

Proceedings of the



4-7 July **2000** – Valencia Spain

These proceedings were printed as a special issue of *WORLD RABBIT SCIENCE*, the journal of the World Rabbit Science Association, Volume 8, supplement 1

**ISSN reference of this on line version is 2308-1910**

*(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)*

**UBILLA E., REBOLLAR P.G., PAZO D.,  
ESQUIFINO A., ALVARIÑO J.M.R.**

**INFLUENCE OF DOE-LITTER SEPARATION ON SEXUAL  
RECEPTIVITY, FERTILITY, PLASMA PROGESTRONE AND  
OESTRADIOL CONCENTRATIONS  
IN LACTATING RABBITS**

Volume A, pages 267-272

# INFLUENCE OF DOE-LITTER SEPARATION ON SEXUAL RECEPTIVITY, FERTILITY, PLASMA PROGESTERONE AND OESTRADIOL CONCENTRATIONS IN LACTATING RABBITS

UBILLA, E., \* REBOLLAR, P.G., \* PAZO, D., \*\* ESQUIFINO, A., \*\* ALVARIÑO, J.M.R.\*

\*Departamento de Producción Animal, E.T.S.I.Agrónomos, Universidad Politécnica, Ciudad Universitaria s/n, 28040 Madrid, Spain.

\*\*Departamento de Bioquímica, Facultad de Medicina, Universidad Complutense, Ciudad Universitaria s/n, 28040 Madrid, Spain.  
eubilla@pan.etsia.upm.es

## ABSTRACT

This study was performed to determine the effects of 48 hours doe-litter separation before artificial insemination (AI) on sexual receptivity (SR), mean plasma progesterone (P) and oestradiol 17- $\beta$  ( $E_2$ ) concentrations. The fertility and litter size obtained was determined after parturition. Control does (n=12) had free access to nursing, whereas biostimulated does (n=12) were separated from their litters for 48 hours before AI. Both groups were inseminated on day 11 after parturition. A rise of  $E_2$  concentrations was observed 48 hours after doe-litter separation, compared to control does and to previous values ( $p < 0.003$ ). Both groups showed similar P concentrations from day 9 to 11 of the postpartum period. A higher proportion of high sexual receptive (HSR) does was observed in the does separated from their litters for 48 hours ( $p < 0.05$ ). This change in the SR may be related to the increased  $E_2$  concentrations showed by this group 48 hours after the separation ( $p < 0.003$ ). Pregnant rabbits of both groups showed increased P concentrations on day 18 of pregnancy. Lower  $E_2$  levels were observed in control does on day 18 of pregnancy compared with biostimulated rabbits ( $p < 0.003$ ). Fertility, litter size and number of dead kits/litter were not affected by the treatment (100% vs. 83,3%;  $9.08 \pm 0.41$  vs.  $9.20 \pm 0.39$  and  $0.51 \pm 0.13$  vs.  $0.43 \pm 0.11$ , in biostimulated and control does, respectively). The results suggest that a transient doe-litter separation causes an increase in the proportion of does showing signs of HSR probably induced by the higher plasma  $E_2$  concentrations and absence of suckling episodes in the biostimulated does before AI. Although the fertility and litter size did not appear to be affected by the treatment, the routine use of a biostimulation method may produce a major impact on the productivity of nursing does.

## INTRODUCTION

Several factors, such as the lactation period and SR may affect the fertility rates obtained in artificially inseminated doe rabbits during the early post-partum period (THEAU-CLÉMENT and ROUSTAN, 1992; UBILLA and REBOLLAR, 1995). Another factor associated with the reduced fertility and oestrus frequency in multiparous lactating does was the abnormal high P concentrations found on the day of AI (BOITI *et al.*, 1996). In rabbits, plasma P concentrations during pregnancy and pseudopregnancy is related to the number of corpora lutea, their synthesis and secretion (MILLS and STOPPER, 1989; HOLT, 1989). The follicular steroidogenic activity is essential for maintaining the corpus luteum life span, as  $E_2$  is the main luteotrophic hormone (McLEAN and MILLER, 1985). Separation of the does from the litters for short periods of time (the so called biostimulation technique) before AI, resulted in increased breeding efficiency in farm conditions when using a large number of animals (MAERTENS 1998; ALVARIÑO *et al.*, 1998; CASTELLINI *et al.*, 1998). Lactating does separated from their litters during 24-48 hours reached similar values to those obtained with hormonal treatments before artificial insemination (COLIN, 1992; FACCHIN *et al.*, 1992; DRAGAN, 1996;). Fertility rates and SR were also improved when rabbit does were

grouped together for short periods of time before insemination (DUPERRAY *et al.*, 1999). It has been postulated that this breeding improvement observed in biostimulated does could be a result of several stimulatory actions and endocrine changes on the ovarian activities and sexual receptivity (CASTELLINI *et al.*, 1998). The aim of the present study was to determine the effects of a 48 hours doe-litter separation before AI on SR, mean plasma P and E<sub>2</sub> concentrations. Fertility and litter size was determined after parturition.

## MATERIAL AND METHODS

### Animals and experimental design

Twenty-four multiparous, lactating doe rabbits of the Californian x New Zealand White crossbreed were used in this study. The animals were housed in individual metal cages, maintained under controlled light/dark cycles (16L: 8D), and fed *ad libitum* with a commercial pelleted diet (Lab Rabbit Chow, Purina Mills Inc., Torrejón de Ardoz, Spain), and had free access to tap water. Litter size was standardised to 8-9 on day 1 after parturition to assure similar lactation conditions during the study. The does were randomly allocated to one of two groups of 12: a *Control group* with free access to nursing, and a *Biostimulated group* in which does were separated from their litters for 48 hours previous to AI. Both groups were inseminated on day 11 of the lactation period using 1 ml/doe of the same fresh semen pooled from 3 bucks and diluted by a commercial extender (MA 24, Ovejero Lab., León Spain). Each dose contained at least  $25 \times 10^6$  spermatozoa/ml. Ovulation was induced by Gonadoreline, a synthetic analogue of GnRH (20 µg/doe i.m.; Ovejero Lab., León Spain), (ALVARIÑO *et al.*, 1998). Biostimulated does were artificially inseminated immediately after nursing and blood sampling. Control does were inseminated after blood sampling. Pregnancies were diagnosed by abdominal palpation on day 12 after AI.

To study the influence of the doe-litter separation on mean plasma P and E<sub>2</sub> 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 hour intervals from day 9 to day 11 of lactation, before AI, and on day 18 after AI.

SR was measured according to the turgidity and colour of the vulva (UBILLA and REBOLLAR, 1995), on day 11 of the lactation period before AI. The does were categorised into three levels: does with high SR (HSR), medium SR (MSR) and low SR (LSR). The fertility and litter size was evaluated after parturition.

### Hormone analyses

Plasma E<sub>2</sub> and P concentrations were determined using commercial <sup>125</sup>I RIA kit (ICN Pharmaceuticals, Inc. Diagnostics Division Costa Mesa, CA 92626 USA, Lot no. E2K9917 and PT9902, respectively) (CEDARS *et al.*, 1990; STOVALL *et al.*, 1991). This study was performed according to the CEE Council Directive 86/609, (1986) for care of experimental animals.

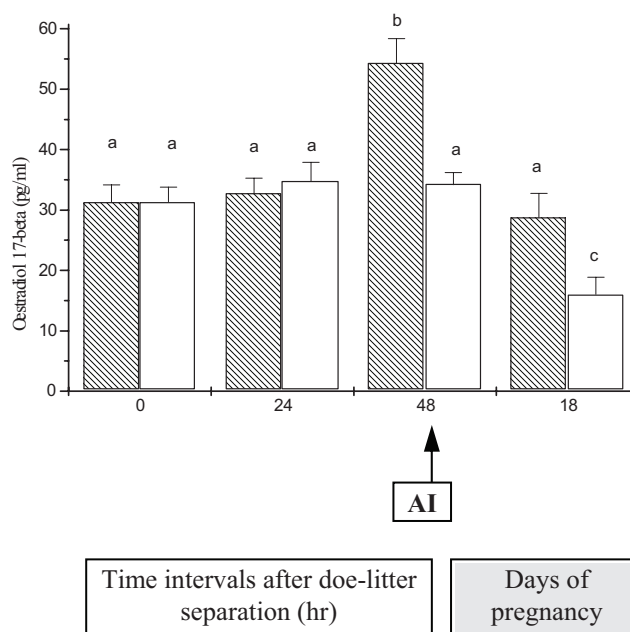
### Statistical analysis

All analyses were conducted using the Statistical Analysis System Software for Windows, version 6.12 (SAS Institute Inc., North Carolina, USA). A non-parametric procedure (PROC CATMOD; SAS, 1990) was used to identify significant effects of treatment on the variables that did not present a normal distribution (plasma P and E<sub>2</sub> concentrations, sexual receptivity and fertility). The means were compared using the Contrast statement. The significant effect of treatment on litter size at birth was analysed by one-way analysis of variance (ANOVA)

using the general linear models (GLM) procedure of SAS (1990). The means were compared using the Duncan multiple range test (SAS, 1990). Values were considered significant if  $p < 0.05$ . Data are presented as mean  $\pm$  SEM.

## RESULTS AND DISCUSSION

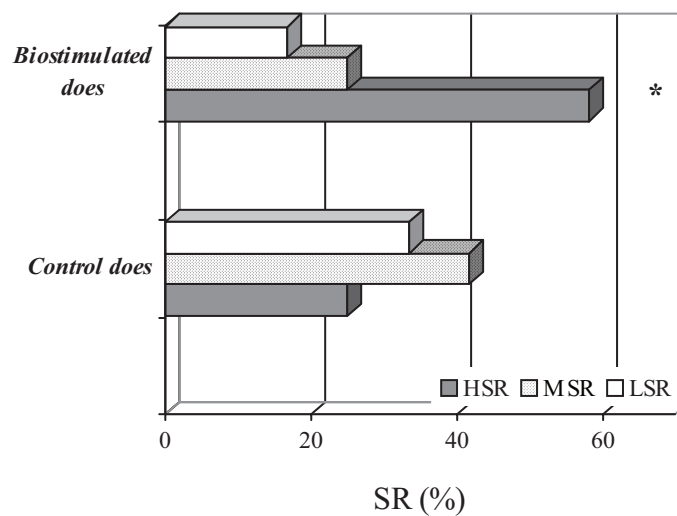
A rise in the mean  $E_2$  concentrations was observed 48 hours after doe-litter separation compared with control does and to previous values showed by both groups ( $p < 0.003$ ), (Fig. 1). The increased plasma  $E_2$  concentrations were similar to those described in does on day 1 and 8 after parturition (REBOLLAR *et al.*, 1992; UBILLA and REBOLLAR, 1995) and may be related to an increased follicular steroidogenic activity (OSTEEN and MILLS, 1980).



**Figure 1.** Plasma oestradiol-17 $\beta$  concentrations in *biostimulated* (▨) and *control* (□) does. The arrow indicates the time of artificial insemination (AI). Each column represents the means  $\pm$  s.e.m. of 12 samples. Significant differences between groups are indicated by different letters ( $p < 0.003$ ).

Low mean plasma P concentrations were found on day 9, 10 and 11 of the postpartum period without differences between groups ranging  $0.21 \pm 0.03$  ng/ml to  $0.36 \pm 0.08$  ng/ml. These P concentrations were lower to those previously observed in does that show oestrus signs during the early postpartum period suggesting the absence of active corpora lutea during this period (BOITI *et al.*, 1996). As a negative relationship between high P levels and SR was previously observed (FORCADA and ABECIA, 1990), no P inhibitory influence on SR before AI could be expected among the does in this study. A higher proportion of HSR does was observed 48 hours after doe-litter separation compared with the control group ( $P < 0.05$ ), (Fig. 2). An increased frequency of sexually receptive does was also observed after shorter doe-litter separations (MAERTENS, 1998), or when doe rabbits were grouped together during 15 minutes before insemination (DUPERRAY *et al.*, 1999; THEAU-CLÉMENT and POUJARDIEU, 1999). In the rabbit, as in other species, the GnRH release and action

appeared to be controlled by ovarian steroids and probably by endogenous opioids released during suckling (ORSTEAD and SPIES, 1987).



**Figure 2.** Sexual receptivity (SR) in *control* and *biostimulated* does. A higher proportion of HSR does were observed in the biostimulated group compared with the control group. (\*  $p < 0.05$ ). HSR = high SR does; MSR = medium SR does; LSR = low SR does.

The absence of suckling episodes together with high  $E_2$  concentrations seen in biostimulated does suggests a stimulatory effect of doe-litter separation on the ovarian activity inducing a higher proportion of HSR does. After AI, pregnant does in both groups showed high mean plasma P concentrations on day 18 ( $7.08 \pm 0.05$  ng/ml and  $6.33 \pm 0.7$  ng/ml, in biostimulated and control does, respectively) and were similar to those previously observed in rabbits with two or more corpora lutea (MILLS and STOPPER, 1989). Non pregnant does showed P concentrations similar to those found in pseudopregnant rabbits on day 18 during the corpus luteum regression (GADSBY, 1989). Mean plasma  $E_2$  concentrations observed in both groups on day 18 of pregnancy are in agreement with those obtained previously in pregnant does (CHALLIS *et al.*, 1973), or pseudopregnant does (DUGRÉ *et al.*, 1989). Control does showed lower  $E_2$  values on day 18 of pregnancy compared with biostimulated rabbits ( $p < 0.003$ ); (Fig. 1). These first results could suggest differences in the secretor activity of large follicles. In rabbits, after ovulation, a temporary depletion of large follicles has been determined increasing again after about 6 days (OSTEEN and MILLS, 1980). The luteal function of rabbit corpus luteum is oestrogen dependent and  $E_2$  has been identified as the main luteotrophic hormone (McLEAN and MILLER, 1985). Nevertheless, it would be necessary to study the relations between follicle populations during pregnancy and fertility in lactating does biostimulated prior to AI, as well as more frequent blood samplings in order to determine the role of the different hormones on the reproductive doe rabbit performance. All biostimulated does became pregnant compared to 10 of the 12 control does (100% vs. 83.3%). These differences were not statistically significant, probably due to the reduced number of does in each group. Litter size and the number of kits dead/litter were not affected by the treatment ( $9.08 \pm 0.41$  vs.  $9.20 \pm 0.39$  and  $0.51 \pm 0.13$  vs.  $0.43 \pm 0.11$ , in biostimulated and control does, respectively). These productivity parameters are in accordance with those previously reported, since high fertility rate was obtained when a large number of lactating does were inseminated after biostimulation for short periods (CASTELLINI *et al.*, 1998; MAERTENS, 1998). These results may indicate a major influence on the productivity of

nursing rabbits if biostimulation techniques are routinely employed prior to AI (THEAU-CLEMENT and POUJARDIEU, 1999; DUPERRAY *et al.*, 1999).

These findings suggest that a transient doe-litter separation causes an increase in the proportion of does showing signs of HSR, probably induced by the absence of suckling episodes and higher plasma E<sub>2</sub> concentrations in the biostimulated does before AI. The basal plasma progesterone levels observed before AI may be related to the absence of luteal influence on SR. The lower E<sub>2</sub> concentrations exhibited by control does on day 18 of pregnancy may suggest differences in the secretion of E<sub>2</sub> by large follicles. Although the fertility and litter size did not appear to be affected by the treatment, the routine used of a biostimulation method may produce a major impact on the productivity of nursing does.

## REFERENCES

- ALVARIÑO, J.M.R., DEL ARCO, J.A., BUENO, A. 1998: Effect of mother-litter separation on reproductive performance of lactating rabbit females inseminated on day 4 or 11 post partum. *World Rabbit Science*, **6**: 191-194.
- BOITI, C., CANALI, C., MONACI, M., STRADAIOLI, G., VERINI SUPPLIZI, A., VACCA, C., CASTELLINI, C., FACCHINI, E., 1998: Effect of postpartum progesterone levels on receptivity, ovarian response, embryo quality and development in rabbits. *Proceedings of the 6<sup>th</sup> World Rabbit Congress*, **2**: 45-49.
- CASTELLINI, C., CANALI, C., BOITI, C. 1998: Effect of mother-litter separation for 24 hours by closing the nestbox or change of cage, on rabbit doe reproduction performance. *World Rabbit Science*, **6**: 199-203.
- CEDARS, M.I., LU, J.K., MELDRUM, D.R., JUDD, H.L. 1990. Treatment of endometriosis with long-acting gonadotrophin-releasing hormone agonist plus medroxyprogesterone acetate. *Obstet. Gynecol.*, **75** (4): 641-645.
- COLIN, M. 1992: Cycles synchronisation in rabbit production *Journal of Applied Rabbit Research*, **15**: 398-406
- CHALLIS, J.R.G., PORTER, D.G., RYAN, K.J. 1973: The concentrations of progesterone, estrone and estradiol 17- $\beta$  in the plasma of pregnant rabbits. *Endocrinology*, **93**: 971-976.
- DRAGAN, N., MUSCALU, G.R., SEICIU, F.L., COCU, F., CIMPEANU, I., BUNACIU, M., DUMITRU, P., STEFANESCU, D., POP, T. 1996: Effect of prostaglandin analogues on sexual receptivity, fecundity and pregnancy of does *6<sup>th</sup> World rabbit Congress*, **2**: 65-67
- DUPERRAY, J., ECKENFELDER, B., THEBAULT, T., PROVOST, J.P. 1999: Effet du regroupement des lapines avant linsemination sur leurs performances de reproduction. *World Rabbit Science (Special Issue)*, **7**: 30-31.
- DUGRE, F.J., LAMBERT, R.D., BELANGER, A., FORTIER, M.A. 1989: Relationship between steroid levels in peripheral serum and uterine tissue during pseudopregnancy in rabbits. *Theriogenology*, **31**(2): 353-359.
- FACCHINI, E., CASTELLINI, C., RASETTI, G., BALLABIO, R. 1992: L'impiego di prostaglandina sintetica (alfaprostol) e di PMSG nella sincronizzazione degli resti e die parti nella coniglia *Rivista di Zootecnia Veterinaria*, **20**: 11-14
- FORCADA, F., ABECIA, J.A. 1990: Circulating progesterone levels, ovulation rate and sexual behaviour in rabbits during pseudopregnancy induced by vasectomized males and HCG injection. *Journal of Applied Rabbit Research*, **13**: 74-79.

- GADSBY, J.E. 1989: Control of corpus luteum function in the pregnant rabbit. *Journal of Reproduction and Fertility*, **37** (Suppl): **45-54**.
- HOLT, J.A. 1989: Regulation of progesterone production in the rabbit corpus luteum. *Biology of Reproduction*, **40**: **201-208**.
- MAERTENS, L. 1998: Effect of flushing, mother-litter separation and PMSG on the fertility of lactating does and the performance of the litter *World Rabbit Science*, **6**: **185-190**
- McLEAN, M.P., MILLER, J.B. 1985: Steroidogenic effect of 17 $\beta$ -estradiol on rabbit luteal cells in vitro: estrogens-induced maintenance of progesterone production. *Biology of Reproduction*, **33**: **459-469**.
- MCNEILLY, A.S., 1988. Suckling and the control of gonadotrophin secretion. In: The Physiology of Reproduction. E. Knobil and J.D. Neill (Editors). Raven Press, NY., pp.2323-2349.
- MILLS, T.M., STOPPER, V.S. 1989: The intraovarian progesterone modulation of follicle development in the rabbit ovary. *Steroids*, **54**: **471-480**.
- ORSTEAD, K.M., SPIES, H.G. 1987. Inhibition of hypothalamic gonadotrophin-releasing hormone release by endogenous opioid peptides in the female rabbit. *Neuroendocrinology*, **46**: **14-23**.
- OSTEEN, K.G., MILLS, T.M. 1980: Changes in the size, distribution and steroid content of rabbit ovarian follicles during early pseudopregnancy *Biology of Reproduction*, **22**: **1040-1046**.
- REBOLLAR, P.G., UBILLA, E., ALVARIÑO, J.M.R., ILLERA, J.C., SILVÁN, G. 1992: Influencia del nivel de receptividad sexual sobre el estradiol plasmático y la respuesta ovulatoria durante el postparto en la coneja *Revista Española de Fisiología*, **48**: **13-18**.
- STATISTICAL ANALYSIS SYSTEM INSTITUTE INC., 1990: SAS User's Guide, *Statistical Analysis System Institute Inc.*, Cary NC.
- STOVALL, T.G., LING, F.W., GRAY, L.A., CARSON, S.A., BUSTER, J.E. 1991. Single-dose methotrexate for treatment of ectopic pregnancy. *Obstet. Gynecol.*, **77** (5): **754-757**.
- THEAU-CLÉMENT, M., ROUSTAN, A. 1992: A study on relationships between receptivity and lactation in the doe and their influence on reproductive performances. *Journal of Applied Rabbit Research.*, **15**: **412-421**.
- THEAU-CLÉMENT, M., POUJARDIEU, B. 1999: Influence d'une separation mere-jeunes, pendant les 24 heures precedant l'insemination, sur les performances de reproduction des lapines et la croissance des lapereaux. *World Rabbit Science (Special Issue)*, **7**: **30**.
- UBILLA, E., REBOLLAR, P.G. 1995: Influence of the postpartum day on plasma estradiol-17 $\beta$  levels, sexual behaviour, and conception rate, in artificially inseminated lactating rabbits. *Animal Reproduction Science*, **38**: **337-344**.