PLASMA OESTRADIOL AND PROLACTIN IN SYNCHRONIZED MULTIPAROUS RABBIT DOES

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ABSTRACT

The effect in plasma levels of prolactin and oestradiol when different methods of oestrus synchronization were applied in multiparous lactating does was evaluated. Forty-eight multiparous rabbit does nursing more than eight kits were randomly allocated in four groups. The treatments were as follows: two groups were synchronized on day 2 post-partum, being separated 48 hours from his litter or hormonally treated with 25 UI of PMSG. A third group was separated from his litter on day 3 post-partum during 24 hours and a fourth one was the control group. All does were inseminated on day 4 post-partum with a pool of fresh semen and ovulation was induced with 1 μg of Busereline (i.m.) immediately after insemination. Blood samples were taken on day 2, 3 and 4 post-partum at 9:00 a.m. Plasma oestradiol was determined by EIA and plasma prolactin by RIA. All does had a plasma oestradiol level similar on day 2 and 3 post-partum (113.24±13.7 and 142.01±13.7 pg/ml, respectively). On day 4 post-partum, does treated with PMSG and separated 24 and 48 hours had a 63.07%, a 73.12% and a 93.47% more mean plasma oestradiol levels than previous values observed on day 3 post-partum (P<0.001). In addition, immediately before artificial insemination rabbit does separated 48 hours had higher plasma oestradiol levels than control does (267.42±27.84 *vs.* 172.33±26.76 pg/ml; P<0.001). There are not differences in mean plasma prolactin between groups or time of sampling. Similar conception rate was obtained in all groups (83. 33 %, 91.67 %, 83.33 % and 66.66 % in does separated 24 or 48 hours, control does and rabbits treated with PMSG respectively). Similar litter size at parturition (9.0 ± 0.9, 10.4 ± 0.8, 9.7 ± 0.9 and 8.9 ± 0.9 kits born alive) and at weaning (25d) (8.4 ±0.5, 8.4 ± 0.5, 7.7 ±0.5 and 7.7 ±0.6 weaned rabbits) were obtained among groups, but a higher number of born dead kits in does treated with PMSG than other groups was observed (1.12 ± 0.3 *vs.* 0.1± 0.3, 0.08 ± 0.25, and 0.1 ± 0.3 young dead rabbits; P<0.05).

Key words: oestrus synchronization, prolactin, oestradiol.

INTRODUCTION

Oestrus behaviour and sexual receptivity at the moment of insemination are related to the fertility parameters of the does (CASTELLINI, 1996; REBOLLAR *et al*. 1994; UBILLA and REBOLLAR 1995). To increase reproductive performance is necessary to apply synchronization methods in lactating rabbit. When a 42-day reproduction rhythm is applied, a 48 hours doe-litter separation is a useful method to increase fertility. Prolactin is essential for the maintenance of lactation (COWIE *et al*., 1969), and the absence of suckling before artificial insemination leads to a decrease in plasma concentrations of prolactin and an increase in plasma oestradiol levels (UBILLA *et al*., 2000). On the other hand, PMSG treatment is the most common means of inducing oestrus. But, when artificial insemination is applied on day 3-4 post-partum (35 day reproduction rhythm), lactating does are less receptive and ovulate less intensively than does on day 12 or 19 post-partum (THEAU-CLÉMENT *et al*., 2000). A 36 or 48 hours doe-litter separation applied on day 3-4 post-partum (ALVARIÑO *et al*., 1998) can improve productivity at birth by 76 to 92%, respectively. The aim of the present study was to determine the effects of different intervals of doe-litter separation and a hormonal treatment (PMSG) on mean plasma prolactin and oestradiol concentration, and on fertility and prolificacy, when multiparous lactating does are inseminated on day 3-4 post-partum.

MATERIAL AND METHODS

Forty-eight multiparous lactating rabbits (4 parturitions and more than 8 kits) of Californian x New Zealand White crossbreed were used in this study. The animals were housed in individual cages, maintained under controlled light/dark cycles (16L: 8D), and fed *ad libitum* with a commercial pelleted diet. The does were randomly allocated to one of four groups of 12: a *Control group* without synchronization, a *BIO48 group* separated during 48 hours from their litters, a *BIO24 group* separated during 24 hours, and a *PMSG group* treated with 25 UI (i.m.), 48 hours before artificial insemination. All does were artificially inseminated (AI) on day 4 post-partum using 0.5 ml /doe of fresh semen diluted in a commercial diluent (MA-24, Lab. Ovejero, (León Spain). Each dose contained at least 20 x 10^6 spermatozoa/ml. An injection of busereline (1μg/doe) was used to induce ovulation immediately after insemination.

To study the influence of treatments on mean plasma oestradiol (E_2) and prolactine (PRL) concentrations, blood samples were collected in all groups from the ear vein into heparinized tubes, and immediately centrifuged at 1000g, for 10 min, at 4 ºC. Plasma was stored at –20 ºC until analysed. The blood samples were collected at 24 hours intervals from day 2 to day 4 of lactation, at 9:00 am, before AI.

Plasma oestradiol was determined using EIA (SILVÁN *et al*., 1993). The sensitivity of assay was 0.1 pg per well. The intra- and inter- assay coefficient of variation was less than 6% and 7%, respectively. Prolactin concentrations were determined by specific RIA methods, using AFP-991086 antibodies for prolactin supplied by the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) and P.A. Parlow (Harbour-UCLA Medical Center, CA). Hormones were labeled with ^{125}I by the Chloramine-T-

method (GREENWOOD *et al*., 1963). The antibody titres used were 1:62,500. All samples were measured in the same radioimmunoassay to avoid inter-assay variations. The intra-assay coefficient of variation was less than 5% and the limit of detection was 0,125 ng/ml.

Statistical analysis of normal variables (hormone concentrations, litter size at birth, born dead at birth and litter size at weaning) was done with a linear model (Proc GLM, SAS, 1998) considering the interactive effect of treatment and time of sampling. Hormone concentrations 48 hours before artificial insemination was used as linear covariate. Non parametric variables (fertility rate) were analysed with a chi-square test (Proc CATMOD) with treatment as the main source of variation.

RESULTS AND DISCUSSION

Mean plasma concentrations of oestradiol were similar 48 and 24 hours before artificial insemination in control and treated does. On day 4 post-partum, does treated with PMSG and separated 24 and 48 hours had higher mean plasma oestradiol levels than mean values observed on day 3 post-partum (217.94 ± 14.03 *vs.* 142.01 ± 13.7 pg/ml respectively; $P<0.001$). Immediately before artificial insemination only rabbit does separated 48 hours had higher plasma oestradiol levels than control does (267.42 ± 27.84 *vs.* 172.33 ± 26.76 pg/ml; $P<0.001$).

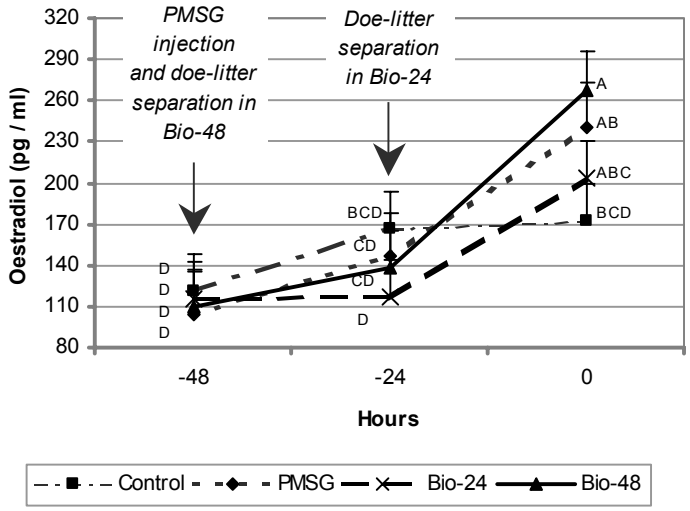

Figure 1. Plasma oestradiol concentrations in control rabbit does and synchronized rabbit does 48 or 24 hours before artificial insemination. Each point represents lsmeans ± sem of twelve animals. Different letters indicate statistical differences between groups ($P<0.001$).

A direct relation between plasma oestradiol mean levels and high or low sexually receptive rabbits has been reported (REBOLLAR *et al*., 1992). The increased plasma

oestradiol levels on day 4 post-partum observed in treated animals in relation to previous days are probably related to hormonal follicle stimulating action. After PMSG treatment, lots of results were published regarding their use in order to improve sexual receptivity and follicles growth (reviewed by MAERTENS *et al*., 1995). On the other hand, changes in plasma concentrations of oestradiol after 48 h of doe-litter separation may induce a higher ovarian activity and could explain a greater maturation of follicles growing in waves after parturition (DÍAZ *et al*., 1987).

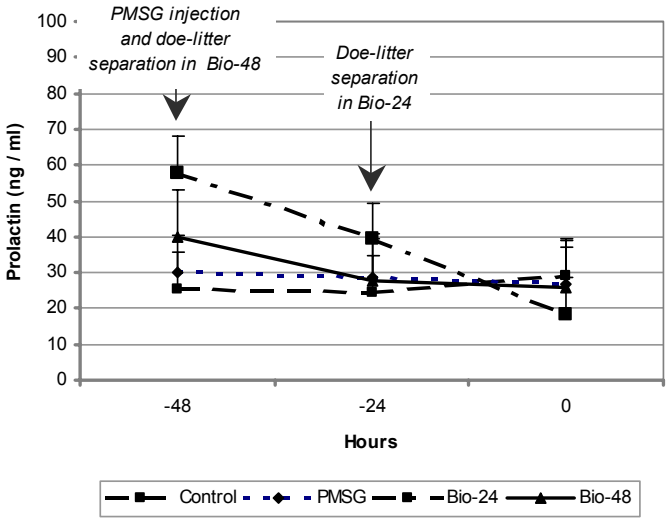

Figure 2. Plasma prolactin concentrations in control rabbit does and synchronized rabbit does 48 or 24 hours before artificial insemination. Each point represents lsmeans ± sem of twelve animals.

There are no differences in plasma concentrations of PRL 48, 24 or 0 hours before artificial insemination among the control and synchronized groups. Our results did not show any significant effect of doe-litter separation on plasma prolactin in contrast with results obtained in rabbit does separated 24 hours at 9 days post-partum (UBILLA *et al*., 2000). In rats, the suckling stimulus suppresses the number of GnRH pituitary binding sites and the degree of suppression is directly related to the intensity of the suckling stimulus (SMITH, 1984). It has been suggested that duration and frequency of suckling are not yet stabilized during the first post-partum day, even in multiparous does (COUREAUD, *et al*, 2000) and it could be possible that prolactin response observed on day 10 post-partum to be higher than response observed on the first days post-partum. In this way, FORTHUN-LAMOTHE and PRUNIER (1999) observed similar ovulation rate in lactating or non lactating full-fed females mated 3 days post-partum, whereas on day 10 post-partum, ovulation rate was lower in lactating ones. Moreover, milk production in the early post-partum is a 60-75% respect to milk production on day 12-19 postpartum (Lebas 1972).

Fertility and prolificacy results are showed in the next table.

Table 1. Fertility and prolificacy in control rabbit does (n=12) and synchronized rabbit does artificially inseminated: Bio24 (separated 24 hours, n=12), Bio48 (separated 48 hours, n=12) and PMSG (treated with 25 UI of PMSG, n=12). Values in the same row with differing superscripts differ (*P*<0.05)

	Control	Bio24	Bio48	PMSG	MSE
Fertility (%)	83.33	83.33	91.67	66.66	-
Total born	9.7	9.0	10.4	8.9	0.42
Born dead	0.1[b]	0.1[b]	0.08[b]	1.12[a]	0.14
Litter Size at weaning	7.7	8.4	8.4	7.7	0.27

The positive effects of PMSG to increase both fertility and prolificacy described by numerous literature reports are not observed, possibly due to the small number of animals inseminated (only 12 per group). Nevertheless, our results are consistent with BOURDILLON *et al*. (1992) and MILANÉS *et al*. (2004), who observed a much less positive effect of PMSG for does having a parity of at least 4 litters.

In agreement with MAERTENS *et al*. (1995) and BOITI *et al*. (1995), the average increase of young born dead/litter observed could be explained by the degenerated embryos obtained in does treated with PMSG.

In summary, the results of the present experiment made in multiparous does with more than 4 parturitions, show that future investigations should be directed to verify endocrinological effects of doe-litter separation during the first parturitions.

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