High body condition score combined with a reduced lambing to ram introduction interval improves the short-term ovarian response of milking Lacaune ewes to the male effect

Nathalie Debus, Ellen Laclef, Amandine Lurette, Moutaz Alhamada, Anne Tesniere, Eliel González García, Jean-Baptiste Menassol, Francois Bocquier

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N. Debus[a,*], E. Laclef[a], A. Lurette[a], M. Alhamada[b,1], A. Tesniere[a], E. González-García[a], J-B. Menassol[b], F. Bocquier[b,2]

[a] SELMET, INRAE, CIRAD, L'Institut Agro Montpellier SupAgro, Univ Montpellier, F-34000 Montpellier, France
[b] SELMET, L'Institut Agro Montpellier SupAgro, CIRAD, INRAE, Univ Montpellier, F-34000 Montpellier, France

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ABSTRACT

The male effect is an effective natural technique to induce off-season ovulation and ultimately mating or artificial insemination in small ruminants. It constitutes an alternative to hormonal treatments in conventional breeding systems and, to shift and organise the yearly production cycle, is currently the only solution complying with European organic standards. However, its associated performances are still heterogeneous, both in terms of the global response and the extent of reproductive synchronisation of the females, due to complex interactions with environmental factors that limit its use on commercial farms. This study was carried out on a French organic farm under commercial conditions to investigate, in the field and across five consecutive years, the main parameters affecting the early ovarian response to a ram effect on Lacaune dairy ewes. While the within-year binary logistic regressions yielded contrasting results, the cross-year mixed-effect binary logistic regression models clearly showed that parameters associated with the nutritional state of the animals have a profound influence on the ovarian response of the ewes. Indeed, the probabilities of a spontaneous resumption of ovarian activity before the ram effect and of an early ovarian response to the ram effect were positively associated with the body condition score, total milk production and the age of the animals, while being negatively associated with the milk production level at the 3rd milk recording. The probabilities of a spontaneous resumption of ovarian activity before the ram effect were positively associated with the interval between lambing and the introduction of the rams. Altogether, these results indicate that the ovarian performances in response to a male effect follow a bell-curve pattern with optimal performances depending upon a complex combination between photoperiodic and nutritional cues. Regarding these latter, this study highlights the major contribution of body reserves and energy balance dynamics.

Implications

Reproductive performances in response to a male effect are variable which limits the use of this technique. We addressed this issue by identifying the main factors involved in the spontaneous and male-induced resumption of ovarian activity during a 5-year study conducted on a commercial organic dairy sheep farm. We identified a set of factors associated with the nutritional status of the ewes has having a significant impact on both parameters, which resulted in reproductive performances following a bell-curve pattern. On-farm performances could be improved by adjusting the timing of the male effect relatively to the nutritional status of the ewes.

Introduction

The seasonality of reproduction in sheep is a constraint for breeders even when raising breeds that exhibit less pronounced seasonal patterns of reproductive activities, such as most Mediterranean breeds. In any case, the spring remains a period unfavourable to reproduction. Hormonal treatments were developed to overcome this limitation and allow not only off-season breeding but also the practice of artificial insemination by stimulating the ewes to have synchronised ovarian cycles. This practice grants

* Corresponding author.
E-mail address: nathalie.debus@inrae.fr (N. Debus).
[1] Present address: Istom, F-49000 Angers, France.
[2] Deceased.

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access to faster genetic progress, simplifies the work of breeders and limits the spread of animal sexual diseases. In the Rayon de Roquefort, the main region of French dairy sheep production located in the south-west of France, animal insemination is a keystone of dairy sheep farming. Thus, in this region, the selection scheme based on this practice is particularly efficient, with a dairy genetic gain of approximately 5–7 litres/year, close to the theoretical maximum (Barillet et al., 2001). However, concerns from various representatives of civil society are growing regarding the environmental and health consequences of these hormonal treatments. Globally, French society is asking for alternative techniques that do not require the use of exogenous hormones to manage reproduction (Brice et al., 2002). European organic farming standards have banned the use of these treatments.

The introduction of rams into a flock of seasonally anoestrous ewes results in an increase in the activity of the hypothalamo-pituitary axis, ultimately leading to ovulation and the resumption of oestrus cycles (Delgadillo et al., 2009). This phenomenon, called the ‘‘male effect”, is a simple cost-effective ‘‘clean, green and ethical” technique for breeding ewes during the anoestrus season (Martin et al., 2004). However, the response of ewes to the male effect is still too variable to consider a systematic use of this technique as an alternative to hormonal treatments. Additionally, the percentage of cyclic ewes within one flock during the anoestrus season, which will affect the response to the male effect, is highly variable.

Although the origins of this global variability have been investigated in several studies, the different factors have generally been studied one by one under controlled experimental conditions. These results obtained from controlled experiments, despite enriching our knowledge about reproductive mechanisms, cannot be used directly to provide practical recommendations for farmers.

When considering the performances of the ovarian response to a male effect, two main parameters have to be taken into account, namely, the proportion of females exhibiting a spontaneous resumption of ovarian activity before male introduction and the proportion of females showing an ovarian response to the male effect (i.e., the response to the male effect *per se*). The relationships between these two parameters are relatively complex. Indeed, while an increase in the first automatically decreases the second, it has also been shown that fertility after a male effect is positively correlated with the percentage of spontaneously cycling females (Folch et al., 2000).

Both of these parameters are impacted by various factors, and nutrition can be considered one of those main factors, with major direct effects on both parameters. Thus, it has been demonstrated that nutrition modulates the timing of seasonal reproductive transitions in sheep (Menassol et al., 2012). Moreover, a reduced nutritional status is associated with a longer postpartum anoestrus (Wright et al., 1990), and a greater live weight is associated with more frequent off-season ovulations (Avdi et al., 2003). In terms of the response to the male effect, a lower nutritional status can be associated with a reduction in the occurrence of ram-induced ovulations and oestrus behaviour (Todorov and Nedelkov, 2015) with contradictory results (Scaramuzzi et al., 2014), as well as an earlier cessation of oestrus cycles (Wright et al., 1990). Therefore, timed nutritional supplementation, known as focus feeding and flushing, is often used as a management technique to improve reproductive performance following a ram effect (Johnson et al., 2011; Ben Khlil et al., 2017).

Other factors closely associated or relatively independent of nutrition may also have an impact on these parameters. Concerning the spontaneous resumption of ovarian activity, the age of the ewes has been shown to increase the occurrence of off-season ovarian cycles (Maatoug-Ouzini et al., 2013). Moreover, this parameter also varies with the breed of sheep (Chanvallon et al., 2011). On the other hand, the ovarian response to a male effect is positively impacted by the timing of ram introduction relative to the females' previous parturition (Ungerfeld et al., 2004) and dry-off (Tournadre et al., 2009). This observation is amplified in milking ewes affected by both postpartum and lactation anoestrus (Cappai et al., 1984), suggesting a link between the dynamics of body energy expenditure and the ovarian response to the male effect. More generally, when observing the behavioural oestrus response of ewes to a male effect, parity, age, time of exposure to the male and sex ratio were also factors described as having multiple impacts on reproductive performances (for a review, see: Rosa and Bryant, 2002; Delgadillo et al., 2009).

The aim of this work was to gain a better understanding, under the commercial breeding conditions of an organic dairy sheep farm monitored for five consecutive years, of how various factors interact to impact the spontaneous and male-induced resumption of ovarian activity and to ultimately develop practical recommendations for hormone-free breeding management.

Material and methods

Location

This experiment was carried out at an organic commercial farm located in the Roquefort area in Aveyron, in southern France (44 387N, 2 9623E). This farm and the associated lands have been certified as organic since 2008, in accordance with the European and French organic farming standards. The farm covers around 80% of its feed needs while the other 20% are purchased from other French organic farms. All animals were cared for as specified within the guidelines of the French National Research Institute for Agriculture, Food and the Environment (INRAE) animal ethics committee, and the experiments were compliant with the Animal Research Act of 1985 in accordance with ethical principles that have their origins in the European Union directive 2010/63/EU.2.1.

Animals and management

The experiment was conducted for five consecutive years (2012–2016) at the time of seasonal anoestrus in the spring on 228–269 adult Lacaune dairy ewes (n = 283, 230, 256, 223 and 226 ewes for each year, respectively), aged from 18 months to 10 years (3.4 ± 0.1, 2.7 ± 0.1, 3.0 ± 0.1, 3.0 ± 0.1 and 3.1 ± 0.1 years for each year, respectively). Due to husbandry conditions and the mating plan of the farm, the number of available rams varied between years. Three to twelve entire mature rams (aged from 5 to 8 years) were used, depending on the year, for the ‘‘ram effect” (n = 8; 3; 6; 9 and 12 rams representing a sex ratio of 1/35; 1/77; 1/43; 1/25 and 1/19 rams/ewes for each year, respectively). The rams could not be weighed, but the sexual activity of the rams was individually monitored using an automated electronic oestrus detector (Alhamada et al., 2017), except for 2012. During the 14 days of the ram effect, an average of 197 ± 27 mounts was recorded for each ram, suggesting that each ram could be considered as sexually active. The ewes were kept as a single flock during all of the experiments.

The ewes were maintained on pasture (alternating temporary and permanent grassland) during the day and in the sheepfold at night. Their diet was complemented with a concentrate feed (depending on their physiological state and in accordance with the dietary recommendations established by INRA (2010)) twice a day at the milking parlour manually or by using an automated concentrate feeder since 2015 and they were given free access to water. Two types of concentrate feed were offered: a complete nitrogen-rich concentrate feed (25–30% of nitrogen-rich extract per kg) and a meslin-based concentrate. The ewes were equally separated into four groups for supplementation as follows:

– Group 1: ewes with the highest milk production, plus ewes in poor body condition and primiparous ewes,
– Group 2: ewes with an average milk production,
– Group 3: ewes with the lowest milk production,
– Group 4: ewes with almost no milk production, plus dry and overweight ewes.

The automated concentrate feeder was programmed to individually deliver mixed amounts of each concentrate according to the average needs of each group.

The rams were kept indoors in a separate building until the start of the experiment. They were maintained under a natural photoperiod and standard husbandry conditions, fed *ad libitum* hay and concentrate feed (depending on their physiological state and in accordance with the dietary recommendations established by INRA (2010)), and were given free access to water. The males were completely isolated from the females for at least 2 months before each male effect.

Experimental design

After the introduction of the rams at Day 0 (**D0**), blood samples were taken from the ewes at D-10, D0 and D+11 and were assayed for plasma progesterone levels to determine the occurrence of cyclicity before (D-10 and D0) and in response (D+11) to the introduction of the rams.

At D0 (23, 18, 17, 17 and 14 April for each year), the rams were equipped with an apron that prevented mating and they were introduced into the flock in the late afternoon, just after milking. They were kept with the ewes for 14 days, only between the daily afternoon and morning milkings (each lasting around 10 hours). After D+14, the ewes were inseminated or mated with one of six different rams.

One week before the introduction of rams, the body condition score (**BCS**, noted on a scale from 0 to 5) (Russel et al., 1969) was evaluated for each ewe by trained technicians. Age, days from previous lambing, litter size, milk yield during the previous lactation (total milk production and milk production level at the third milk recording just before the introduction of the rams) were also collected for each ewe and across each year.

Blood samples and hormone assays

Blood samples (3 mL), taken by jugular venepuncture, were used to determine the concentrations of progesterone. They were collected in the morning after milking and placed in heparinised tubes (17 IU/mL sodium heparin, Vacutainer; Becton Dickinson and Company, Franklin Lakes, NJ). After sampling, the plasma was separated by centrifugation (3 600 rpm for 20 min at 4 °C) and stored at −20 °C.

Progesterone was assayed in duplicate measures of 10 μL of plasma using a double-antibody ELISA (Canépa et al., 2008). The sensitivity of the assay averaged 0.22 ng/mL (28 assays), the intra-assay CV was 14%, and the inter-assay CV was 12%.

Data analysis

Cyclicity determination

A ewe was considered to be cyclic before the introduction of the rams (defined as a spontaneous resumption of ovarian activity) if the plasma progesterone concentration was above 1 ng/mL in at least one of the two blood samples taken before the introduction of the rams (D-10 and D0). Only ewes that were categorised as not cyclic before the introduction of the rams were considered as being able to respond to the male effect. Thus, a given ewe was considered to be responding to the male effect, if categorised as non-cyclic before and cyclic after (plasma progesterone concentration at D+11 > 1 ng/mL) the introduction of the rams. This single blood sampling at D+11 allowed to monitor both main patterns of resumption of ovarian activity in response to a ram effect (Lassoued et al., 1997).

Zootechnical parameters

Four continuous and two categorical explanatory zootechnical variables were included in the data analysis: the age of the animals (age, in days), the interval between lambing and male introduction (**LME** interval, in days), total milk production (**TMP**, in L), milk production at the third milk recording (3rd milk recording, in mL), the size of the last litter (litter size, from 1 to 4 depending on the year) and the category of the body condition score (high: BCS > 3, medium: 2 < BCS ≤ 3 and low: BCS ≤ 2). These parameters are presented according to the BCS categories and for each year in Table 1.

Statistical models

Within years' model

Binary logistic regressions were performed for each year to determine the contribution of the three categories of BCS and the five other factors listed above (age, LME interval, TMP, 3rd milk recording, litter size) for the probability of being cyclic before the male effect and for the probability of responding to the male effect. The binary variables to be explained each have two modalities: Yes or No, which corresponded to the success and failure of the events "The ewe is cyclic before the male effect" and "The ewe is responding to the male effect". These analyses were performed using the glm function of the glm2 package of R software (Marschner, 2011). Given the large number of explanatory variables, only their simple effects were analysed.

Between years' model

Five years of monitoring resulted in a longitudinal dataset with a repetition of measurements for some individuals in the flock. To analyse the five-year effect of the variables defined above, mixed-effect binary logistic regression models were performed using the glmer function of the lme4 package of R software (Bates et al., 2015). In these models, BCS, age, LME interval, litter size, TMP and 3rd milk recording were fitted as fixed factors, while individual and year were fitted as random factors. Since the sex ratio varied with year, it was fitted within the models as a random factor nested within year. Only the simple effects of the explanatory variables were analysed.

The models took the following form:

$$Log(Odds) = Log\left(\frac{P}{1-P}\right) = Constant + \sum Coeff_i * Variable_i + \varepsilon,$$

where *Odds* is the ratio between the probability of success and the probability of failure of the event "The ewe is cyclic before the male effect" and "The ewe is responding to the male effect", *P* is the probability of being cyclic before the male effect and for the probability of responding to the male effect, *i* is one of the zootechnical parameters, *Coeff* is the regression coefficient associated with each variable *i* and $\mathcal{E}$ *is* the model random error.

The statistical associations between the events to be explained and the explanatory variables were obtained by observing the odds ratio (**OR**). These ORs are equal to the exponential value of the regression coefficients. When referring to a continuous variable, ORs significantly higher (or lower) than 1 (*P*-value < 0.05) indicate an increased (or reduced) probability of the events being explained

Table 1
The zootechnical characteristics of the ewes according to their respective body condition score categories.

Year	n[1] (nL,nM,nH)	Age (years)			3rd milk recording (mL)			TMP (L)			LME interval (days)			Litter size		
		BCS low	BCS medium	BCS high	BCS low	BCS medium	BCS high	BCS low	BCS medium	BCS high	BCS low	BCS medium	BCS high	BCS low	BCS medium	BCS high
		Mean (±SEM)														
2012	283	3.0	3.4	3.9	1 250	1 025	658	218	250	256	105	137	182	1.1	1.2	1.4
	(29,219,35)	(±0.30)	(±0.10)	(±0.20)	(±55.0)	(±25.9)	(±52.1)	(±10.9)	(±5.0)	(±9.5)	(±7.3)	(±3.2)	(±4.9)	(±0.06)	(±0.03)	(±0.09)
2013	230	2.6	2.7	3.8	1 239	979	728	222	250	248	107	146	178	1.2	1.3	1.3
	(14,203,13)	(±0.40)	(±0.10)	(±0.30)	(±95.4)	(±19.5)	(±59.1)	(±11.6)	(±4.8)	(±19.8)	(±2.1)	(±2.7)	(±7.9)	(±0.11)	(±0.03)	(±0.13)
2014	256	2.9	2.9	4.7	1 237	942	486	156	199	162	94	145	181	1.4	1.3	1.3
	(17,232,7)	(±0.40)	(±0.10)	(±0.40)	(±57.2)	(±18.1)	(±41.3)	(±12.2)	(±4.3)	(±21.8)	(±6.5)	(±3.0)	(±8.1)	(±0.12)	(±0.03)	(±0.29)
2015	223	2.9	3.1	3.6	929	790	534	198	231	199	253	195	193	1.1	1.2	1.4
	(18,198,7)	(±0.50)	(±0.10)	(±0.70)	(±77.3)	(±17.7)	(±53.4)	(±10.8)	(±4.1)	(±26.3)	(±34.8)	(±5.8)	(±2.2)	(±0.08)	(±0.03)	(±0.20)
2016	226	7.0	2.9	3.8	1 130	1 057	805	253	248	277	135	151	195	1.5	1.4	1.3
	(2,178,46)	(±0.00)	(±0.10)	(±0.20)	(±230.0)	(±23.8)	(±25.0)	(±66.2)	(±5.6)	(±6.3)	(±40.0)	(±3.7)	(±1.5)	(±0.50)	(±0.04)	(±0.07)

BCS = body condition score; TMP = total milk production; LME interval = lambing to male effect time interval.
BCS low: BCS $\leq$ 2; BCS medium: 2 < BCS $\leq$ 3; BCS high: BCS > 3.
[1] n: total number of ewes; nL: number of ewes in the low BCS class; nM: number of ewes in the medium BCS class; nH: number of ewes in the high BCS class.

with each 1-unit increase in the value of the explanatory variable. For the categorical variables, ORs significantly higher (or lower) than 1 (P-value < 0.05) indicate an increased (or decreased) probability of the events being explained when compared with a given level of the explanatory variable. It should be noted that for the categorical variable BCS, the reference category used was the Low class. Thus, for each regression, the OR associated with BCS Low is equal to one.

Statistical analyses were performed using R software (R Core Team, 2021). The data are presented as the mean ± SEM.

Results

Cyclicity before the male effect

The progesterone results showed that the cyclicity before the male effect was highly variable and ranged from 29 to 61% depending on the year (35, 30, 29, 61 and 61% for each year, respectively) and varied with BCS (17, 39 and 71% for low, medium and high BCS, respectively).

Within years' results

The results of the binary logistic regression models performed for each year are presented in Supplementary Table S1.

Within years, the results were highly heterogeneous, with few consistent effects across years. The occurrence of cyclicity before the introduction of rams was positively associated with the LME interval (P < 0.01) only during 2013 and 2014. During 2016, age and TMP were positively associated (P < 0.01 and P < 0.05, respectively) with the proportion of cyclic ewes before the male effect, while the 3rd milk recording was negatively associated (P < 0.05) with this same parameter.

Between years' results

The results of the mixed-effect binary logistic regression model over the 5 years of the experiment are presented in Table 2 and Fig. 1. The contributions of the individual variables to the predicted probabilities of the spontaneous resumption of ovarian cycles are presented in Fig. 2.

The variables BCS (class High), age, 3rd milk recording, TMP and LME interval significantly contributed (P < 0.05 and P < 0.001 for first two and last three variables, respectively) to the probability of being cyclic before the male effect, whereas the contribution of litter size was not significant (Table 2). Among these variables, the high BCS level made the greatest contribution to the outcome of the model. Thus, this model shows that a gain in BCS class multiplied the probability of responding to the male effect by 3.3 (from BCS medium to high; Table 2). As shown in Fig. 1, the ewes that were most likely to be cyclical combined high BCS, low 3rd milk recording and long LME interval values. Age also had a positive linear effect on the probability of being cyclical (Fig. 2).

Cyclicity in response to the male effect

Progesterone results ranged from 31 to 85%, showing that the response of ewes to the male effect was highly variable between years (51, 31, 41, 85 and 52% for each year, respectively). This parameter also varied according to BCS (17, 50 and 90% for low, medium and high BCS, respectively).

Within years' results

The results of the binary logistic regression models performed for each year are presented in Supplementary Table S2.

Once again, the within-year results were highly variable, and consistent results across years could not be found. Among all vari-

Table 2
Five-year logistic regression model for the event "The ewe is cyclic before the Male Effect".

	OR[1]	95% CI[1]	*P*-value
BCS			
Low	Reference		
Medium	1.803	0.830–3.913	0.14
High	3.333	1.307–8.502	<0.05
Age	1.112	1.006–1.230	<0.05
3rd milk recording	0.998	0.998–0.999	<0.001
TMP	1.007	1.004–1.010	<0.001
LME interval	1.008	1.005–1.011	<0.001
Litter size	0.947	0.683–1.313	0.70

BCS = body condition score; TMP = total milk production; LME interval = lambing to male effect time interval.
BCS low: BCS $\leq$ 2; BCS medium: 2 < BCS $\leq$ 3; BCS high: BCS > 3.
[1] OR = odds ratio – CI = confidence interval.

ables, 3rd milk recording (years 2014 and 2015, $P < 0.05$ and $P < 0.01$, respectively) and BCS (class medium: years 2015 and 2016, $P < 0.05$ and $P < 0.01$, respectively; class high: year 2012, $P < 0.05$) more often showed significant contributions to the models (2 out of 5 models). Depending on the year, the variables age (year 2016, $P < 0.01$) and LME interval (2013, $P < 0.05$) were positively associated with the proportion of ewes responding to the male effect, while litter size (year 2014, $P < 0.05$) was negatively associated with this same parameter.

Between years' results

The results of the mixed-effect binary logistic regression model over the 5 years of the experiment are presented in Table 3 and Fig. 3. The contributions of individual variables to the predicted probabilities of the early ovarian response to the male effect are presented in Fig. 4.

The variables BCS (class medium and high), Age, 3rd milk recording and TMP significantly contributed (all $P < 0.001$) to the probability of responding to the male effect, whereas the contribution of LME interval and litter size was not significant (Table 3). Among these variables, BCS made the greatest contribution to the outcome of the model. Thus, this model shows that a gain in BCS class multiplied the probabilities of responding to the male effect by a 6.4 or 19.0 factor (from BCS low to medium and BCS medium to high, respectively; Table 3).

As shown in Fig. 3, the ewes that were most likely to respond to the male effect combined a high BCS and age with low 3rd milk recording values. Moreover, the negative impacts of 3rd milk recording values on the probabilities of responding to the male effect were reduced with age.

Discussion

The results of this study highlight the importance of farm management practices on the short-term ovarian response of milking ewes to a male effect. Two main parameters were considered to characterise this response, *i.e.*, the occurrence of ovarian cycles before and after the introduction of rams, corresponding to a "potential of response" and the response *per se*. Hence, the probability of an early spontaneous resumption of ovarian activity before the introduction of rams was positively associated with BCS, TMP, the interval between LME Interval and the age of the animals,

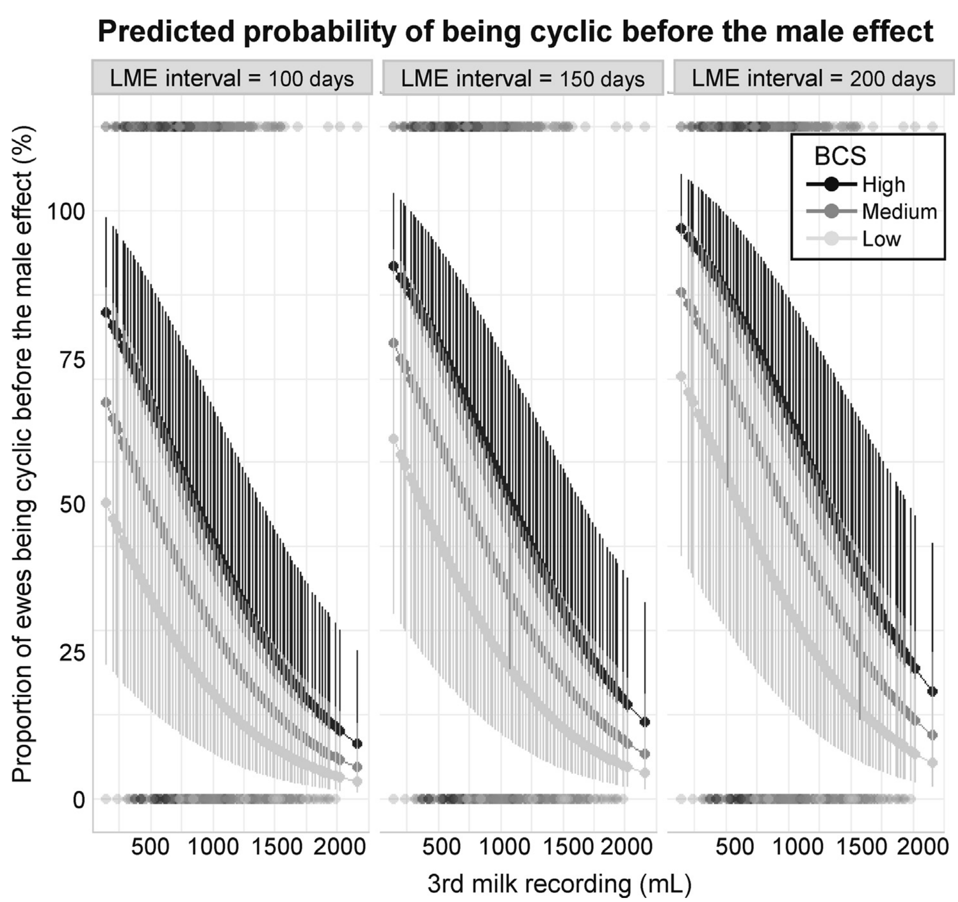

Fig. 1. Predicted probabilities (probabilities ± 0.95 confidence interval) for the ewes of being cyclic before the male effect according to the body condition score class (BCS) (Low: light grey circle, Medium: dark grey circle and High: black circle), the production level at the 3rd milk recording (3rd milk recording) and the lambing to male effect time interval (LME interval). BCS classes: Low $\leq$ 2; 2 < Medium $\leq$ 3; High > 3.

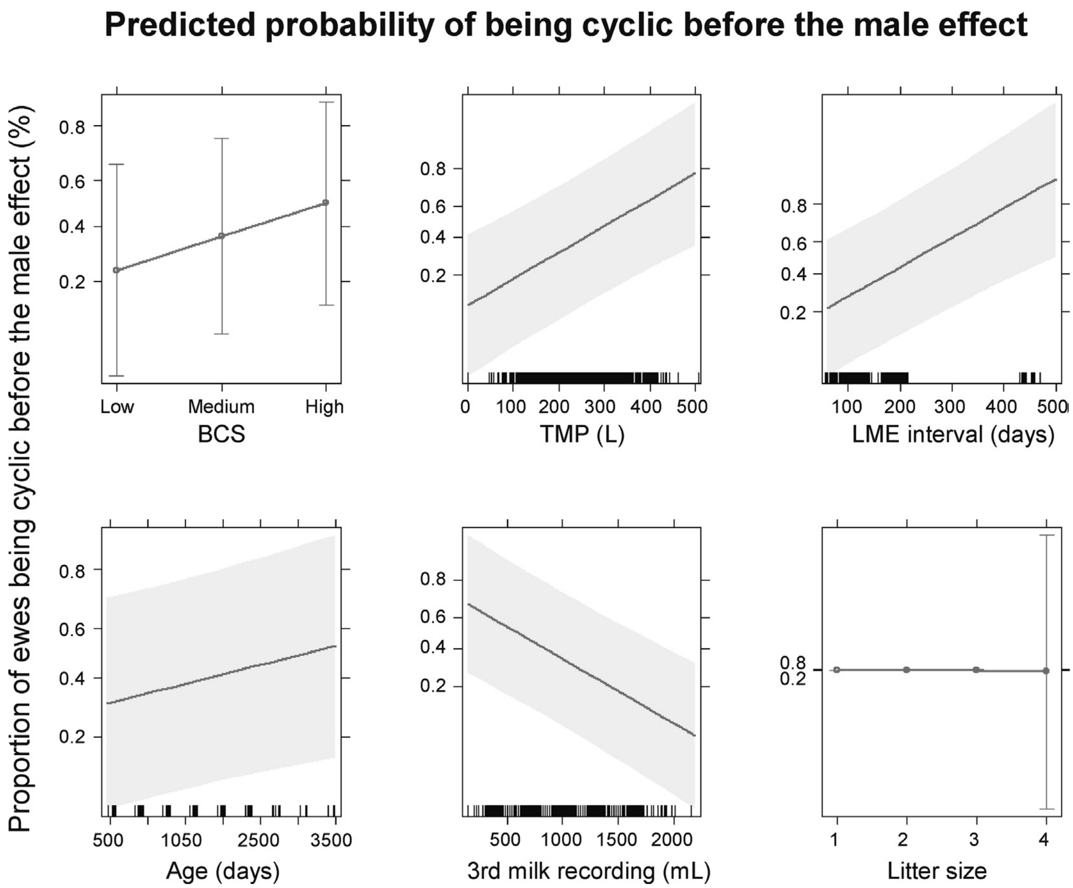

Fig. 2. Predicted probabilities (probabilities ± 0.95 confidence interval) for the ewes of being cyclic before the male effect according to the zootechnical characteristics: body condition score class (BCS), total milk production (TMP), the interval between lambing and the male effect (LME interval), age, milk production at the third milking recording (3rd milk recording) and litter size. BCS classes: Low ≤ 2; 2 < Medium ≤ 3; High > 3.

Table 3
Five-year logistic regression model for the event "The ewe is responding to the male effect".

	OR[1]	95% CI[1]	*P*-value
BCS			
Low	Reference		
Medium	6.402	2.591–15.820	<0.001
High	19.46	3.868–97.880	<0.001
Age	1.326	1.160–1.516	<0.001
3rd milk recording	0.997	0.997–0.998	<0.001
TMP	1.011	1.007–1.014	<0.001
LME interval	1.000	0.996–1.004	0.90
Litter size	0.861	0.542–1.368	0.50

BCS = body condition score; TMP = total milk production; LME interval = lambing to male effect time interval.
BCS low: BCS ≤ 2; BCS medium: 2 < BCS ≤ 3; BCS high: BCS > 3.
[1] OR = odds ratio – CI = confidence interval.

while the milk production level at the 3rd milk recording was negatively associated with this same parameter. The same associations were observed for the probability of a short-term ovarian response to the male effect, except for the LME Interval, which had no significant influence on this parameter.

Concerning the spontaneous acute or persistent resumption of ovarian activity within one flock, both being indistinguishable in our study, our results are in line with previous studies performed in sheep establishing a positive relationship between age, live weight and the time since lambing with spontaneous off-season ovarian activity (Avdi et al., 2003) and between body condition score (Forcada et al., 1992; Menassol et al., 2012) or the time since a previous lambing (Mitchell et al., 2010) and early resumption of ovarian activity. On the other hand, regarding the factors explaining the early ovarian response to a male effect, our results are also in agreement with other studies, such as the experiment reported by Dzabirski and Notter (1989), where the frequency of ovulation after a male effect, realised in spring, was lower for Dorset ewes (meat sheep) that had previously lambed during the winter season in comparison with Dorset ewes that had previously lambed during the fall season (21 ± 9 vs 59 ± 8%, respectively). Therefore, an extended interval between lambing and introduction of the rams (approximately 4 vs 8 months) had a dramatic positive impact on the proportion of ewes exhibiting an early ovarian response. A study performed in a commercial organic farm raising Limousine meat sheep (Tournadre et al., 2009) confirmed these results. Similar conclusions were drawn in a study conducted on the expression of oestrus following a male effect on 22 commercial farms breeding Merino sheep in South Australia (Kleemann et al., 2006).

Body condition score has also been identified as a factor impacting the short-term ovarian response to a ram effect in a Mediterranean breed such as the Barbarine ewe (Khaldi and Lassoued, 1991) as well as in a more seasonal breed such as the Île-de-France ewe (Johnson et al., 2011), both being sheep breeds raised for meat production. In dairy sheep, Luridiana et al. (2015), in a study conducted on two commercial farms, identified BCS and age as factors positively correlated with early oestrus expression in response to a male effect in Sarda ewes. Similar conclusions were drawn in a study conducted on six Bulgarian farms raising dairy, meat and multi-purpose breeds of sheep, where a positive correlation was established between BCS and the percentage of

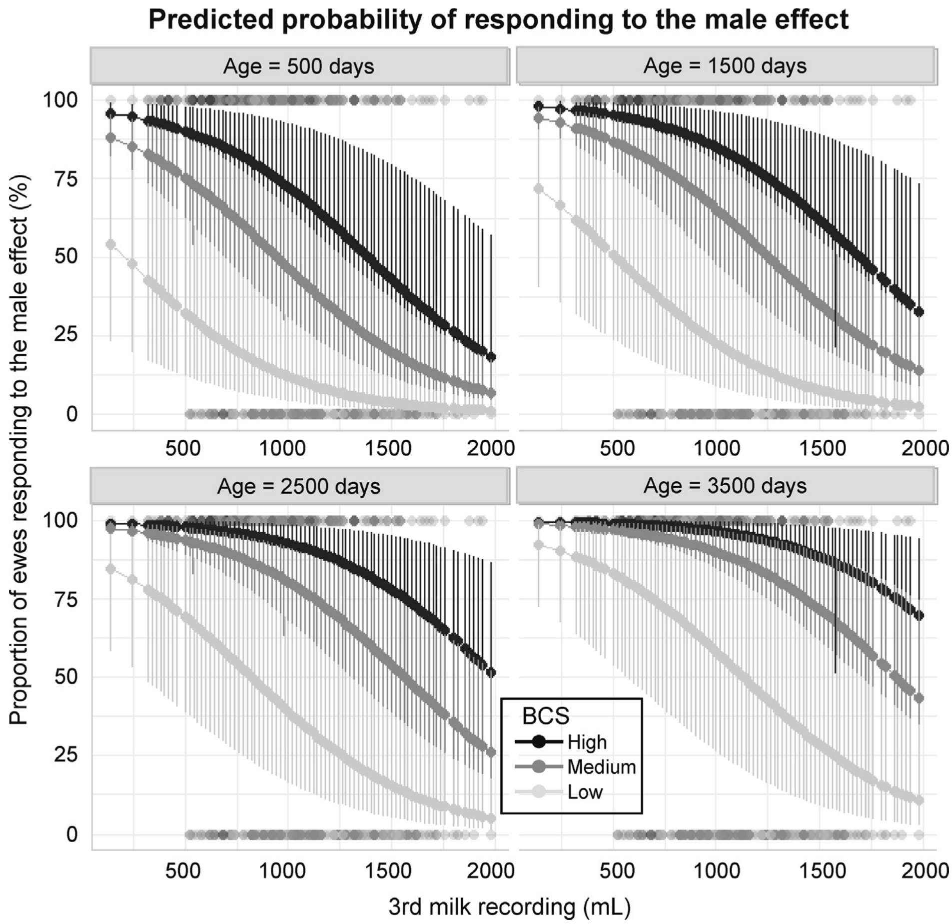

Fig. 3. Predicted probabilities (probabilities ± 0.95 confidence interval) for the ewes of responding to the male effect according to the body condition score class (BCS) (Low: light grey circle, Medium: dark grey circle and High: black circle), the production level at the 3rd milk recording (3rd milk recording) and the lambing to male effect time interval (LME interval). BCS classes: Low ≤ 2; 2 < Medium ≤ 3; High > 3.

ewes displaying oestrus behaviour in response to a male effect (Todorov and Nedelkov, 2015).

Regarding this matter, it is noteworthy that the effects of nutrition might be mediated on oestrus expression independently of direct effects on the early ovarian response following ram introduction. This was suggested by a study performed on Merino ewes, where low live weight and BCS associated with dietary restriction beginning during mid-pregnancy had negative effects on oestrus expression (77.5 ± 2.5 vs 30.0 ± 22.5%) but not on ovulations in response to a male effect (Wright et al., 1990). Finally, a positive correlation of age with all of the reproductive performances associated with the male effect (early ovarian response, oestrus expression, ovulation and conception rates) was also found but only when comparing 1-year nulliparous ewes with multiparous ewes (Ungerfeld, 2016).

This study confirms that nutrition acts as a major factor modulating the ovarian response to a male effect and that it is one of the main factors to be taken into account when this technique is employed by farmers to maximise reproductive performance on commercial farms. Indeed, each factor having a significant contribution to our statistical model is associated with body reserves and the energy balance of the animals. For instance, it is well known that in ruminants, a negative state of negative energy balance associated with high milk production, especially at the beginning of lactation, has global adverse effects on reproduction (Gootwine and Pollott, 2000). Additionally, there is a negative genetic correlation between total milk production and fertility that induces lower fertility in adult ewes (David et al., 2008). The apparent contradictory effects of milk production variables, total milk production (positively associated) and milk production at the third milk recording (negatively associated), on the probabilities of ewes exhibiting ovarian cycles spontaneously before the introduction of rams and in response to the male effect highlight the importance of the effects of metabolic dynamics on reproductive functions. In accordance with the effects of the interval between lambing and introduction of the rams (LME Interval), this result indicates that the negative effects associated with high milk yields on reproductive functions are time-dependent, with high milk production levels close to the introduction of rams being associated with fewer spontaneous ovarian cycles and a lower proportion of ewes responding to the male effect.

In our study, despite similar manifestations, the effects of nutrition on the resumption of ovarian activity before (spontaneous) or after (induced) the introduction of rams are probably mediated through different pathways. While the timing of the spontaneous resumption of ovarian activity originates from the interaction between nutrition and photoperiodic cues at the central level (Menassol et al., 2012), the integration of nutritional cues along the pathways involved in the interpretation of the sociosexual signals associated with the male stimulus is less known. This interaction acts on the emission and interpretation of these sociosexual signals (Delgadillo et al., 2009) and probably involves both central and peripheral levels (Johnson et al., 2011; Scaramuzzi et al., 2014). These different neurophysiological pathways could explain why there is an effect of the LME interval on the spontaneous resumption of ovarian cycles before the introduction of rams and not in response to the male effect. From the results of our study, we suggest that the factors associated with nutritional cues have synergetic effects that shape an optimal time window, with interactions with the photoperiodic signal, for the induction of early ovarian cycles in response to the male effect (Fig. 5). In other words, the sociosexual signals associated with the ram stimulus can disrupt the inhibitory photoperiodic signal depending on its amplitude and the nutritional status of milking ewes. This asser-

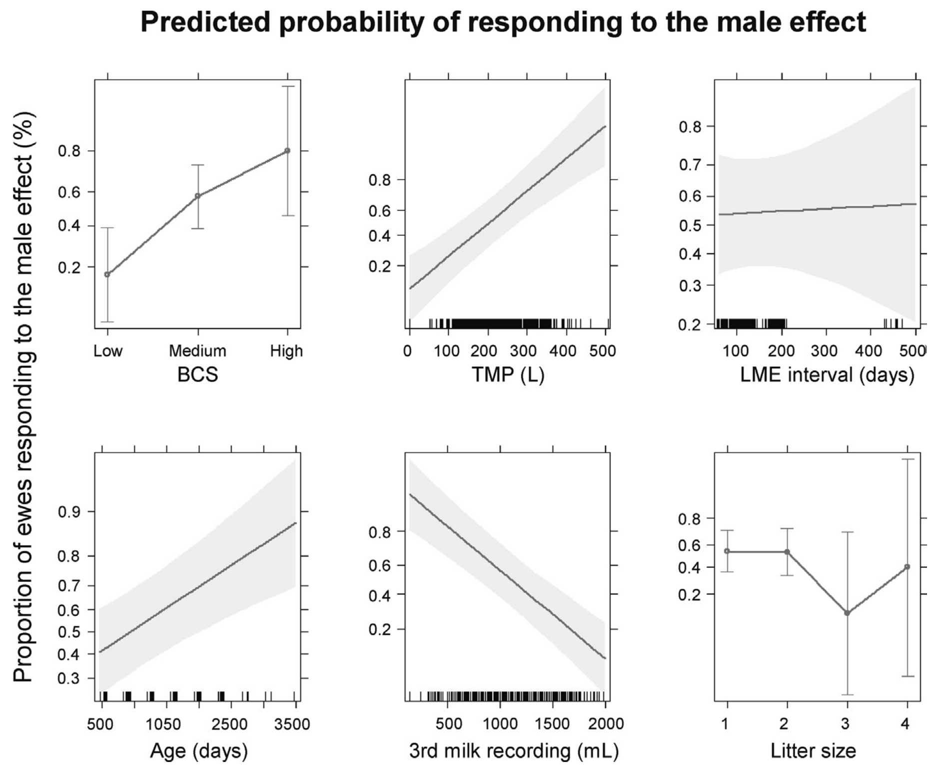

Fig. 4. Predicted probabilities (probabilities ± 0.95 confidence interval) for the ewes of responding to the male effect according to the zootechnical characteristics: body condition score class (BCS), total milk production (TMP), the interval between lambing and the male effect (LME interval), age, milk production at the third milking recording (3rd milk recording) and litter size. BCS classes: Low ≤ 2; 2 < Medium ≤ 3; High > 3.

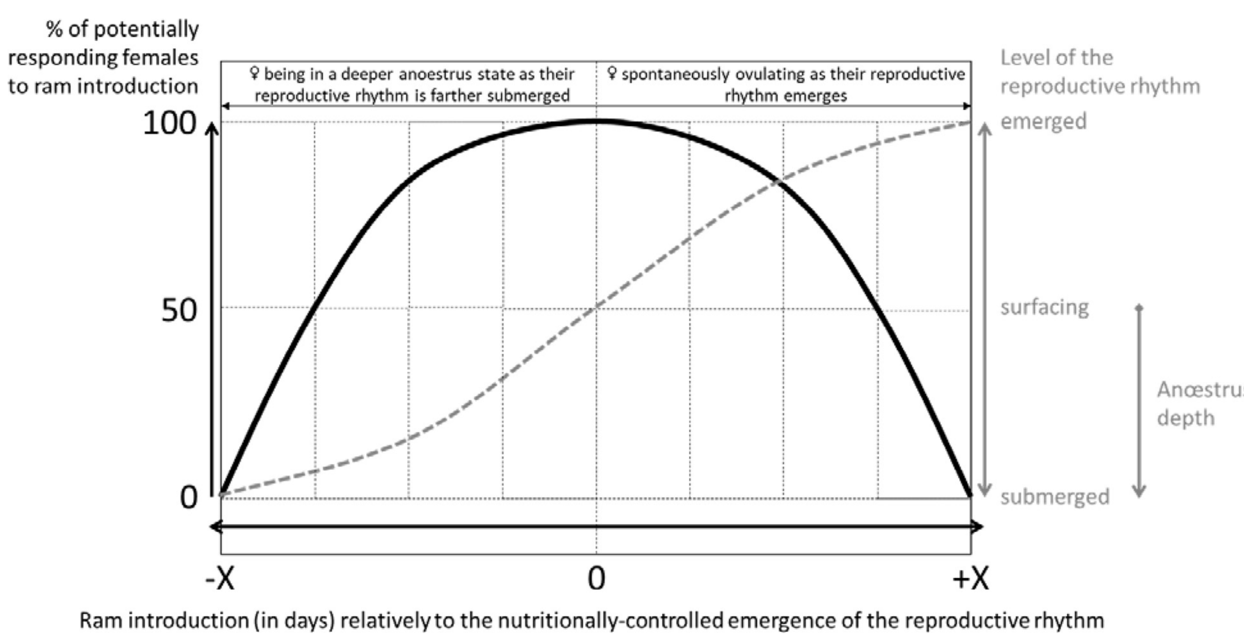

Fig. 5. Schematic representation of the proportion of females potentially responding to a male effect (solid line, primary Y-axis) given the time of introduction of the rams relative to the spontaneous resumption of ovarian cycles. Nutritional and photoperiodic cues interact to control the emergence of each individual reproductive rhythm (dashed line, secondary Y-axis). From [-X; 0] (left side of the figure), the alleviation of the anoestrus deepness gradually allows a greater proportion of ewes to be able to respond to the ram effect until a theoretical maximum is reached. From [0; +X] (right side of the figure), the gradual increase in ewes spontaneously resuming their ovarian cycles limits the proportion of ewes being able to respond to the ram effect. This global pattern illustrates the occurrence of an optimal time window for the realisation of a ram effect associated with greater success in the induction of ovarian cycles.

tion prolongs an earlier observation of a positive correlation between fertility after the male effect and the percentage of ewes exhibiting spontaneous ovarian activity within one flock (Folch et al., 2000), as well as a previous interpretation proposed by Scaramuzzi et al. (2014) suggesting that the photoperiodic signal could override potential nutritional impacts on the reproductive response to a male effect.

In comparison with other studies, some specific conditions in the protocol of realisation of the male effect for our study can be discussed. Regarding the duration of the rams' presence with the ewes, a factor known to impact the performances of the male effect, it is conventionally recommended a continuous presence of the male to maximise the endocrine or ovulatory responses of females to the male stimulus (Oldham and Pearce, 1983; Hawken and Beard, 2009). In our study, the rams were kept with the ewes only between the afternoon and morning milkings, corresponding to a daily average presence of 10 hours. This practice is specific to the dairy systems of the Roquefort production area where rams are usually kept indoor. We found that this management practice did not downgrade the reproductive performances of the ewes in response to a male effect (personal data) and therefore, it was maintained throughout the experiment. On the other hand, it was shown in goats that 1 or 2 daily hours of contact with sexually active males is sufficient to stimulate ovulatory activity in non-cyclic goats (Bedos et al., 2014). The sex ratio, another important factor that can affect the response to the ram effect (Rosa and Bryant, 2002; Delgadillo et al., 2009), was highly variable between years in our study (from 3 to 12 depending on the year). This was due to husbandry conditions and the mating plan of the farm. In order to take into account this variability, this factor was included in each of our statistical models as a random factor nested within the year of the experiment. The contrasted results observed between years could also be explained by other uncontrolled environmental factors and the usual adaptations of management practices by the farmer including sex ratio. We consider that this variability between years is a strong argument in favour of a longitudinal study and cross-year analysis to extract and interpret the significant factors under such on-farm conditions.

In practical terms, the results of this study highlight several factors that have to be prioritised in farm management practices associated with the realisation of a male effect. They highlight that to maximise the reproductive response of ewes to the male effect and the associated objectives of an off-season synchronised reproductive response, a compromise including BCS, milk production and the timing of the introduction of the rams relative to the date of lambing has to be identified. These management issues are particularly important in organic farm conditions where the ban on any hormonal treatment and the legal requirements for local feed production impose a greater sensitivity of the reproductive rhythm to local environmental and nutritional conditions.

In conclusion, we found that a combination of factors linked with body reserves and energy balance defines a time window associated with optimal early reproductive performances of milking ewes in response to a male effect. The originality of this work is that it was conducted on a commercial farm over several years, which allowed us to surpass the variability between years to draw consistent conclusions that prolong previous studies in this field. This confirms the importance of precise nutritional management of farm animals to improve their reproductive performance and limit the variability associated with the male effect, a key technique of hormone-free breeding programmes in small ruminants. The implementation of automated concentrate feeders in milking parlours is a valuable tool that can be used in response to our findings depending on the objectives of the farmer. Complementary investigations are currently being conducted to analyse the factors affecting the behavioural oestrus response and the associated pregnancy rates under similar breeding conditions.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100519.

Ethics approval

All animals were cared for in accordance with the guidelines of Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) animal ethics committee and the experiments were compliant with the Animal Research Act of 1985 in accordance with ethical principles that have their origins in the European Union directive 2010/63/EU.2.1.

Data and model availability statement

None of the data were deposited in an official repository.

The data that support the study findings are available to reviewers.

Author ORCIDs

N. Debus: https://orcid.org/0000-0001-5380-7455.
E. Laclef: https://orcid.org/0000-0003-0506-8197.
A. Lurette: https://orcid.org/0000-0001-9638-4472.
M. Alhamada: https://orcid.org/0000-0002-0258-7859.
A. Tesniere: https://orcid.org/0000-0003-1430-9458.
E. González-García: https://orcid.org/0000-0001-9232-1941.
J-B. Menassol: https://orcid.org/0000-0002-3947-1067.

Author contributions

N. Debus: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Project administration, Validation, Visualization, Writing – original draft

E. Laclef: Conceptualization, Formal Analysis, Visualization, Writing – original draft

A. Lurette: Conceptualization, Formal Analysis, Visualization, Writing – original draft

M. Alhamada: Investigation

A. Tesniere: Investigation, Validation, Visualization

E. González-García: Writing – review & editing

J-B. Menassol: Conceptualization, Formal Analysis, Visualization, Writing – original draft

F. Bocquier: Conceptualization, Funding acquisition, Project administration, Supervision

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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