's body condition score (BCS): At lambing, trained stockpersons assessed BCS on a scale from 0 (no fat and very little muscle on the backbone and ribs) to 5 (backbone or ribs cannot be felt) (Russel et al., 1969). BCS has been related to peri- and post-natal lamb loss (Kenyon et al., 2012).

- All the lambs were weighed at birth.
- Lamb rectal temperature was taken immediately on arrival at the nursery, using a digital thermometer.
- Vigour score: Ten minutes after arriving at the nursery, each lamb was put on its legs in a  $2 \text{ m} \times 1.50 \text{ m}$  pen, observed for a few seconds, and scored on vigour of a four-point scale (inspired from the scoring system used in Matheson et al., 2011).

### 2.3. Behavioural reactivity indicators (Table 1 and 2)

- Handling score: Immediately after the vigour test, the lamb was

placed on its back, in a V-shaped device (two 52 cm-long × 27 cm-high wooden boards positioned in a V shape), observed over 10-second period, and scored on ease of handling. This test is inspired from a test developed in piglets to early evaluate coping dispositions in an aversive situation (more or less struggling on a four-point scale, Zebunke et al., 2015).

- Sucking score: Lastly, the lamb was held in the stockperson's arms, offered milk from a bottle fitted with a rubber teat for one minute, and scored on sucking activity (four-point score: full acceptance with high, weak or no sucking to rejection).

- Reactivity in an arena test at 21 days: One third of the lambs (n = 202) were randomly selected at 21 ± 4 days old and submitted to a two-phase test to characterise their behavioural reactivity. The lambs were transported by group in a small cart from the nursery to the test room located in another building nearby (distance: 15 m). There, they were placed in a group of 25 lambs (the maximum number that could be tested each day) in a holding pen before being tested individually. The first phase measured the lamb's reaction to social isolation in an unknown environment (phase 1). The lamb was introduced into the middle of a 6-m × 1-m blind-walled corridor (divided into 6 equal zones) and observed for 2 min through a video camera. The second phase followed immediately after and measured the lamb's reactions to the presence of an unknown person entering and standing without moving at one end of the corridor for two minutes (phase 2). The standing location was changed at each animal tested to exclude potential position-related bias. At the end of the test, the lambs were placed with familiar pen-mates in a second holding pen before being herded together and taken back to the nursery. (Table 1).

Vocalisations, locomotion, exploratory behaviours, and time spent in the zones where the human was located were all recorded (Table 2). Locomotion was assessed by measuring the number of zones crossed during each phase. Proximity to the human was calculated using the following formula:

Proximity index = time spent in zone 1 / 6) + time spent in zone 2 / 5) + time spent in zone 3 / 4) + time spent in zone 4 / 3) + time spent in zone 5 / 2) + (time spent in zone 6 / 1). The human was in zone 6.

## 2.4. Survival and growth rate

Lamb mortality was recorded up to one week after weaning, and most of the lambs that died were autopsied by a veterinarian (analysis from governmental laboratory for the Department of Cher, Bourges, France). The post-mortem examination aimed to identify the causes of mortality based on macroscopic observation of damage of the digestive tract, cardiovascular and respiratory systems. Further bacteriological or parasitological examinations were also performed if there was any

**Table 1**

Definitions of the behavioural scores on the lambs on arrival at the nursery.

Score	Description	Score name
<b>Vigour score</b>		
1	Lying, no tonus, unable to stand up, flat flanks	Weak
2	Standing immobile, with flat flanks	Weak
3	Standing mobile, with flat flanks	Liv_empty
4	Standing mobile, with distended flanks	Liv_filled
<b>Handling score</b>		
1	No movement	Immobile
2	Calm movements of the legs	Wiggle
3	Struggling intensively without escaping from the device	Struggle
4	Immediately jumps out of the device	Struggle
<b>Sucking score</b>		
1	Rejects the teat (mouth closed); insistence needed	No suck
2	Accepts the teat in the mouth, but no sucking	No suck
3	Accepts the teat with weak sucking activity	Weak suck
4	Accepts the teat and sucks vigorously	Good suck

**Table 2**

Description of the behavioural categories used during the test.

Behavioural categories	Description
<b>Vocalisations</b>	Bleats emitted by the tested lamb
<b>Zones crossed</b>	A lamb was considered to have crossed a zone once it stepped over a zone boundary with its two front legs
<b>Look other</b>	Head directed towards something other than the human and more than 5 cm from a wall, from the ground.
<b>Sniff environment</b>	Nose 5 cm or less from its environment (ground, walls)
<b>Jumps</b>	Lamb jumps with two legs against the wall
<b>Look human</b>	Head directed towards human and more than 5 cm from the human
<b>Vigilance posture</b>	Lamb stood motionless, head in upright position and ears perpendicular to the head
<b>Human zones*</b>	Zones located at each end of the corridor (1 m x 1 m)

Locomotion was assessed by measuring the number of zones crossed during each phase

Proximity index = 1/6 \* time spent in zone 1 + 1/5 \* time spent in zone 2 + 1/4 \* time spent in zone 3 + 1/3 \* time spent in zone 4 + 1/2 \* time spent in zone 5 / 2) + time spent in zone 6. The human was in zone 6. \*The standing location was changed at each test

suspicion of infectious or parasitic origin of the death.

Lambs were weight at weaning, and the difference was used to compute average daily gain (ADG).

## 2.5. Statistical analysis

The statistical analyses aimed to determine the indicator factors (early-life indicators and behavioural reactivity) in lamb survival and growth rate until weaning. The analyses were performed on three datasets: 1) neonatal measures taken on all 567 lambs for survival analysis, 2) 501 lambs that survive until weaning for growth analysis and 3) the 202 lambs that were submitted to the reactivity test at 21 days also for growth analysis.

### 2.5.1. Set of early-life indicators

Table 3 presents the ten early-life indicators used for the statistical analyses. Behavioural scores that were expressed in less than 5% of cases were pooled together in order to have less unbalanced classes. In order to be used as predictors in regression analysis of lamb survival and growth rate until weaning, we discretised dam age and BCS and lamb birth weight and rectal temperature into three classes.

### 2.5.2. Factors reflecting lamb reactivity at 21 days of age

When a variable was recorded in phases 1 and 2, it was analysed as a repeated measure with a mixed linear model using the lme4 package with test phase, lamb sex and order within the test as fixed effects and the lamb as random effect. The two-way interaction phase\*sex being non-significant was not kept in the model. We graphically checked the

**Table 3**

Early-life indicators.

Variable	Levels
<b>Dam age</b>	2–3 years: 52%; 4–5 years: 32%; 6–8 years: 16%
<b>Dam Body Condition Score</b>	≤ 2: 29%; between 2 and 3: 39%; ≥ 3: 32%;
<b>Litter size at birth</b>	2 lambs: 14%; 3 lambs: 71%; 4 lambs: 15%
<b>Lamb sex</b>	Male: 52%; Female: 48%
<b>Lamb birth weight</b>	< 2.5 kg: 14%; 2.5–3.5 kg: 46%; ≥ 3.5 kg: 40%
<b>Lamb rectal temperature</b>	< 38 °C: 12%; 38–38.5 °C: 16%; ≥ 38.5 °C: 72%
<b>Lamb vigour score</b>	Weak: 21% <sup>(a)</sup> ; Liv_empty: 46%; Liv_filled: 34%
<b>Lamb handling score</b>	Immobile: 48%; Wiggle: 38%; Struggle: 14% <sup>(b)</sup>
<b>Lamb sucking score</b>	No suck: 26% <sup>(c)</sup> ; Weak suck: 29%; Good suck: 45%

<sup>a</sup> **Vigour:** Weak 21% results from 2.29% with a vigour score '1' on the four-point scale and 18.52% with a score '2'

<sup>b</sup> **Handling:** Struggle 14% results from (= 14.11% (score 3) + 0.16% (score 4))

<sup>c</sup> **Sucking:** No suck: 26% results from (= 4.61% (score 1) + 21.42% (score 2))



normality of the residuals. As for the variables ‘proximity’ and ‘time in human zone’ the plot of residuals was not equivalent for phase 1 and 2, and that for these 2 variables, the random effect of animal was not significant, we performed a robust regression with ‘robustbase’ package.

To integrate the behavioural variables recorded during the two phases of the reactivity test, we carried out a principal component analysis (PCA) for each phase of the test in order to obtain synthetic reactivity variables. For phase 1, the variables involved in the PCA were: frequency of vocalisations, sniff environment, look other, jumps, and number of zones crossed. For phase 2, the variables involved in the PCA were: time in human zone, frequency of vocalisations, sniff environment, look other, look human, vigilance postures and number of zones crossed. Given their share of explained variance (over 40%), the first principal components of each phase (PCA1-P1 for phase 1, PCA1-P2 for phase 2) were kept as synthetic variables to also serve as predictors in regression analysis on ADG for the subpopulation of lambs submitted to the reactivity test ( $n = 202$ ). PCA was performed by using the Facto-MineR package. We also calculated Spearman’s correlation between PCA1-P1 and PCA1-P2.

### 2.5.3. Analysis of lamb survival

A  $\chi^2$  test was performed on autopsy data to test whether the causes of death differed between the first week of life (neonatal stage) and the rest of the rearing period.

To determine the effect of several indicators on the odds of survival of the lambs, we conducted a survival analysis using a Weibull model. Given the low mortality rate found in the present study, in the first week of life, we performed a global analysis running up until the end of the study, i.e. one week after weaning. For lambs that died during this period, age at death was used as the time variable in the Weibull regression. Lambs that were alive a week after weaning were censored and their current age was used as the time variable. Survival analysis tested all 10 early-life indicators (dam age, BCS and litter size; stockperson recording the scores; lamb sex, birth weight, rectal temperature, handling score, vigour score, and sucking score).

Each of the survival models was built in three steps.

The first step consisted in filtering the 10 indicators. For each indicator, we used a univariable parametric survival regression model to analyse the association between survival and each of the 10 indicators. The indicators with a  $p$ -value  $\leq 0.15$  were kept to build a full multivariate model. The second step consisted in excluding the highly inter-correlated indicators. Correlations were estimated between each dyad of indicators filtered via the previous step. As the indicators studied in this study are only qualitative, we used the Cramér’s V intercorrelation coefficient (threshold set at 0.6). Finally, from the set of highly-correlated indicators, those with the best predictive capacity (estimated by AIC) were kept to construct the full models. The third step consisted in simplifying each of the full models by bidirectional stepwise elimination according to AIC. The simplification procedure was stopped once no indicator that would decrease the AIC value could be added or removed (Burnham et al., 2011). We also only kept indicators that have potential effect (i.e.  $p$ -value  $< 0.10$ ).

Calculations were done using R 3.6.0 (R Core Team, 2018). The vcd package (Meyer et al., 2017) was used to calculate Cramér’s V inter-correlation coefficient. The survival package (Therneau and Grambsch, 2000) and the SurvRegCensCov package (Hubeaux and Rufibach, 2015) were used to compute the survival curves.

### 2.5.4. Analysis of lamb growth rate

ADG between birth and weaning was modelled by linear regression. We built the model in three steps, in the same way as for lamb survival with the same initial indicators (see above). Normality of the residuals was checked by quantile-quantile plot, and homogeneity of the variance was checked graphically (the plot of residuals vs. fitted values and the plot of square root of the residuals vs. fitted values). For the

subpopulation of lambs that also underwent the reactivity test, we added PCA1-P1 and PCA1-P2 to the set of early-life indicators in the regression. Calculations were done using R 3.6.0 (R Core Team, 2018). The car package (Fox and Weisberg, 2019) was used for graphical checks. The vcd package (Meyer et al., 2017) was used to calculate Cramér’s V intercorrelation coefficient.

## 3. Results

### 3.1. Lamb mortality

Over the three-year-long study, 66 out of the 567 lambs died at a mean age of  $16.3 \pm 14.2$  days. Cumulative death rate was 5% at one week and reached 11.6% at the end of the artificial rearing period. Forty-nine out of the 66 dead lambs were autopsied. The analyses showed a clear preponderance of digestive causes and difficulties adapting to the automatic milk feeder in lambs scored as “weak”: 37% having digestive disorders (infectious enteritis, enterotoxaemia, and abomasal pathologies), 33% died of starvation which corresponded to lambs that did not feed (intestinal and gastric vacuity), 10% suffered respiratory problems, and the remaining 20% for various causes (such as omphalitis, septicaemia, trauma). Main cause of death was starvation (11/23) when death occurred during the first week, and digestive disorders in older lambs (15/26) ( $P < 0.05$ ).

### 3.2. Early-life indicators as predictors of survival and growth rate

Results for descriptive statistics of the six early-life indicators (dam age, BCS, litter size at birth, and lamb sex, birth weight, and rectal temperature) are reported in Table 3. The criteria, lamb vigour score, handling score and sucking score, all showed significant variability. At least 15% of the lambs were scored into each class of score for all indicators.

#### 3.2.1. Prediction of lamb survival

The multivariable Weibull regression with AIC stepwise selection showed that sucking score, rectal temperature and dam age were additive predictors of risk of death (Table 4). By contrast, both handling scores and vigour scores were not kept in the final model. Compared to lambs scored “Good suck”, those that scored “No suck” or “Weak suck” were nearly twice as likely to die ( $P = 0.09$  and  $P = 0.011$ , respectively). The risk was lower when rectal temperature on arrival at the nursery was between  $38^\circ\text{C}$  and  $38.5^\circ\text{C}$  ( $P < 0.05$ ) or above  $38.5^\circ\text{C}$  ( $P < 0.001$ ) than when below  $38^\circ\text{C}$ . Dam age was also associated with higher risk for death, as lambs from dams aged 4–5 years had a 2.15-times higher Hazard Ratio for death ( $P < 0.01$ ) than lambs from dams aged 2–3 years or 6–8 years.

#### 3.2.2. Prediction of lamb growth rate

ADG until weaning was  $248 \pm 66.9$  g for the whole population ( $n = 567$  lambs) and  $253 \pm 70.3$  g for the 202 lambs that were submitted to the behavioural reactivity test. The sub-sample is representative of the whole population, as its average fits in the 95% confidence interval (242–254 g).

Lamb’s sucking score, vigour score, birth weight, and sex were the four indicators that best predicted ADG (adjusted  $R^2 = 21.25\%$ ; Table 5). Handling score was not kept in the final model. Lambs scored “Good suck” did not differ from those scored “No suck” for ADG ( $P > 0.1$ ). But “Good suck” lambs had a significant higher ADG than the “Weak suck” ones ( $+13.9$  g/d,  $P < 0.05$ ). Lambs scored ‘standing mobile’ either with flat or distended flanks (Liv\_empty and Liv\_filled) had both a higher ADG (about 20 g/d,  $P < 0.01$ ) compared to lambs scored “Weak” in vigour. Compared to lambs weighing less than 2.5 kg at birth, ADG was nearly 40 g/d higher in lambs weighing between 2.5 and 3.5 kg ( $P < 0.001$ ) and more than 80 g/d higher in lambs weighing above 3.5 kg ( $P < 0.001$ ). Finally, sex was a variable kept in the final model with male having a

**Table 4**

Adjusted hazard ratios and 95% CI on independent variables for lamb survival by Weibull regression (n = 567).

Variable	n	Estimate	Hazard ratio	95% CI	P-value <sup>†</sup>
Sucking score	Lambda	0.011			
	Gamma	0.815			
	Good suck (Ref)	257			
	No suck	146	1.75	0.91–3.37	0.099
Rectal temperature	Weak suck	164	2.17	1.23–3.84	0.011
	< 38 °C (Ref)	68			
	38–38.5 °C	91	0.476	0.24–0.94	0.037
Dam age	≥ 38.5 °C	407	0.178	0.1–0.32	< 0.001
	2–3 years (Ref)	294			
	4–5 years	183	2.15	1.29–3.58	< 0.01
	6–8 years	90	0.687	0.28–1.67	0.41

Ref = the level used as reference in the model

**Table 5**

Outcome of the multivariable linear regression model for average daily gain (g/day) in artificially-reared lambs (n = 501).

Variable	Estimate	95%CI	P-value <sup>†</sup>
Sucking score	Intercept	189	170–207
	Good suck (Ref)		
	No suck	-4.9	-17.85 to -8.05
Vigour score	Weak suck	-13.9	-26.67 to -1.19
	Weak (Ref)		< 0.05
	Liv. empty	19.7	4.77–34.59
Birth weight	Liv. filled	22.2	6.03–38.34
	< 2.5 kg (Ref)		< 0.01
	2.5–3.5 kg	39.1	22.07–56.12
Sex	≥ 3.5 kg	80.1	62.53–97.76
	Male (Ref)		< 0.001
	Female	-7.97	-18.56–2.62

Ref = the level used as reference in the model; 95%CI: 95% Confident Interval

higher ADG than female lambs suggesting a possible influence ( $P < 0.15$ ).

### 3.3. Reactivity in arena test and its additional contribution to prediction of growth rate

#### 3.3.1. Characteristics of lamb's reactivity

Lambs moved less, vocalised less and spent more time in the “end zones” in the presence of the unknown human than when they were alone in the test device (phase 2 vs phase 1,  $P < 0.001$ , Table 6). When the lamb was on its own, the first axis of the PCA (PCA1-P1; 43.2% of variability) characterised activity or agitation and had positive values (Fig. 1A) associated with locomotor activity variables (number of crossed zones, jumps) and negative values associated with exploration variables (look, sniff the environment). When the unknown person was present, the first axis (PCA1-P2; 41.3% of variability) characterised response to the human and had positive values (Fig. 1B) associated with the time spent close to the person and negative values associated with

agitation variables (number of zones crossed, look at the person, vigilance). The correlation between PCA1-P1 and PCA1-P2 was nearly 0 ( $r_s = 0.04$ ,  $P > 0.05$ ). These components are named “general activity in social isolation” for PCA1-P1 and “affinity towards humans” for PCA1-P2 in the following sections.

#### 3.3.2. Contribution of reactivity in the arena test to growth rate prediction

Lamb general activity in social isolation, affinity towards humans, handling score, birth weight and rectal temperature were the five indicators that predicted best ADG (adjusted  $R^2 = 34.73\%$ ; Table 7). Regarding the arena test, lambs with higher general activity in social isolation had a higher ADG ( $P < 0.05$ ) and those with a stronger affinity towards humans had a significantly lower one ( $P < 0.001$ ). Compared to lambs scored “Immobile” in the handling score, lambs scored “Wiggle” did not differ on ADG ( $P > 0.01$ ) but lambs that “Struggle” tended to have a lower ADG ( $P = 0.07$ ).

Considering the other variables kept in the final model, ADG was more than 30 g/d higher in lambs weighing 2.5–3.5 kg ( $P < 0.05$ ) and more than 80 g/d higher for lambs weighing more than 3.5 kg ( $P < 0.001$ ) compared to lambs weighing less than 2.5 kg at birth. In the same way, lambs with a rectal temperature of 38–38.5 °C or  $\geq 38.5^\circ$  had a higher ADG (more than 40 g/d,  $P < 0.05$  and  $P < 0.01$  respectively) compared to lambs with a rectal temperature lower than 38 °C.

## 4. Discussion

The present study suggests that considering some early behavioural reactivity indicators of the lambs in an artificial system may improve their survival or growth predictability, at least until weaning in addition to more standard indicators such as body temperature and birth weight. Our results show that weak sucking activity for the first milk bottle, low rectal temperature (below 38 °C), and a dam age (4 and 5 years) are good indicators to predict early mortality. Likewise, weak sucking activity for the first milk bottle and low vigour scores appeared to predict low lamb growth rate in addition to low birth weight and female sex. The results of lamb behavioural reactivity in arena test evaluated at 21 days of age are particularly interesting as they significantly contribute to

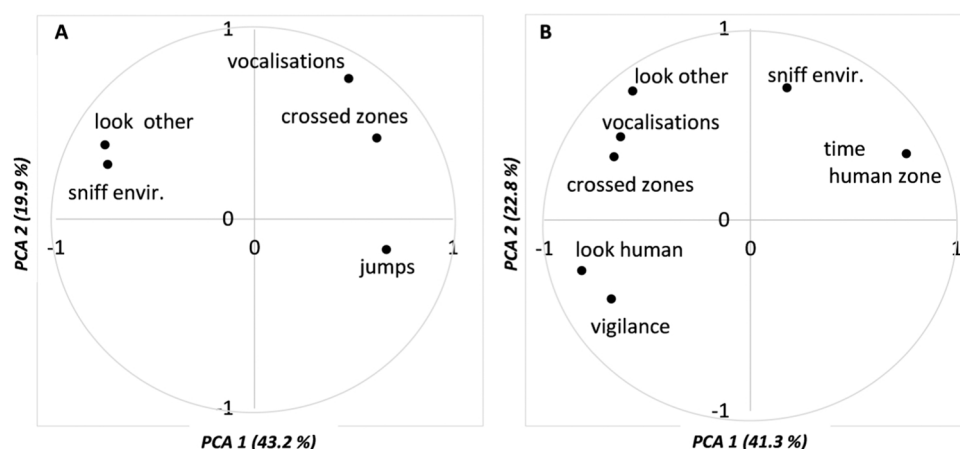
**Table 6**

Outcome of the multivariable linear regression models between behavioural measures assessed in the arena test and some indicators (lamb's sex, phase of the test (phase 2 = presence of an unknown human) and order within the day of test)).

	Intercept	Sex	Phase				Order			
		Estimate sex female	SE	P-value	Estimate phase 2	SE	P-value	Estimate	SE	P-value
Number of zones crossed	57.33	1.68	2.24	0.454	-30.81	1.69	< 0.001	-0.13	0.13	0.338
Number of vigilance postures	12.52	0.55	0.51	0.284	-3.19	0.39	< 0.001	0.06	0.03	0.064
Time in human zone (s)	19.78	-0.35	2.72	0.897	24.66	7.08	< 0.001	-0.22	0.16	0.164
Proximity index	41.00	0.64	1.75	0.712	22.22	2.25	< 0.001	-0.13	0.10	0.175
Number of vocalisations	36.06	3.52	1.10	0.002	-16.70	0.64	< 0.001	-0.13	0.06	0.053

Locomotion was assessed by measuring the number of zones crossed during each phase

Proximity index =  $1/6 \times$  time spent in zone 1 +  $1/5 \times$  time spent in zone 2 +  $1/4 \times$  time spent in zone 3 +  $1/3 \times$  time spent in zone 4 +  $1/2 \times$  time spent in zone 5 + time spent in zone 6.



**Fig. 1.** Principal component analysis (PCA) on lamb behaviours during the isolation phase (A) and the phase with exposure to an unknown human (B) in the arena reactivity test.

**Table 7**

Outcome of the multivariable linear regression model for average daily gain (ADG in artificially-reared lambs with reactivity factors (n = 202).

Variable		Estimate	95%CI	P-value
General activity in social isolation	Intercept	167	135–199	
		10.1	2.07–18.09	0.015
	Affinity towards humans	-18.1	-26.28 to -9.84	< 0.001
	Handling score			
	Immobile (Ref)			
	Wiggle	3.32	-14.4–35.2	0.713
	Struggle	-21.6	-45.1 to -1.93	0.0736
Birth weight	< 2.5 kg (Ref)			
	2.5–3.5 kg	30.6	6.72–54.4	0.013
	≥ 3.5 kg	87.3	62.85–111.7	< 0.001
Rectal temperature	< 38 °C (Ref)			
	38–38.5 °C	43.3	8.87–77.68	0.0146
	≥ 38.5 °C	43.7	14.61–72.84	0.0037

Ref = the level used as reference in the model; 95%CI: 95% Confident Interval

the final model of prediction of growth rate. They suggest that lambs expressing a higher agitation in social isolation in an unknown environment would have better growth rate. By contrast, those with a higher affinity to humans would have a reduced one.

Newborn lambs sucking activity when arriving at the nursery appears to be a fairly variable indicator. Lambs displaying no or few sucking bouts are particularly at risk. They represented 55% of our population and had a higher risk of death. Lambs that showed weak sucking activity had a lower growth rate. Our results are fully in line with studies performed on mothered lambs where efficient early-life sucking behaviour was shown to be an important factor for lamb survival and growth (Dwyer et al., 2016; Odevci et al., 2021). Low neonatal sucking activity may have several explanations, some related to physiology/fitness. Some lambs have an inborn poor sucking reflex, which in mammals is developed in utero and is crucial for survival (Woisard, 2006). A weak sucking activity may also reflect a low level of body reserves, inadequate nursing by the dam in the first hours of life, or a poor quality of colostrum (Dwyer et al., 2016). They may also not be motivated to suck an artificial teat as they had already suckled their dam and experienced the texture of the natural udder (Napolitano et al., 2008). However, in line with our hypothesis, this could reflect variations in behavioural reactivity among lambs to the challenge of the artificial feeding context (e.g., absence of the mother as a secure base or of a

natural teat). They could be distressed by the absence of their dam, more or less stressed by human handling, or confused by the artificial rubber teat or milk-replacer taste (Beauchamp and Mennella, 2009; Belanche et al., 2019). This would jeopardize the lamb's ability to adapt to the artificial feeding system and therefore its subsequent growth and survival, which is consistent with the fact that starvation was the main cause of lamb death during the first week of life. Further information should be sought to explore these various hypotheses in more details, in particular in relation to learning abilities in such a system.

Lamb vigour when entering the nursery also emerged as a promising predictive indicator for growth rate, but not survival. A weak vigour score (as found in 1 out of 5 lambs in our population) was related to a lower growth rate. Our results are in line with Matheson et al. (2012) who showed that vigour recorded 5 min after birth was positively related to 8-week weight in mothered lambs. Our study performed in artificially-fed lambs shows that the vigour assessment is still discriminant at 24 h of age and is predictive of later growth rate.

In addition to early-life behavioural indicators, our analysis confirms that lamb rectal temperature and dam age were predictive of survival. Lambs with a rectal temperature below 38 °C when entering the nursery (12% of the lambs in our population) had a higher hazard ratio for death. Lower rectal temperature at birth in mothered lambs is known to be associated with lower survival (Barlow et al., 1987; Brien et al., 2010; Hegarty et al., 2017). In the most extreme cases, the lambs that cannot thermoregulate will inevitably die (Dwyer and Morgan, 2006), as appeared to be the case in this study. Our results also show that lambs born from 4 to 5-year-old dams have a lower survival rate compared to lambs born from younger dams. The effect of dam age on lamb survival is hard to unravel, especially as it can vary among breeds (Gama et al., 1991).

Lamb birth weight was further predictive of growth rate in addition to early-life behavioural indicators. Lambs with a birth weight below 2.5 kg were significantly penalized with a lower ADG compared to heavier lambs. Our results are in agreement with the positive phenotypic correlation ( $r_p = 0.34$ ; Bibé et al., 2002) between birth weight and ADG from birth to weaning at 70 days in mothered lambs. In artificial rearing, there is generally a competition among lambs for a same teat, whatever the number of teats. When one uses an automatic self-feeder, a ratio of four to five lambs per rubber teat is recommended (Ontario Ministry of Agriculture, Food and Rural Affairs, 2021). This was the case in our study and may have created competition between the lambs, and hence favoured the heaviest and most vigorous. Indeed, Van Welie et al. (2016) observed that heavy-born lambs nursed by their mothers were more efficient feeders compared to other lambs within triplet litters and that this lower estimated milk intake is persistent over time. It is not known whether the persistence of this lower suckling efficiency also exists in

artificially-fed lambs.

The second aim of this study was to evaluate whether lamb behavioural reactivity to social isolation in a novel environment and to unknown human presence are also additively predictive of individual growth rate variability. Previous research has shown that lamb responses to the test procedure used in the current study have been consistent through repetitions (Tallet et al., 2006). In this study, we found a near-null correlation between general activity during social isolation and affinity towards the human. With a different protocol, Hazard et al. (2016) showed that response to social isolation and response to humans are two different and genetically-heritable dimensions of lamb behavioural reactivity. While genetic factors play a significant role in determining behavioural reactivity, it's important to note that environmental influences and life experiences during development, especially in the context of the human-animal relationship, also contribute to this trait (Nowak and Boivin, 2015).

In this study, we found a positive relationship between animal reactivity in social isolation in a new pen and growth rate. Our results are in line with Beausoleil et al., (2008, 2012) who suggested that this reactivity could also reflect the general activity of the lamb regardless of the situation, as the most active animals were also bolder and had lower cortisol release after an arena test. If Beausoleil et al. (2008) hypothesis holds true, the most active lambs, could also be the most active and efficient in teat competition during suckling, resulting in a better growth rate. This hypothesis could be validated in future work designed to record direct observations of lamb behaviour during feeding when using an electronic automatic milk feeder, such as the system used by David et al. (2014) that records feeding activity. A second hypothesis is that lambs that are more active in the arena test are more social and thus more easily disturbed by social separation. Ligout et al. (2011) observed that the lambs that were more reactive to social isolation in an arena test had lower inter-individual distances on pasture, thus confirming stronger social motivation. As a result, our lambs might have been more responsive to social facilitation and learned better to suckle from a rubber as mentioned in previous work (Veissier and Stefanova, 1993), in which case they would ingest more milk and have a better growth rate.

Regarding affinity towards humans, we expected a positive correlation between degree of proximity to the human experimenter during the test and later growth rate. Several studies in several species have found that lower fear responses to humans are associated with higher performances: animals that are more fearful to humans could be so sensitive to regular human disturbances that it induces chronic stress and consequently poor growth performances (Mota-Rojas et al., 2020). However, in the current study, we found an inverse relationship between lamb reactivity and growth rate. Our results indicate that lambs that stayed calmer near the human had lower rates of growth until weaning. Proximity towards a stationary person is considered to be a sign of reduced fear of humans when it is also associated with reduced vocalisation (Nowak and Boivin, 2015). Our results bring the novel insight that this affinity may make some lambs more dependent on human presence, thereby penalizing their growth rate in standard husbandry conditions. In the artificial feeding context, humans act as a 'substitute mother', and the human-animal bonds that develop can be strong or weak depending on the individual lamb's characteristics and the quality of the human-animal interactions (see Nowak and Boivin, 2015 for review). A lamb's association and affinity to humans will be driven by at least two strong motivations: feeding and social contact. The lambs showing the highest affinity towards the human may have needed more help from the stockperson in order to adapt to artificial feeding and, ultimately, to survive. Other lambs may have needed more social contacts due to the loss of their dam as their most relevant social model (Napolitano et al., 2008), and so they may have developed stronger attachment to humans in the absence of their dams. However, unlike the maternal ewe, the human is not permanently with them, so the lambs might experience chronic social stress when the human leaves them, which could penalize their welfare and possibly also their growth rate, as suggested by our

results. Further studies are needed to explore these mechanisms more deeply, particularly during the critical period of adaptation to artificial feeding.

## 5. Conclusions

This study, performed on a large number of lambs reared under the same conditions and cared for by the same people over three years, provides valuable indicators for predicting the future of artificially-fed lambs in term of growth and/or survival. In addition to birth weight and rectal temperature at arrival in the nursery, behavioural indicators such as lamb acceptance of sucking and vigour score also appeared important indicators to record. Our findings on lamb behavioural reactivity also reveal a potential risks tied to inducing over-dependency on humans in lambs reared by artificial feeding. This study opens up new perspectives for the management of artificially-fed lambs. The early-life indicators presented in the current study are easy for farmers to use but need to be further investigated on larger scale in commercial farm systems. Farmers should also implement specific practices for lambs who are considered at risk, in particular by trying to reduce the risk of lamb over-dependency on human stockperson.

## CRediT authorship contribution statement

Marie-Madeleine Mialon: conception and design of the study, analysis and interpretation of data, drafting the article, Raymond Nowak: conception and design of the study, analysis and interpretation of data, revising article critically for important intellectual content, Patricia Falourd: conception and design of the study, acquisition of data, Didier Marcon: conception and design of the study, acquisition of data, Romain Lardy: analysis and interpretation of data, revising article critically for important intellectual content, Xavier Boivin: conception and design of the study, interpretation of data, revising article critically for important intellectual content.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work was supported by a grant from the Centre-Val de Loire Regional Council for the 'Ovin2A' project. We thank the staff at the INRAE experimental farm (UE 0332, Bourges) for their committed animal care and E Delval and C Mallet for performing the behavioural reactivity tests. We thank Metaform Langues for English-language proof-editing support.

## Ethics statement

The experiment was carried out in accordance with the French code of practice for animal care and use. All the procedures were approved the Val-de-Loire (France) institutional animal care and use committee (agreement No. 00820.31 of 08 July 2014) and performed by appropriately trained personnel.

## Software and data repository resources

None of the data were deposited in an official repository.



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