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PRELIMINARY STUDY OF THE INDIVIDUAL VARIABILITY OF THE SEXUAL RECEPTIVITY OF RABBIT DOES

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Abstract: The aim of this preliminary experiment was to investigate the individual variability of the sexual receptivity of a rabbit doe in the presence of a buck (lordosis position, 0-1 variable). Twenty primiparous does maintained without reproduction were tested over 4 mo (3 tests per week at a 2- or 3-day interval) after their first kindling. Out of 48 tests, the receptivity rate was 52.5±50.0% on average and varied from 20.0 to 73.3% depending on the test day. The does were lactating at the beginning of the test period and a strong receptivity decrease was revealed at the peak of lactation. Receptivity did not vary according to the tester buck or to the test operator. The individual receptivity of does varied from 8.6 to 81.3%; three of them had a receptivity rate lower than 30% and four of them a receptivity rate greater than 70%. No relationship was revealed between average receptivity and body weight or body weight variations around first litter weaning. The repeatability of sexual receptivity of non-lactating does was 23.2%. Lowly receptive does had a shorter average oestrus time (<2 tests) and a longer dioestrus time (≥6 tests), whereas highly receptive does had a longer oestrus time (>4 tests) and a shorter dioestrus time (≤3 tests). The correlation between average receptivity and average oestrus time was 0.80. These results indicate a fairly high individual variability of the expression of rabbit sexual receptivity and of its duration, and justify the exploration of an eventual genetic origin in a subsequent experiment.

Key Words: rabbit, sexual receptivity, oestrus behaviour.

INTRODUCTION

It has long been assumed that the rabbit doe is in permanent oestrus. However, Moret (1980) revealed the existence of alternate periods of acceptance of mating (oestrus) and rejection of mating (dioestrus) in nulliparous rabbit does, the length of which varies considerably between animals. Thus, the female rabbit does not have an apparent and regular oestrous cycle. A doe is said to be "sexually receptive" and consequently in oestrus when it manifests mating acceptance behaviour in the presence of a male (lordosis position). It is possible to test the sexual receptivity of a rabbit doe at any given time by placing the female in the buck's cage and observing whether it exhibits mating acceptance or rejection behaviour.

Receptive does at insemination produce 3 to 4 times more kits than lactating and non-receptive ones (Theau-Clément, 2008). Accordingly, in rabbit farms, receptivity is often induced by injection of pregnant mare serum gonadotropin (equine chronic gonadotrophin) and/or alternatives to the use of hormones, referred to as "biostimulation" (Theau-Clément, 2008; Renouf and Klein, 2008). In the search for more sustainable farming systems, another alternative would be to take advantage of the genetic pathway to increase the level of receptivity of does at insemination. The

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efficiency of this strategy depends on the heritability of sexual receptivity. A prerequisite is to check the individual variability of the expression of this behaviour, which is the objective of this study, and to compare our results with those of Moret (1980), but using a larger population size and a longer test period.

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 breeding system

Twenty INRA 1777 primiparous rabbit does were used after their first kindling, for which litters were homogenised (8 kits). Eighteen vasectomised INRA 2266 bucks were housed in the same room as the females for the receptivity tests. Animals were housed in individual flat-deck cages, under a constant 16L:8D lighting programme. Rabbit does were maintained without any insemination throughout the entire experiment. To avoid excessive weight gain, females received 140 g/d of a commercial pellet diet containing 17.3% crude protein and 15% crude fibre. Water was provided *ad libitum*.

Experimental design

To avoid the peak of receptivity *postpartum* (D0 and D1 *postpartum*), sexual receptivity of primiparous does was tested as of D2 *postpartum* by presentation to a vasectomised buck, as described by Theau-Clément *et al.* (2005), on the Monday, Wednesday and Friday of each week for 4 mo (from November 2008 to February 2009). The time lag between consecutive tests was therefore 2 or 3 d.

A total of 48 tests were performed. During the first 12 tests, rabbit does were lactating (weaning took place at 28 d). During the subsequent tests, rabbit does were neither pregnant nor lactating, as no insemination was performed. The body weight and the health status of the does were checked every 14 d to eliminate data recorded during unhealthy periods, as health status may interfere with receptivity.

Statistical analysis

To guard against interactions between health status and oestrus behaviour, the result of tests the week before a rabbit's death or removal were eliminated from the analysis (shaded area in Figure 1). In order to analyse the kinetics of sexual receptivity, the experiment time was divided into four phases of 12 tests each. The percentage of receptive does was analysed as a Bernoulli variable (0/1) by analysis of variance that included the fixed effects of the rabbit doe (20 levels), of the phase (4 levels), of the tester buck (18 levels), of the operator (technician in charge of the tests: 4 levels) and significant interactions taken two-by-two. The SAS® GLM procedure was used.

The analyses of the relationship between receptivity at day 4 and at day 18 post-weaning, on the one hand, and the current body weight or the body weight variations since previous weighing (10 d before weaning) on the other, were performed by analysis of variance including the operator as a fixed effect and the current weight and the weight changes as covariates. The relationship between the average individual receptivity (excluding the lactation phase) was studied with respect to the body weight at day 4 and at day 18 post-weaning, and with respect to weight variations between these 2 stages, using these traits as covariates in an analysis of variance, including the phase as a fixed effect (3 levels).

To estimate the repeatability of receptivity, the PROC VARCOMP procedure from SAS® was used, including the does as a random effect, plus 2 fixed effects, the tester buck and the operator. Because of a strong effect of lactation on the expression of sexual receptivity, this analysis was performed only on data recorded after the lactating period.

For each rabbit doe, the sequence of the test results shows a series of positive tests, separated by a series of negative tests that will be referred to as "pauses". As soon as there was a negative test, it was considered that there was a pause. As a synthetic descriptive analysis of the test sequences, we calculated the average and maximum duration of

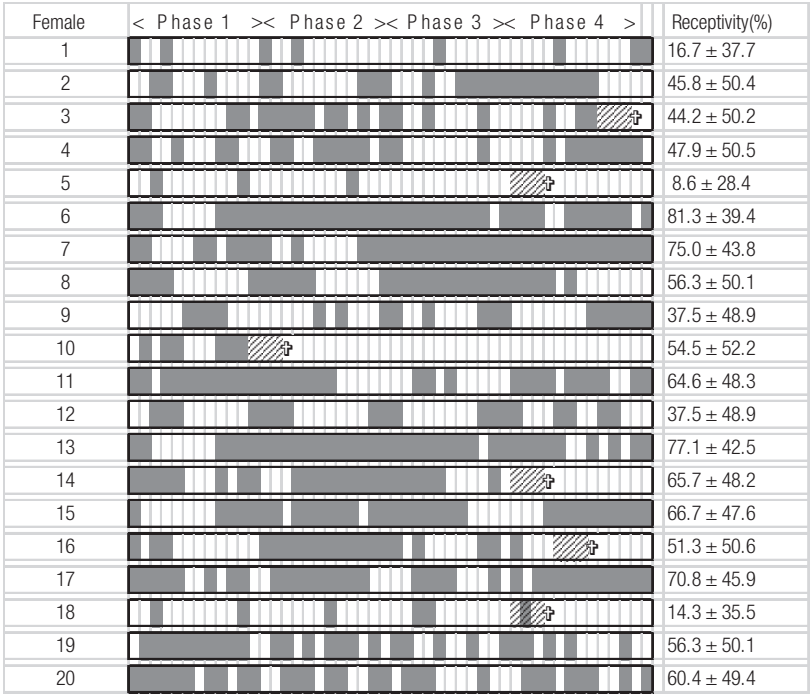

Figure 1: Temporal variations in the receptivity of rabbit does after the first kindling. Grey boxes represent positive tests. The shaded areas correspond to observations made the week before the death or removal of rabbit does, and the corresponding data were eliminated from the analysis.

positive tests, and the average duration of pauses, expressed as a number of tests. A Pearson test made it possible to test the significance of the correlation between average doe receptivity and average duration of oestrus.

RESULTS AND DISCUSSION

Six females died or were removed during the experimental period. A total of 870 receptivity tests were analysed.

Kinetics of receptivity after the first kindling

The percentage of receptive does was 52.5±50.0% on average and varied from 20.0 to 73.3%, depending on the day of the test (Figure 2). The percentage of receptive does did not vary according to the interval with the previous test (2 d: 52.0%, 3 d: 52.8%). During lactation (first phase), a drastic drop in receptivity was observed between 3 and 14 d *postpartum*, and the frequency of receptive does increased from 18 d *postpartum* until weaning. The kinetics of sexual receptivity seemed to be opposed to that of milk production, which increases until about the 20th d of lactation and then decreases (Cowie, 1969; Lebas, 1972; Casado *et al.*, 2006). Indeed, at the physiological level, Ubilla *et al.* (1992) showed that plasmatic prolactin concentration (the hormone responsible for milk production) is very low during the first 3 d of lactation (<10 ng/mL), followed by high mean prolactin levels from day 7 to 19 of lactation (maximum: 35 ng/mL) and low mean prolactin levels from day 19 to 28 of lactation (17 ng/mL at 28 d of lactation). Their observations illustrate an antagonism between the expression of sexual receptivity and prolactin secretion necessary for milk production, reflecting a global partial antagonism between reproduction and lactation.

The percentage of receptive does fluctuated less after the lactation phase.

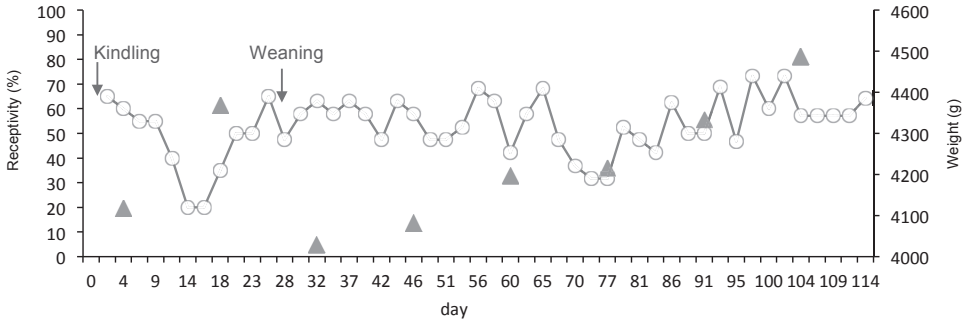

Figure 2: Kinetics of the average sexual receptivity of rabbit does after their first kindling (48 observations) until day 114 *postpartum*. The experiment was divided into 4 phases of 12 tests each. During the first 12 tests, rabbit does were lactating (phase 1). –O– Receptivity, ▲ Weight.

The changes in the average body weight of rabbit does throughout the experimental period are reported in Figure 2. Changes in body weight might in fact interfere with receptivity. The recovery of live weight, particularly in young rabbit does during and after their first lactation, is known to be critical to fertility (Parigi-Bini and Xiccato, 1993). Figure 2 illustrates that the global changes in average body weight were not related to the changes in receptivity rate: after the lactation phase, a steady weight intake was observed, whereas receptivity fluctuated.

Receptivity variation factors

The selected statistical model explains 48% of the variability in receptivity.

Even if the pattern of the receptivity variations was different in phase 1 compared to the subsequent ones, there was no significant phase effect (49.0±50.1 62.0±48.7 52.6±50.0 and 59.6±49.2% for phases 1, 2, 3 and 4, respectively), highlighting the lack of long-term effects of test repetitions on the expression of sexual behaviour (Table 1). In fact, it was feared that the practice and repetition of receptivity tests involving the presence of a buck could induce a pseudopregnancy and therefore interfere with the results of the subsequent tests. Pseudopregnancy is a physiological state due to uncontrolled ovulations (independent of mating or gonadotropin-releasing hormone injection), known to decrease sexual receptivity and, consequently, fertility (Boiti *et al.,* 1996; Theau-Clément *et al.*, 2000; Boiti *et al.,* 2006; Theau-Clément *et al.*, 2008a).

Table 1: Factors of variation or rabbit doe receptivity: results of the analysis of variance.

Factors of variation	Receptivity (%)
Main effects	
Female	$P<0.001$
Phase	NS
Tester buck	NS
Operator	NS
Interactions	
Female×Phase	$P=0.003$
Female×Tester buck	NS
Female×Operator	NS
Phase×Tester buck	NS
Phase×Operator	$P=0.040$
Tester buck×Operator	NS

NS: non-significant.

The significant female-by-phase interaction indicates that the ranking of rabbit does for receptivity may vary depending on the *postpartum* phase. The changes in live weight of rabbit does around the first weaning could explain the different receptivity trends in relation to the phase, as the recovery of live weight is known to influence subsequent fertility (Parigi-Bini and Xiccato, 1993) and perhaps receptivity. Nevertheless, no influence of the body weight variations around weaning was observed on receptivity at 4 and 18 d post-weaning or on average receptivity, suggesting that the weight variations do not significantly contribute to female-by-phase interaction.

The tester buck and the operator did not influence the percentage of receptive does. Nevertheless, it must be emphasised that the significant phase by operator interaction shows that the ranking of the operators for receptivity may vary depending on the phase.

Concerning the relationship between body weight or body weight changes around weaning and receptivity at either 4 and 18 d post-weaning, the analysis failed to show any relationship between receptivity and body weight traits. Similarly, after removal of the lactation phase, no relationship was found between the average receptivity on the one hand and body weight or weight variations around weaning on the other. In consequence, the average body weight corresponding to positive tests was similar to that of negative ones (4228±383 and 4227±387 g for positive and negative tests, respectively).

Individual variability of sexual receptivity of rabbit does

Figure 1 illustrates the sequence of receptivity tests for each female and gives their average receptivity. This figure shows the high variability in the expression of receptivity of rabbit does: some females are nearly always receptive, whereas some others are rarely receptive. Indeed, out of 48 tests, the average receptivity rate of the females ranged from 8.6 to 81.3%. Three rabbit does had a receptivity rate lower than 30% and 4 others had a rate greater than 70%. The results of the variance analysis confirmed the high effect of the female on the receptivity rate ($P<0.001$). Another indicator of individual variability of receptivity is repeatability. This coefficient is the correlation between repeated receptivity records on the same female and encompasses genetic and permanent environmental effects. It constitutes the upper limit of the heritability coefficient. After eliminating data collected during the lactation period (corresponding to phase 1), the repeatability of receptivity was estimated at 23.2%. This value has a magnitude similar to that of the repeatability of litter size in rabbits (between 0.10 and 0.20) (Gomez *et al.*, 1996; Rochambeau, 1998; Garreau *et al.*, 2000; Garcia *et al.*, 2002a, 2002b; Argente *et al.*, 2003). This estimate indicates a fairly high individual variability and encourages the exploration of an eventual genetic origin in a further experiment.

Moret (1980) had tested the receptivity of 15 nulliparous rabbit does (aged from 19 to 22 wk, 5 observed in October-November, 5 in November-December and 5 in March-April), following a sudden transfer the day before the experiment, from a building with a 12L:12D lighting programme, to a building with a 16L:8D lighting programme. The females were presented daily to a male for 30 consecutive days. The experiment also showed a great individual variability in the expression of oestrus behaviour (receptivity rates ranging from 7 to 90%). In that experiment, the receptivity rate was higher than in our study (from 51 to 66%, depending on the season) and could be the result of light stimulation at the beginning of the experiment related to the sudden change from 12 to 16 h of daily lighting (Theau-Clément *et al.*, 2008b).

Individual variability of average oestrus duration

The average oestrus period was 3.7±2.1 tests, the maximum oestrus period was 10.8±7.8 tests, and the average pause period was 3.6±1.8 tests (Table 2). Infrequently receptive rabbit does (<30% of the tests) had a short mean oestrus period (<2 tests) and a long average pause period (≥6 tests). Frequently receptive females (>70%) had a longer mean oestrus period (>4 tests) and a shorter pause period (≤3 tests). A significant correlation was observed between the average receptivity and the average oestrus period (r=0.80, $P<0.001$). However, several cases were observed (female 3, for example) where a medium receptivity (44.2%) co-existed with a short oestrus period (<2 tests), compensated for by a high cycle number (n=10). These results are in agreement with those of Moret (1980), who observed that out of a total of 15 rabbits studied, 3 does were receptive for at least 27 consecutive days and 4 does were receptive for only 1 to 4 d consecutively.

Thus, our results for receptivity obtained on primiparous does maintained in a constant environment and tested over a long period indicate a fairly high variability in the expression of oestrus behaviour in our breeding system and confirm the preliminary observations of Moret (1980).

Table 2: Individual variability in the average oestrus period of rabbit does.

Female	Tests number	Series of positive tests	Average oestrus period (No. tests)	Max oestrus period (No. tests)	Average pause period (No. tests)
1	48	7	1.1	2	6.7
2	48	6	3.7	13	3.7
3	43	10	1.9	5	2.4
4	48	9	2.6	7	2.8
5	35	3	1.0	1	8.0
6	48	5	7.8	25	2.3
7	48	5	7.2	27	3.0
8	48	4	6.8	16	5.3
9	48	7	2.6	6	4.3
10	11	3	2.0	3	1.7
11	48	7	4.4	16	2.8
12	48	6	3.0	4	4.3
13	48	6	6.2	24	2.3
14	35	5	4.6	14	2.4
15	48	5	6.4	10	4.0
16	39	6	3.3	13	3.2
17	48	8	4.3	11	2.0
18	35	4	1.3	2	6.0
19	48	12	2.3	10	1.6
20	48	12	2.4	6	1.7

CONCLUSION

The results of this preliminary experiment show the high individual variability in the expression of oestrus behaviour of primiparous rabbit does and encourage the exploration of a possible genetic determinism of this variability. If this hypothesis is correct, then using females selected for their ability to come into oestrus would decrease the use of exogenous hormones or biostimulation in the preparation of rabbit does for artificial insemination.

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