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GENETIC AND NON-GENETIC FACTORS AFFECTING RABBIT DOE SEXUAL RECEPTIVITY AS ESTIMATED FROM ONE GENERATION OF DIVERGENT SELECTION

THEAU-CLÉMENT M.^{*†‡}, SÉCULA A.^{*†‡}, SALEIL G.^{*†‡}, MONNIAUX D.[#], BRECCHIA G.[§],
BOITI C.[§], BODIN L.^{*†‡}, BRUN J.M.^{*†‡}

^{*}INRA, GenPhySE (Génétique, Physiologie et Systèmes d'Élevage), F-31326 CASTANET-TOLOSAN, France.

[†]Université de Toulouse, INPT ENSAT, GenPhySE (Génétique, Physiologie et Systèmes d'Élevage), F-31326 CASTANET-TOLOSAN, France.

[‡]Université de Toulouse, INPT ENVT, GenPhySE (Génétique, Physiologie et Systèmes d'Élevage), F-31076 TOULOUSE, France.

[#]INRA, UMR 0085 PRC Physiologie de la Reproduction et des Comportements, Centre de recherche Val de Loire, NOUZILLY, France.

[§]Dipartimento di Medicina veterinaria, Laboratorio di Biotecnologie fisiologiche, Università di Perugia, PERUGIA, Italy.

Abstract: Sexual receptivity of rabbit does at insemination greatly influences fertility and is generally induced by hormones or techniques known as “biostimulation”. Searching for more sustainable farming systems, an original alternative would be to utilise the genetic pathway to increase the does' receptivity. The purpose of the present study was to identify genetic and non-genetic factors that influence rabbit doe sexual receptivity, in the context of a divergent selection experiment over 1 generation. The experiment spanned 2 generations: the founder generation (G0) consisting of 140 rabbit does, and the G1 generation comprising 2 divergently selected lines (L and H lines) with 70 does each and 2 successive batches from each generation. The selection rate of the G0 females to form the G1 lines was 24/140. The selection tests consisted of 16 to 18 successive receptivity tests at the rate of 3 tests per week. On the basis of 4716 tests from 275 females, the average receptivity was 56.6±48.2%. A batch effect and a test operator effect were revealed. The contribution of females to the total variance was 20.0%, whereas that of bucks was only 1.1%. Throughout the experiment, 18.2% of does expressed a low receptivity (< 34%), 50.7% a medium one and 33.1% a high one (>66%). Some does were frequently receptive, whereas others were rarely receptive. The repeatability of sexual receptivity was approximately 20%. The results confirmed the high variability of sexual receptivity of non-lactating rabbit does maintained without any biostimulation or hormonal treatment. A lack of selection response on receptivity was observed. Accordingly, the heritability of receptivity was estimated at 0.01±0.02 from an animal model and at 0.02±0.03 from a sire and dam model. The heritability of the average receptivity of a doe was calculated as 0.04. In agreement with the low estimated heritability, the heritability determined was no different from zero. Nevertheless, the occurrence of pseudopregnancies due to uncontrolled ovulations and the presence of *corpora lutea*, as assessed by progesterone titrations, could have interfered with receptivity. Further studies would be necessary to confirm the low heritability of female rabbit receptivity.

Key Words: rabbit, sexual receptivity, divergent selection, pseudopregnancy.

INTRODUCTION

In European rabbit meat production, reproduction is generally carried out by artificial insemination (AI), associated with a management system known as “single batch”. In this system, all rabbit does undergo a specific intervention on the same day (e.g., all does are inseminated on the same day), and the next intervention takes place 6 wk later. In this context, the economic value of fertility is increased, as a doe that was not fertilised at one insemination series remains unproductive for 6 wk. It has long been assumed that the rabbit doe is in permanent oestrus. However, it has been

Correspondence: M. Theau-Clément, Michele.Theau-Clement@toulouse.inra.fr. Received June 2015 - Accepted July 2015.
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demonstrated that does alternate periods of acceptance (oestrus) and periods of refusal of mating (dioestrus), whose durations are highly variable between animals (Moret, 1980; Theau-Clément *et al.*, 2011). A female rabbit is said to be 'receptive' when she accepts mating, indicated by her lordosis posture in the presence of a buck. Receptive does at insemination produce three to four times more rabbits at weaning than non-receptive ones, particularly when they are lactating (Theau-Clément, 2008). Consequently, receptivity is often induced by injection of pregnant mare serum gonadotropin (equine chronic gonadotrophin) and/or by alternatives to hormone use, known as "biostimulation" (Renouf *et al.*, 2008; Theau-Clément, 2008). In the research context of more sustainable farming systems, one alternative would be to take advantage of genetic selection to increase the receptivity level of the does at insemination.

The aim of the present experiment was: (i) to identify non-genetic factors that influence sexual receptivity on a large scale; and (ii) to study the genetic variability of rabbit doe receptivity within the context of a divergent selection experiment on sexual receptivity over one selection cycle and two generations.

MATERIALS AND METHODS

All procedures were conducted in accordance with the guidelines for the Care and Use of Animals in Agricultural Research and Teaching (French Agricultural Agency and Scientific Research Agency; approval number of the PECTOUL experimental farm: A 31 113 16).

Animals and experimental design

The selection experiment used the INRA1777 strain (New Zealand White breed). Two generations were involved, based on the same pattern (Figure 1). G0 was the founder generation (line F) and a divergent selection procedure gave rise to the high (H) and low (L) receptivity lines in G1. In each generation, 140 primiparous does were used. They were distributed into 2 batches at a 6-wk interval. Their 1st insemination occurred at 19.6 wk of age. In each generation, from 59 to 66 vasectomised INRA2266 bucks were housed in the same room as the females for the receptivity tests. These bucks were scattered throughout the room and used to test the 2 batches of does during 2 successive test periods. After the 1st kindling, the females were tested for receptivity once a week until weaning 35 d *post partum*. They were then submitted to intensive testing for 6 wk, with three receptivity tests per week, leading to a total of 18 tests that were used for selection. The test consisted in observing the behaviour of the female for 2 min after it was

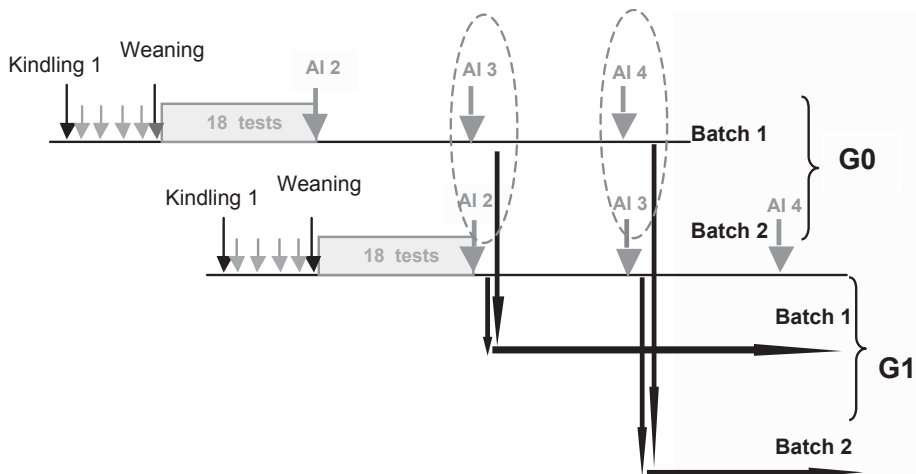


Figure 1: Experimental design (G0: founder generation; G1: 1st generation of selection). From the 1st kindling until weaning of rabbit does: one receptivity test/week (grey arrows); between weaning and the 2nd AI: 3 tests/wk for a total of 18 tests. The 2 generations were tested on the same model.

introduced into a tester buck cage. If the female was receptive, the variable receptivity was coded '1'. If not, a second trial was performed with another buck, the result of which was '0' or '1'. Five operators were in charge of the tests, each of them involved in each generation and each batch. Neither biostimulation nor hormonal treatment was applied to induce sexual behaviour. After the testing period, the does continued their reproductive life with a production phase (3 artificial inseminations at a 42-d interval: AI2, AI3 and AI4). Each batch of G1 was procreated from both batches of G0 (Figure 1) from the selected breeders. The estimated breeding values for receptivity (EBV) were calculated for females and males after the test period. The estimation was performed by BLUP with an animal model based on the results of the intensive testing, using PEST software (Groeneveld and Kovac, 1990). The 24 G0 females with the highest EBV and the eight best G0 males were selected to produce both batches of the G1 high receptivity line (H line). These females were inseminated (16 were efficient) at the 3rd and 4th artificial insemination of their reproductive phase (Figure 1) by semen from the selected males, avoiding full-sib mating. The two batches from the G1 low receptivity line (L line) were produced in a similar way to the H line, selecting and mating the same number of males and females (14 were efficient), but with the lowest EBV. To avoid penalising their reproduction, all the does, whatever the line, received a subcutaneous injection of 25 IU of eCG (Chronogest-Intervet) 2 d before AI.

For the 2nd batch of G1, blood samples were collected at AI2 (immediately after the testing period) and at AI3 (after a period without any test) in order to assess the functional status of the ovarian *corpora lutea* and detect occasional pseudopregnant does on the basis of progesterone concentrations. All blood samples were collected by venipuncture of the marginal ear vein and placed in ethylenediaminetetraacetic acid (EDTA)-containing tubes and immediately centrifuged. Plasma was stored at -20°C until assay for progesterone concentrations, measured by radioimmunoassay according to the procedure described by Boiti *et al.* (1974). Progesterone was extracted from plasma samples with ethyl ether. For extraction, 0.2 mL of plasma was used and each sample was assayed in duplicate. The assay sensitivity was 0.08 ng/mL; intra- and inter-assay coefficients of variations were 5.3% and 10.2%, respectively. Complete luteolysis was interpreted as a decline of plasma progesterone concentrations to values of 1.0 ng/mL or less (Browning *et al.*, 1980).

Statistical analyses

Genetic and non-genetic factors influencing oestrus behaviour. Only the results of the intensive test periods were analysed. Receptivity was coded as a binary trait (0=female not receptive; 1=receptive female) and analysed as a continuous variable using 2 linear mixed models: an animal model and a sire and dam model. Parameters were estimated in both models using the REML (Restricted Maximum Likelihood) method (Neumaier and Groeneveld, 1998) using the ASReml software (Gilmour *et al.*, 2009), taking the same fixed effects into account but different random effects. The fixed effects were the combination between the generation and the line, referred to as the 'generation-line' (3 levels: F, L, H, where F=founder G0 population, and L and H=low and high receptivity line in G1, respectively), the batch (2 levels: B1 or B2), the test operator (5 levels) and the generation-line per batch interaction. The random effects that were included in both models made it possible to estimate the variance component for the tester buck, for the permanent environmental effect (σ_p^2) and for the residual (σ_e^2); in addition, the variance component of the individual tested female (σ_a^2) was estimated in the animal model, whereas the sire (σ_s^2) and dam (σ_d^2) variance components were estimated in the other model.

Genetic parameters. In the animal model, heritability was calculated as $h_a^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$ and repeatability as

$r_a^2 = \frac{\sigma_a^2 + \sigma_p^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$, whereas in the sire and dam model, heritability could be calculated in two ways:

$h_s^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_d^2 + \sigma_p^2 + \sigma_e^2}$ and $h_{sd}^2 = \frac{2(\sigma_s^2 + \sigma_d^2)}{\sigma_s^2 + \sigma_d^2 + \sigma_p^2 + \sigma_e^2}$, in order to check for the presence of maternal

(and/or dominance) effects on the performance. Finally, the heritability of the average receptivity of the rabbit doe over the whole testing period could be calculated with the formula $h_{\mu}^2 = \frac{nh_a^2}{1 + (n-1)r_a}$, where h_a^2 and r_a are the parameters calculated above and n is the number of records ($n=18$).

The heritability realised was calculated according to the formula $h_R^2 = R/S$, where R is the response to selection after 1 cycle of divergent selection, i.e., the difference between the average receptivity of the 2 lines H and L at G1, and S is the selection differential, i.e., the difference in average receptivity between the top and bottom selected dams in G0.

Progesterone titrations. For does in Batch 2 at G1, receptivity and fertility were studied using an analysis of variance that took account of the fixed effect of the plasma progesterone (P4) concentration (3 levels: $P4 < 1$ ng/mL, $1 < P4 \leq 3$ ng/mL, and $P4 > 3$ ng/mL), the number of AI (2 levels: AI2 immediately after a period of 18 tests, and AI3, 6 wk after AI2 without any test) and the interactions between these 2 factors. The effects of the line and the lactation status were not included in the model, as a preliminary study did not reveal any effect of these factors (AI2: non-lactating does; AI3: lactating and non-lactating does).

RESULTS

Kinetics of the sexual receptivity of rabbit does after the first weaning

Sexual receptivity was studied in 275 rabbit does based on records obtained from 16 to 18 tests. The average receptivity was $56.6\% \pm 48.2$. The changes in receptivity over time according to generation, line and batch are shown in Figure 2. In G0, the receptivity level considered over the 2 batches fluctuated between 45 and 67%, except for the last 3 tests, which revealed a drop in receptivity. In G1, the changes in receptivity appeared to be different in the 2 batches. In Batch 1, receptivity fluctuated slightly from 50 to 72%, regardless of the line. In contrast, the changes seemed to depend on the line in Batch 2. For the L line, there was a general downward trend, generating a lower level of receptivity than that observed in Batch 1. For the H line, excluding the 1st test, the level of receptivity was also lower than in Batch 1 on average, but did not show any downward trend. At first glance, the L and H lines showed some differences in the first phase of Batch 2, with a higher level of receptivity in the L line.

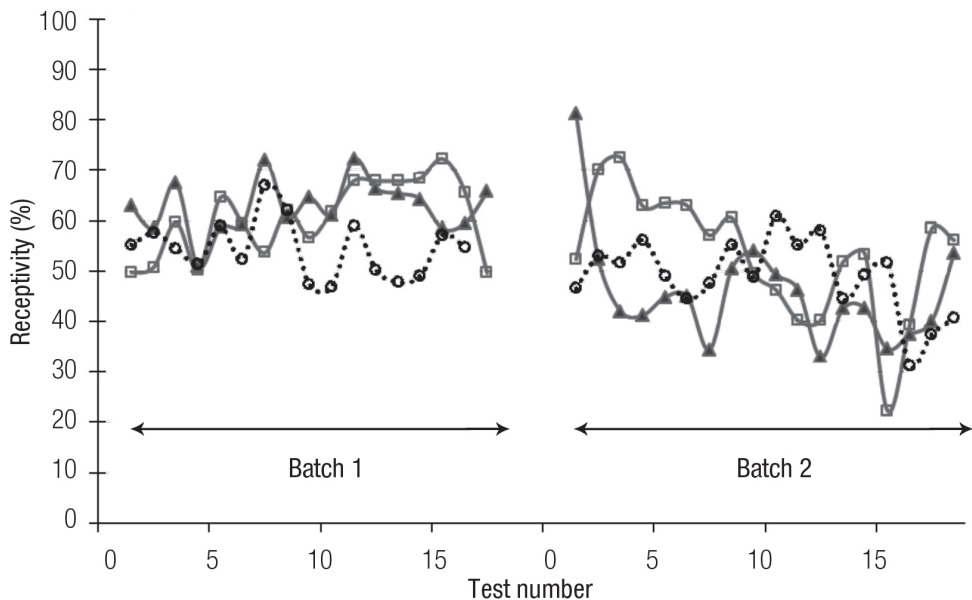


Figure 2: Changes in rabbit does' receptivity over time according to generation, line, and batch (F: founder population (G0); L: low G1 line; H: high G1 line). —□— L; —▲— H; —○— F.

Non-genetic factors influencing sexual receptivity

The estimates of the fixed effects, based on 4716 tests, were similar in both the animal and the sire/dam model. They are presented in Table 1.

Generation-line effect. The average receptivity was not significantly different according to the generation-line (F: 57.6%; L: 59.5%; and H: 54.2%).

Batch effect. Compared to Batch 2, the average receptivity was significantly higher in Batch 1 (62.1 vs. 52.2%).

Generation-line by batch interaction. The descriptive results above concerning the differential kinetics of the lines for sexual receptivity were not statistically approved. Indeed, the generation-line by batch interaction was not significant: the ranking of the lines was not statistically different in the 2 batches.

Operator effect. The effect of the person in charge of the tests was revealed when a specific operator was involved at each generation and each batch.

Tester buck effects. The variance component due to the tester bucks was low ($\sigma_{Buck}^2 = 0.003$), and their contribution to the total variance of receptivity was 1.1% (results not shown).

Individual variability of sexual receptivity

At G0, four females were never receptive and only one at G1. Thirteen and 6 does were always receptive at G0 and G1, respectively. Three classes of receptivity of equal amplitude were defined. The low receptivity class (<34%) corresponded to 18.2% females, the medium one to 48.7%, and the highest one (>66%) to 33.1% (Figure 3).

Genetic factors influencing sexual receptivity

Estimated heritability. The variance components and the estimated heritability coefficients are shown in Table 2. In both models, estimated variances associated with genetic effects (animal, sire or dam) were very low and not significantly different from 0. Consequently, heritabilities were also low and not significant. There were no differences between model estimations, and although the estimation of the dam component of the variance seemed to be higher than the sire one, they were not significantly different. In both models the permanent environmental effect accounted for about 19% of the total variance, leading to a repeatability coefficient of receptivity of 0.20.

Table 1: Estimates of the fixed effects on rabbit does' sexual receptivity (least squares means±standard error).

Effects	Number	Receptivity (%)
Average	4716	56.6
Fixed effects		
Generation-Line		
F	2285	57.6±2.5
L	1231	59.5±3.5
H	1200	54.2±3.4
		P=0.850
Batch		
B1	2163	62.1± 2.6 ^a
B2	2553	52.2± 2.4 ^b
		P=0.002
Operator		
1	773	60.9±2.4 ^a
2	1690	52.8±2.1 ^b
3	1298	60.7±2.2 ^a
4	580	57.1±2.6 ^{ab}
5	375	54.0±3.0
		P<0.001
Generation-Line*batch		
F B1	1002	60.5±3.3
F B2	1283	54.7±3.1
L B1	589	62.9±4.6
L B2	642	56.1±4.5
H B1	572	62.7±4.6
H B2	628	45.7±4.5
		P= 0.301

F: founder population (G0); L: low G1 line; H: high G1 line. Means with different superscripts in the same column are significantly different (P<0.05).

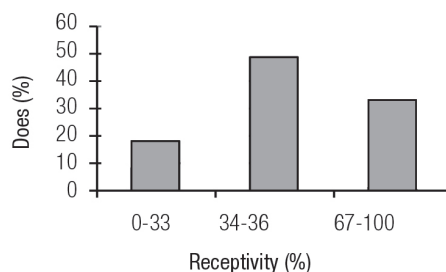


Figure 3: Percentage of rabbit does with low (<34%), medium (34-66%) or high (>66%) sexual receptivity.

Table 2: Variance components and genetic parameters (\pm standard error) of rabbit does' sexual receptivity estimated by the animal and sire/dam models.

Component		Animal model	Sire/dam model
Animal	σ_a^2	0.003 \pm 0.005	-
Sire	σ_s^2	-	0.0005 \pm 0.002
Dam	σ_d^2	-	0.002 \pm 0.004
Permanent environment	σ_p^2	0.047 \pm 0.007	0.047 \pm 0.006
Residual	σ_e^2	0.1912	0.1912
Repeatability	r	0.20 \pm 0.02	0.20 \pm 0.02
Heritability	h^2	$h_a^2 = 0.01 \pm 0.02$	$h_s^2 = 0.01 \pm 0.03$ $h_{sd}^2 = 0.02 \pm 0.03$

Using the parameters provided by the animal model, the heritability of the average of the 18 repeated tests was $h_{\mu}^2 = 0.04$.

Realised heritability. The selection differential, i.e., the difference between the top and bottom selected females, was 91.2–19.1=72.1%. The selection response was: H–L=54.2–59.5=–5.3%, which was no different from zero. As a consequence, the realised heritability was no different from zero ($h_R^2 = -0.07$).

Influence of test repetition on rabbit sexual receptivity

Because of the high difference in fertility between AI2 and AI3 observed in G0 batch 1 (70 vs. 90%), G0 batch 2 (56 vs. 84%) and G1 batch 1 (51 vs. 95%, respectively), for the 2nd batch of G1 we decided to collect blood samples immediately before insemination at AI2 (after the testing period) and at AI3 (after a period without any test) to carry out progesterone titrations and detect eventual pseudopregnant females. The percentage of does with basal P4 concentrations (<1 ng/mL) was only 13.2% for AI2 vs. 60.9% for AI3. In contrast, 41.2% had high levels of P4 (>3 ng/mL) at AI2 vs. only 18.8% for AI3. We also observed that out of 26 does with high P4 levels (>3 ng/mL) at AI2, only 2 of them had the highest progesterone concentration at AI3, while all the others had a P4 level lower than 3 ng/mL. Receptivity and fertility significantly decreased when plasma progesterone concentration increased, particularly beyond 3 ng/mL (Table 3). Receptivity and fertility also significantly decreased for AI2 compared to AI3 when inseminations were performed at the end of a series of 18 tests. However, these 2 factors interacted; the P4 level significantly influenced both receptivity and fertility only for AI2, after 6 wk of intensive receptivity tests (Figure 4).

Table 3: Influence of progesterone level and AI number¹ on rabbit does' receptivity at insemination and subsequent fertility, estimated in batch 2 of generation 1.

	Number	Receptivity (%)	Fertility (%)
Average (standard deviation)	132	68.2 (46.8)	67.4 (47.0)
Progesterone level			
P4<1 ng/mL	48	78.6 \pm 7.3 ^a	96.2 \pm 6.6 ^a
1<P4 \leq 3 ng/mL	44	82.6 \pm 6.5 ^a	79.4 \pm 5.9 ^{ab}
P4>3 ng/mL	40	54.8 \pm 6.8 ^b	34.5 \pm 6.1 ^b
		$P=0.008$	$P<0.001$
AI number			
AI2	68	58.8 \pm 5.5	61.6 \pm 5.0
AI3	64	85.3 \pm 5.6	78.4 \pm 5.1
		$P=0.001$	$P=0.021$
Progesterone level \times AI number		$P<0.001$	$P=0.008$

¹AI2: immediately after a period of 18 tests; AI3: 6 wk after AI2, without previous test.

^{a,b}Means with different superscripts are significantly different ($P<0.05$).

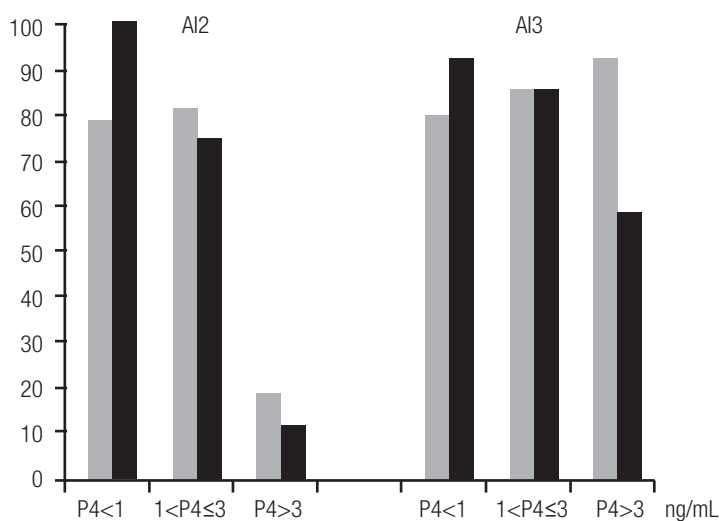


Figure 4: Interaction between progesterone levels (P4) and artificial insemination (AI) number on rabbit doe receptivity at insemination and subsequent fertility observed on G1, Batch 2. AI2, immediately after a period of 18 tests; AI3, 6 weeks after AI2, without previous test. ■ Receptivity (%), ■ Fertility (%).

DISCUSSION

Kinetics and non-genetic factors influencing sexual receptivity

The average receptivity examined at frequent time intervals over 6 wk was not significantly different according to the generation-line, but, quite unexpectedly, was influenced by the batch number, being much higher in Batch 1 than in Batch 2. It should be emphasised that the ranking of the 2 batches was the same in both generations. How to explain the different receptivity observed between the 2 batches? As they were conducted at a 6-wk interval, a season effect could be invoked. However, at each generation, the second batch was subject to more favourable natural photoperiodic conditions compared to the first: in G0, B2 took place from February to March i.e. during increasing day length, unlike B1; in G1, B1 took place from October to December i.e. during decreasing day length, unlike B2. Therefore, a seasonal effect can be ruled out. Another hypothesis is the bucks' fatigue, as the same bucks were used in both batches. If the bucks' effect was decisive, a similar effect would have been observed in the 3 lines, which was not the case. In the founder line, the drop in receptivity was observed only for the last 3 tests. In line L, except during the last tests, a steady decrease was observed in B2, whereas in line H the drop was observed immediately after the 1st test of B2. Therefore, buck fatigue could not be considered as decisive.

Individual variability and heritability of sexual receptivity

The repeatability of sexual receptivity of around 20% corresponds to previous results (Theau-Clément *et al.*, 2014; 23.2%); some does were frequently receptive, whereas others were rarely receptive. Our study confirms the high individual variability of sexual receptivity of non-lactating rabbit does maintained without any biostimulation or hormonal treatment (Moret *et al.*, 1980; Theau-Clément *et al.*, 2011). Our study demonstrates that in our experimental conditions, the contribution of females to the total variance of receptivity was about 20-fold higher than that of bucks, which was very low (20.2 vs. 1.1%).

The lack of any difference in sexual receptivity between the H and L lines was quite surprising, as their dams had huge receptivity differences (19.1% for the L line and 91.2% for the H line). This result clearly illustrates the lack of

response to selection. Realised and estimated heritability were in agreement and lead us to conclude that receptivity is a lowly heritable trait. To our knowledge, this is the first genetic study on rabbit sexual receptivity using artificial insemination. This result was a strong argument for deciding to stop the selection experiment at the end of G1, even if one generation may be insufficient to assess the inheritance of a trait. The differences between females were therefore not due to additive genetic effects. This result is not so surprising, given the high phenotypic relationship between receptivity and fertility (Theau-Clément, 2008), and the low heritability of fertility (Piles *et al.*, 2004). The differences in receptivity between females were mostly accounted for by permanent environmental effects, not excluding the possibility of dominance effects. What could constitute these permanent environmental effects? Several causes were hypothesised: an early conditioning effect of the 1st receptivity tests experienced by the rabbit does on subsequent receptivity was not confirmed by our research. Permanent environmental effects could also be due to the micro-environment of the cage where the does were raised, but our attempts to identify such effects were fruitless.

Influence of test repetition on sexual receptivity

Our results raised the question of the biological significance of the trait measured. Is the sexual receptivity measured following this protocol (18 tests repeated at 2 or 3-d intervals) the same trait as receptivity measured once only? In other words, was receptivity altered by the multiple successive tests? A troubling result was that fertility scores at AI performed just after the test series were systematically lower (50-70%) than those at AI performed 6 or 12 wk later (80-95%), after a period without any test. This reduced fertility could be ascribed to pseudopregnancy, a physiological status due to uncontrolled ovulations (in the absence of any mating or GnRH injection) and the presence of *corpora lutea*, just like during pregnancy (Boiti *et al.*, 2006). These ovulations that occurred during the testing protocol could be a consequence of these successive tests and could interfere with subsequent receptivity and fertility. The lower fertility would not have been compensated by eCG due to its inefficiency in pseudopregnant does, as suggested by Theau-Clément *et al.* (2008). Blood samples were collected at AI2 and AI3 from the same rabbit does (G1, Batch 2). As the only difference between the 2 groups of inseminations was the practice of receptivity tests, our results suggest a strong relationship between the occurrence of tests and receptivity and, hence, fertility, and confirms the results of Boiti (2006) and Theau-Clément (2008). However, the presence of pseudopregnant does could be enhanced by the fact that primiparous does (AI2) have a higher sensitivity to pseudopregnancy, as demonstrated by Theau-Clément *et al.* (2008). This could explain why for AI2, when progesterone concentration was higher than 3 ng/mL, receptivity and fertility were highly depressed. Conversely, for AI3 (secondiparous does), there was no relationship between progesterone concentration, receptivity and fertility.

Consequently, in our experimental design, receptivity would have been altered by the residual effects of previous tests. This raises the question of the biological significance of the measured "receptivity phenotype": it could be a composite trait encompassing true receptivity and sensitivity to pseudopregnancy. Nevertheless, as mentioned previously, it must be noted that the level of progesterone did not depend on the line. The question therefore concerns our estimate of heritability. It should be emphasised that in a preliminary experiment that served as a basis for the present one, and where a high number of repeated tests were also performed (n=48), no pseudopregnancies were revealed.

CONCLUSION

Our protocol, designed to measure the sexual receptivity of rabbit does, consisted of performing a series of 16-18 tests at a 2 or 3-d interval. A batch effect (2 successive batches at each generation) and a test operator effect were revealed. Our results confirmed the fairly high individual variability of sexual receptivity of non-lactating rabbit does maintained without any biostimulation or hormonal treatment: the females accounted for 20% of the variability. Surprisingly, despite this variability, the sexual receptivity of the female rabbits does not appear to be heritable, consequently leading to a lack of selection response. Nevertheless, the occurrence of pseudopregnancies due to uncontrolled ovulations and the presence of *corpora lutea* could have interfered with receptivity. If the observation of the behaviour of rabbit does in the presence of a buck at the moment of insemination is an efficient tool to measure sexual receptivity, our results suggest that the repetition of these tests could generate pseudopregnancies. The measured phenotype for receptivity would thus be a combination of true receptivity and sensitivity to pseudopregnancy. Further

studies would be necessary to confirm the low heritability of rabbit sexual receptivity. However, knowing the potential negative effect of measuring receptivity on the subsequent test results and the value of the repeatability coefficient, it would be interesting to design an experiment that would place more emphasis on the number of females tested and less on the number of tests per female (i.e., only one test per doe), thus avoiding the bias due to test repetition.

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