Effects of doe–litter separation on endocrinological and productivity variables in lactating rabbits

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Abstract

The objective of this study was to explore the effects of a doe–litter separation for 48 h before artificial insemination (AI) on various endocrinological and productivity variables determined in 24 doe rabbits. The control group (C) (n = 12) had free access to nursing and the separated group (S) (n = 12) were kept apart from their litters for 48 h prior to AI on day 11 of the lactation period. Lower plasma prolactin (PRL) concentrations were observed 24 h after the doe–litter separation (\(P = 0.04\)). The S does had increased plasma oestradiol (E\(_2\)) concentrations 48 h after the doe–litter separation, compared with preseparation values of the same group and with C does (\(P < 0.0001\)). A higher proportion of does showing high sexual receptivity was observed among the S group compared with the C group (\(P < 0.05\)). The suckling episode ranged from 3 to 5 min in S does. Teat stimulation by suckling caused an immediate increase in PRL in S does (\(P < 0.0001\)). PRL remained high until 7 h after suckling in S does, compared with C does (\(P < 0.04\)). The doe–litter separation resulted in a mean (±S.E.M.) reduction in litter weight of 217 (±22.1) g (\(P < 0.02\)). No kits died in either group during the separation period. The litter weight on day 21 after parturition and milk production/doe were not affected by treatment. When analysing the subsequent litters, no differences were observed between S and C does in kindling rate, litter size at birth, number of dead kits/litter, litter size at weaning and number of kits dead from birth to weaning. The results suggest that a transient doe–litter separation causes an increase in the proportion of does showing signs of oestrus and may have a major influence on fertility. These effects may be induced by the absence of suckling episodes together with the higher plasma E\(_2\) and lower PRL concentrations seen in the S does prior to AI. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The reproductive performance of lactating doe rabbits is characterised by irregular oestrous and anoestrous periods, constituting a disadvantage when attempting to establish synchronised reproduction and the use of artificial insemination (AI). Kindling rates (KR) obtained in artificially inseminated lactating doe rabbits appear to be affected by frequent variations in sexual receptivity (SR) during the lactation period (Castellini et al., 1998). Receptive does are reported to have high plasma oestradiol17-β...
(E$_2$) concentrations (Ubilla and Rebollar, 1995); these show higher KR and litter size at birth than non-receptive does (Theau-Clément and Roustan, 1992). The reduction in fertility and SR rates observed during the lactation period appeared to be influenced by hormonal antagonisms between blood prolactin (PRL) and gonadotrophin concentrations (Fortun and Bolet, 1995; Ubilla and Rebollar, 1995).

Recently, several studies on stimulating ovarian activity in lactating doe rabbits have demonstrated the influence of the separation of does from their litters for short periods of time prior to AI (Pavois et al., 1994; Alvarino et al., 1998; Maertens, 1998). These methods, especially after a doe–litter separation of at least 36 h before AI, improved KR and litter size at birth, with results similar to those obtained when administering hormonal treatments, such as prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) or pregnant mare serum gonadotrophin (PMSG), before AI (Facchin et al., 1992; Pavois et al., 1994). Litter size at birth appeared to be reduced when AI was performed on day 4 after delivery in normal lactating does, compared with does separated from their litters for 48 h before AI (Alvarino et al., 1998). These results indicate an antagonism between reproduction and lactation during the first few days of the lactation period (Alvarino et al., 1998).

The above references suggest that a transient doe–litter separation in lactating doe rabbits may induce several endocrinological changes that could affect reproductive performance, probably by stimulating the activity of the pituitary and ovary. Nevertheless, the mechanisms involved in these events are not yet clearly understood.

This study was therefore designed to investigate the changes occurring in plasma PRL and E$_2$ concentrations after the separation of does from their litters for a short period of time before AI, as well as the influence on SR, change in litter weight, and litter survival. Several other reproductive variables obtained after parturition were also determined.

2. Materials and methods

2.1. Animals

This study was performed with 24 multiparous, lactating, Californian × New Zealand White cross-breed doe rabbits. Animals were housed in research facilities of the Animal Production Department. They were maintained under controlled light–dark cycles (16:8, L:D), housed in individual metal cages, fed ad libitum using a commercial pelleted diet (Lab Rabbit Chow, Purina Mills, Torrejón de Ardoz-Madrid, Spain) and had free access to tap water. On day 1 after parturition, litter size was standardised to 8–9 by adding or removing kits to assure similar lactation conditions during the experiment.

2.2. Experimental design

The lactating does were randomly allocated to one of two groups of 12 animals: (1) a control (C) group with free access to nursing and (2) a separated (S) group in which the does were separated from their litters by means of a metal screen for 48 h prior to AI (Luzi and Crimella, 1998), i.e. from day 9 until day 11 of the lactation period.

Both groups were inseminated on day 11 of the lactation period using 1 ml/doe of the same fresh semen pooled from several males and diluted using a commercial extender (MA 24, Ovejero, León, Spain). Each dose contained at least $25 \times 10^6$ spermatozoa/ml. Ovulation was induced by gonadorelin, a synthetic analogue of gonadotrophin-releasing hormone (GnRH; 20 µg/doe, i.m.; Ovejero) (Alvarino et al., 1998). Separated does received AI immediately after nursing and blood sampling (about 10 min after the separation period ended). Control does nursed their young at least 4 h before AI and received AI after blood sampling. Pregnancies were diagnosed by abdominal palpation on day 12 after AI.

In order to study the influence of separation on plasma E$_2$ and PRL concentrations, blood samples were collected in both groups from the marginal ear vein into heparinized tubes, and immediately centrifuged at 1000 g for 10 min at 8°C. Plasma was stored at −20°C until analysed. The blood samples were collected at 24-h intervals from day 9 to 11 of the lactation period. To study the PRL secretion pattern after suckling, sampling was also performed at the following times: 1, 1.5, 2, 2.5, 3, 3.5, 4 and 7 h after AI. In S does the first sample obtained on day 11 of the lactation period was collected prior to AI but after nursing. To measure basal plasma PRL concentrations, both groups of does nursed their
litters at least 4 h before sampling on day 9. All C rabbits nursed their litters at least 4 h before the second and third blood samples were collected (on days 10 and 11, respectively). No does of either group nursed their litters after AI until the last sample was collected 7 h later.

Sexual receptivity was assessed based on the turgidity and colour of the vulva (McNitt and Moody, 1989; Ubilla and Rebollar, 1995) before AI. The does were categorised into three levels: high SR (HSR), medium SR (MSR) and low SR (LSR) (Rodriguez et al., 1989; Ubilla and Rebollar, 1995).

This study was performed according to the CEE Council Directive 86/609 (1986) for care of experimental animals.

2.3. Variables measured in the current litters

The influence of the transient doe–litter separation on litter survival was observed every 8 h (from 09:00 h on day 9 to 09:00 h on day 11 of lactation). The change in litter weight after separation was evaluated by comparing the litter weights on day 9 versus day 11 of the same group. The duration of the suckling episode before AI on day 11 was measured in the S group at the end of the separation period. The litter weight was determined on day 21 after parturition in both groups. Milk production/doe was estimated for a lactation period of 30 days in both groups by means of the following regression: \( y = 276.91 + 1.831x; \ r^2 = 0.846, \ (P<0.001); \) (\( y = \) milk production for a lactation period of 30 days; \( x = \) litter weight on day 21 after parturition) (Torres et al., 1979).

2.4. Variables measured in the subsequent litters

The KR, litter size at birth, number of dead kits/litter, litter size at weaning and number of kits dead from birth to weaning was checked after parturition.

2.5. Hormone analyses

Plasma PRL concentrations were measured in duplicate samples by a specific radioimmunoassay (RIA) method (Ubilla et al., 1992) using AFP-991086 antibody supplied by the National Institute of Health (NIH, Bethesda, MD, USA) and Dr. A.F. Parlow (Harbour-UCLA Medical Center, CA, USA). The titre of antibody used was 1:62,500. The PRL standard used was RbPR-1. Hormone was labelled with \(^{125}\text{I}\) by the chloramine-T method (Greenwood et al., 1963). The volume of plasma for PRL determinations was 10 µl. \( \textit{Staphylococcus aureus} \) (prepared by the Autónoma University, Department of Plant Physiology, U.A.M., Madrid, Spain) was used to precipitate the bound fraction (Ubilla et al., 1992). All samples were measured in the same assay run to avoid inter-assay variations. The sensitivity of the assay for PRL was 0.125 ng/µl and the intra-assay coefficient of variation was <5%. The intra-assay coefficient was calculated using one pool of plasma measured ten times in the same assay. The mean (±S.E.M.) concentration was 106.9±4.1 ng/µl. Plasma E\(_2\) concentrations were determined using a commercial \(^{125}\text{I}\) RIA kit (ICN Pharmaceuticals Diagnostics Division, Costa Mesa, CA, USA; Lot no. E2 K9917). Plasma E\(_2\) concentrations were expressed as pg/ml. All samples were analysed in the same assay. The sensitivity of the assay was 0.74 pg/ml, and the intra-assay coefficient of variation was <7%. The intra-assay coefficient was calculated using one pool of plasma measured 10 times in the same assay. The mean (±S.E.M.) concentration was 35.8±2.5 pg/ml.

2.6. Statistics

All analyses were conducted using the statistical analysis system (SAS, version 6.12, 1990). A non-parametric procedure (PROC CATMOD) was used to identify significant effects of treatment on the variables that did not approximate to a normal distribution (PRL and E\(_2\) concentrations, SR, KR, number of dead kits/litter, litter size at weaning, number of kits dead from birth to weaning, and change in litter weight after the separation from the does). Means were compared using the CONTRAST procedure. Milk production/doe and litter size at birth variables were analysed by one-way analysis of variance followed by the Duncan multiple range test (SAS, 1990).

3. Results

No differences over time were observed in plasma PRL concentrations among C does during the period...
studied. Separated does showed lower plasma PRL concentrations 24 h after doe–litter separation compared with preseparation values of the same group and with the C does (P<0.04). Higher plasma PRL concentrations were observed in S does after nursing compared with the C does (P<0.0001). PRL concentrations remained high from 1 to 7 h after suckling compared with C does (P<0.001 to P<0.04) (Fig. 1).

Mean plasma E2 concentrations were similar among C does during the period studied. The S does showed increased plasma E2 concentrations 48 h after the doe–litter separation compared with the preseparation values of the same group and with the C does (P<0.0001) (Fig. 2).

A higher proportion of HSR does was observed among the S does compared with the C does (P<0.05) (Fig. 3).

The doe–litter separation before AI resulted in a mean reduction (P<0.02) in litter weight, while litter weight increased (P<0.02) from days 9 to 11 of the lactation period in C does.

No kits died in either group during the separation period before AI. No differences were observed between S and C does in litter weight on day 21 after parturition and milk production/doe (Table 1). The suckling episodes ranged from 3 to 5 min in S does. No differences were observed between S and C does in KR, litter size at birth, number of dead kits/litter, litter size at weaning and number of kits dead from birth to weaning in the subsequent litters (Table 1).

4. Discussion

Plasma PRL concentrations measured in both groups are consistent with those previously reported during the lactation period (Fuchs et al., 1984; Ubilla...
et al., 1992). Control does showed low PRL concentrations during the sampling period since these animals suckled at least 4 h before blood samples were collected on days 9, 10 and 11. Fuchs et al. (1984) reported similar PRL concentrations 5 h after suckling on days 5–30 of lactation. McNeilly and Friesen (1978) observed basal PRL concentrations 1–2 h after suckling or manual teat stimulation on day 11 of lactation. Decreased PRL concentrations were observed 24 h after the doe–litter separation, probably due to the absence of suckling episodes that stimulate PRL release (McNeilly and Friesen, 1978). These endocrine changes observed in S does may have stimulatory effects on ovarian activity and SR since high plasma PRL concentrations can inhibit the oestrogenic effect on SR (Theau-Clement et al., 1990; Ubilla et al., 1992). The low PRL concentrations observed could be related to high follicular steroidogenic activity and may explain the increased plasma $E_2$ concentrations found in this study 48 h after the doe–litter separation. These increased $E_2$ concentrations may be directly related to ovarian activity and were similar to those found previously in does on day 1 after parturition, associated with the first follicular growth wave (Ubilla and Rebollar, 1995) and high follicular activity (Rebollar et al., 1992). In the rabbit, as in other species, the release and action of GnRH by the hypothalamus appeared to be controlled by ovarian steroids and probably by endogenous opioids (Orstead and Spies, 1987). Previously it was suggested that the increased secretion of $\beta$-endorphin produced by suckling in the ewe may inhibit the secretion of gonadotrophins during the lactation period (McNeilly, 1988). However, in this study, $\beta$-endorphin should be decreased as suckling stimuli was absent in S does. The absence of suckling episodes together with the high $E_2$ concentrations and low PRL concentrations observed in S does may have a major influence on the SR and the fertility of these does. These stimuli may enhance the SR in rabbits previously separated from their litters by inducing a higher number of oestrus

Fig. 2. Plasma oestradiol-17$\beta$ ($E_2$) concentrations in control (□) and separated (■) does. The arrow indicates the time of artificial insemination (AI). Each column represents the mean±S.E.M. of 12 samples. The separated does had higher $E_2$ concentrations 48 h after the doe–litter separation compared with the preseparation values of the same group and with the control does (****, $P<0.0001$).
Fig. 3. Sexual receptivity (SR) in control and separated does. A higher proportion of high SR (HSR) does were observed in the separated group compared with the control group (*, $P<0.05$) (MSR = medium SR does; LSR = low SR does).

Table 1
Effect of doe–litter separation for 48 h before artificial insemination on mean (±S.E.M.) reproductive and productive variables in nursing doe rabbits

<table>
<thead>
<tr>
<th></th>
<th>Separated does</th>
<th>Control does</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current litter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter survival after separation (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Change in litter weight after separation (g)</td>
<td>$-217.6±22.1^*$</td>
<td>$+302±27.1^*$</td>
</tr>
<tr>
<td>Litter weight on day 21 after parturition (g)</td>
<td>$2402.1±129.4$</td>
<td>$2234.3±140.7$</td>
</tr>
<tr>
<td>Milk production/doe (g)</td>
<td>$4675±150.4$</td>
<td>$4368±135.1$</td>
</tr>
<tr>
<td><strong>Subsequent litter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kindling rate (%)</td>
<td>100</td>
<td>83.3</td>
</tr>
<tr>
<td>Litter size at birth (g)</td>
<td>$9.08±0.41$</td>
<td>$9.20±0.39$</td>
</tr>
<tr>
<td>Number of dead kits/litter</td>
<td>$0.51±0.13$</td>
<td>$0.43±0.11$</td>
</tr>
<tr>
<td>Number of kits dead from birth to weaning</td>
<td>$1.17±0.09$</td>
<td>$1.67±0.11$</td>
</tr>
<tr>
<td>Litter size at weaning (on day 30 after parturition)</td>
<td>$7.4±0.19$</td>
<td>$7.1±0.24$</td>
</tr>
</tbody>
</table>

*, Litter weights of the same group were compared between day 9 and 11 ($P<0.02$).

periods (Maertens, 1998). These effects could explain the higher proportion of HRS does found in the S group. Similar findings were observed after a 40-h doe–litter separation or after PMSG treatments (Maertens, 1998).

After suckling, mean plasma PRL concentrations rose to 10 times the previous mean values (detected on day 10 of lactation) in S does, confirming the PRL release during suckling that occurs in response to physical stimulation of the nipples (McNeilly and Friesen, 1978). This response was followed by sustained high PRL concentrations in S does until 7
h after suckling. Similar PRL secretion patterns during the early lactation period were previously observed after a once daily suckling episode (Muccioli et al., 1982; Fuchs et al., 1984). The suckling episode intervals observed in S does are in agreement with previous results (Grosvenor et al., 1967). Compared with other animals, rabbit does normally nurse their litters once daily and the kits suckle during a 2–5 min uninterrupted suckling period (Grosvenor et al., 1967; McNitt, 1992), whereas the rat nurses several times per day and the litters suckle in an intermittent mode (Wakerley and Lincoln, 1971). In the rat, PRL secretion declines as soon as suckling stops (Grosvenor and Whitworth, 1974). Therefore, the differences in PRL secretion between these species could be a response to different suckling patterns (Fuchs et al., 1984). The sustained PRL release observed in the rabbit may explain the essential lactogenic role that this hormone plays in the maintenance of lactation (Deis et al., 1980; Fuchs et al., 1984). Frequent suckling episodes with short periods of elevated PRL release are necessary to start and maintain lactation in other animals (Tolis et al., 1974; Djiane et al., 1977) whereas, in doe rabbits, the sustained PRL release appears to be able to induce and maintain milk yield throughout lactation (Fuchs et al., 1984). Other relevant aspects involved with maternal behaviour may be induced by the sustained PRL secretion, since does showing lower plasma concentrations after parturition refuse to nurse their litters (McNeilly and Friesen, 1978). In this study, the sustained PRL release observed in S does was detected until the last sampling, 7 h after suckling. These results suggest a longer PRL release in does previously separated from their litters for a short period of time, than that found after suckling during a normal lactation (Fuchs et al., 1984; Ubilla et al., 1992).

The KR and litter size at birth were not affected by the doe–litter separation, and were consistent with previous results (Alvarino et al., 1998). Although no statistical differences for KR were obtained, all S does became pregnant after AI compared with 10/12 C does. This could be significant if a greater number of animals were inseminated, since increased KR was indeed obtained when a large number of lactating does were artificially inseminated after a separation from their litters for short periods (Castellini et al., 1998; Maertens, 1998). A Doe±litter separation for 48 h before AI causes an increase in the proportion of does showing HSR signs and may have a major influence on the fertility of these does. These effects are probably due to the absence of suckling episodes together with the higher plasma E2 and lower PRL concentrations seen in the S does. The suckling episodes ranged from 3 to 5 min and provoked a sustained PRL release of longer duration than that observed in normally lactating does. This sustained PRL release may be involved with the maintenance of lactation and maternal behaviour. Although the litter weight decreased after the doe–litter separation, all kits survived and the effect of separation on litter weight was not detected on day 21 after parturition. Similarly, Maertens (1998) found that the loss of weight of the litters was 7–8% after 40 h of separation. Several authors have suggested that the loss in the litter weight observed could be partially compensated for during the fattening period (Alvarino et al., 1998). Our study did not show any effect of the treatment on milk production/doe.

The litter size at weaning and the number of kits dead from birth to weaning was not negatively affected by the treatment. These results are in agreement with those described by other authors (Castellini et al., 1998; Maertens, 1998).

In summary, the results suggest that a doe–litter separation for 48 h before AI causes an increase in the proportion of does showing HSR signs and may have a major influence on the fertility of these does. Further information on the ovarian activity induced by a transient separation of does from their litters appears to be necessary in order to confirm the stimulatory actions of separation on SR and fertility.

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References


